OPEN ACCESS

Ghana Alternative Medicine Journal (GAMJ)

Abbreviated Key Title: Gha alt Med Jrnl
ISSN 2756-7176 (Print) Open Access
Journal homepage: https://saspublishers.com/journal/gamj/home

Antiplasmodial Potential of Ethanolic Extract of *Moringa oleifera Lam* Seeds in PLASMODIUM Berghei-Infected Mice

Chinenye Chinaza Okorie[1], Kemzi Nosike Elechi-Amadi[2*], Ojoye Ngoye Briggs[2], Onengiye Davies-Nwalele[2], Lynda K. Giami[2], Joseph O. Ebifa[3]

[1]Zorel Pharmacy, Port Harcourt, Nigeria
[2]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria
[3]Department of Medical Laboratory Services, Federal Medical Centre, Nnebisi Road, Isieke 320242, Asaba, Delta, Nigeria

DOI: 10.36347/gamj.2023.v04i01.003 | **Received:** 24.11.2022 | **Accepted:** 31.12.2022 | **Published:** 07.01.2023

***Corresponding author:** Kemzi Elechi-Amadi
Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria

Abstract **Original Research Article**

This study evaluated the curative and prophylactic antiplasmodial potential of ethanol extract of Moringa oleifera seed. Seventy mice, weighing 20-30grams were used for the study. The mice were divided into seven groups (A-G) of five rats each. Groups A-F were the treatment groups, while group G was the control group. The mice were inoculated with *Plasmodium berghei* and then treated with standard antimalarial drugs (A, B and C) and 200mg/kg, 300mg/kg and 500mg/kg (D, E and F). The mice were treated for three days. Thereafter, blood films were made from the mice, dried, stained and viewed using x10 lens. The % parasitaemia and chemosuppression were determined. The ethanol extract significantly reduced the % parasitaemia in the treated mice, compared to the control group. However, the effects of the extract at the doses used for the study were less comparable to those of the standard drugs used for this study. The ethanol extract of Moringa seed has both curative and prophylactic antiplasmodial potential. However, these effects were less comparable to those of the standard drugs used for the study. It is recommended that the extract be considered for use as an antiplasmodial agent.

Keywords: Antiplasmodial, moringa, malaria, mice, malaria, plasmodium berghei.

INTRODUCTION

Malaria is a disease of great concern especially in African countries. It is reported that annually, about 300 million acute cases of the disease, leading to more than a million deaths, mostly in the sub-Sahara Africa where the disease is endemic [1]. It has been estimated that about 3.3 million people of the world population reside in malaria endemic zones and Africans are the most affected with about 90% of all malaria death [2]. Malaria disease is caused by malarial protozoa parasite from the genus *Plasmodium. It is* transmitted through the bite of female *Anopheles* mosquito. Malaria contributes to the disease burden, especially in developing countries like Nigeria. The management of malaria has been through case management using antimalarial drugs.

One of the major challenges associated with malaria disease is that of resistance. It has been reported that malaria parasite has developed resistance to antimalarial drugs, except the artemisinin [3]. Regrettably, due to its short half-life, artemisinin has been reported to produce fast recrudescence when used alone to treat malaria infection. For this reason, it is used in combination with other antimalarial substances, a combination called Artemisinin Combination Therapy (ACT) [4], which is the recommended therapy for the management of malaria infection. Also, the resistance of mosquitoes to insecticides has led to an increase in severe malaria, which has complicated the eradication of the disease as well as the resurgence of malaria [5]. Furthermore, many of the antimalarials used currently have high toxicities, which is also has its effect on patients' health [6].

The medicinal value of the parts of Moringa can never be overemphasized, however over an extended period, a different part of the plant was used

in folk medicine to cured certain diseases [7]. A study [8] has confirmed that the plants is used as traditional medicine for many purposes that enhance the body health. Several active constituents of Moringa can be used as modern medicine [9]. Every part of the moringa plant (that is, seeds, leaves, bark, roots, sap, and flowers) are edible, and also possess a variety of attributes; and these have been used in traditional medicine [9].

Studies indicate that Moringa possesses has a number of properties including anti-diabetic, antiinfammatory, anti-cancer [10], anti-ischemic [11] and even anti-plasmodial properties [12].

MATERIALS AND METHODS

A. Experimental Animals

A total of 70 healthy Swiss albino mice (*Mus musculus)* of both sexes, 6 to 8 weeks old and weighing 20-30 grams, were used for this study. They were gotten from the University of Port Harcourt Animal House. All mice were housed in regular animal cages in the laboratory in the Department of Pharmacology, and had access to feed and water *ad libitum*. The mice were kept in the laboratory to acclimatize to the environment for one week before experimentation. The cages were cleaned and beddings changed every 24 hours.

B. Collection and Preparation of *Moringa Oleifera* Seed Extract

A large quantity of mature seeds *Moringa oleifera* plant was plucked from a local garden in Port Harcourt, Rivers State. The plant was identified and authenticated at the Herbarium, Plant Science Department, University of Port Harcourt, Rivers State with the voucher specimen number, UPS/PSB/2016/025, given. It was dried under room temperature for 2weeks, and the seeds were cleaned thoroughly to remove dirt, the seed coats and wings were manually de-shelled. The seeds were dried in the shade at room temperature for a week at the Pharmacology Laboratory, Department of Pharmacology, University of Port Harcourt, Rivers State. The white kernels were ground to a fine powder using an electric blender and the powdered seeds were packaged in an air-tight plastic container until extraction.

The extraction was done using the cold extraction technique as described by another study [13]. Briefly, 200g of the Moringa powder was measured using an electrical measuring scale (The Electronics Counts, The Digital Weighing Balance, Golden-Mettler, USA) and poured into 500ml beaker containing 300ml of 90% ethanol solvent. The mixture was swirled for 10 minutes, kept for 24 hours and then filtered using Whatmann No1 filter paper. The pulp was then transferred into the beaker and another 300ml Ethanol solvent was added to it and kept for another 24 hours before filtering. The whole procedure was carried out for 72 hours and the whole liquid filtrate was evaporated using rotary evaporator at optimum temperature and concentrated at 60°C water bath. The seed extract was obtained as oil with weight and volume of 22.65g and 23mls respectively, transferred into a sterile McCartney bottle and stored in a cool, dry place. 1ml of stock seed extract is equivalent to 1gram since density of Moringa seed oil equals 0.9057g/cm^3 or 0.909g/cm^3 [14].

C. Determination of Phytochemical Components of *Moringa Oleifera* Seeds

Preliminary phytochemical screening of *Moringa oleifera* seeds were carried out using the standard methods.

D. Acute Toxicity Test (LD_{50}) of Moringa Seed Oil Extract

This was done using the modified Arithmetic method of Karber in another study [15]. Nine (9) mice, divided into three groups of three mice, were administered with 150, 500 and 1000 mg/kg body weight of ethanol extract of Moringa seeds. The animals were studied for 24 hours for signs of toxicity.

E. Source of Rodent Parasite and Inoculation of *Plasmodium Berghei*

The Chloroquine-sensitive *Plasmodium berghei* (*NK 65* strain) parasite-infected mice were sourced from the National Institute of Medical Research, Lagos, Nigeria. The parasite density of the mice was determined according to the method adapted from another study [16]. Briefly, on day 5 after the parasite inoculation, the parasite density was determined to be within 14- 20 parasite/field. The inoculated mice were kept and observed for similar symptoms of malaria infection seen in human.

The mice were then sacrificed after they were anesthetized using Ketamine. The blood was collected through cardiac puncture into a test tube containing 0.5% trisodium citrate (an anticoagulant).

The infected Blood was diluted with Phosphate buffer saline (PBS) to produce 1x 10^7 *P. berghei* parasitized red blood cells in every 0.2ml of the inoculation volume.

F. Experimental Design

The study investigated both the curative and prophylactic potentials of the Moringa seed extract.

i. Curative potential: The curative test for the antimalarial activity of ethanol extract of *Moringa oleifera* seeds on established *Plasmodium berghei* infection was conducted using the method adapted from another study [17]. Thirty five mice of both sexes were randomly divided into seven groups of five mice each (Groups A – G). They were inoculated with 0.2ml of 1x10^6 *P. berghei*

parasitized erythrocyte intraperitoneally on the first day (D_0), according to the method of Al-Adhroey *et al.*, 2010. Seventy – two hours after inoculation (D_3), a pre-treatment blood smear was collected from each mouse.

Groups A, B and C were then treated orally with Artemether/ Lumefantrine (20/120 mg/kg body weight), Artesunate/Mefloquine (300/375 mg/kg body weight) and Chloroquine (10mg/kg) respectively. The dosages were extrapolated from the human dose, using Paget and Barnes (1964) conversion table [18]. Groups D, E and F were treated orally with 200, 300 and 500mg/kg body weight of ethanol extract of Moringa seeds respectively, while Group G was treated orally with 0.2ml of sterile distilled water. Each mouse were treated orally once daily for three consecutive days (D_3-D_6) post inoculation and the thin film of blood smear made daily from the tail blood of each mouse before treatment.

ii. Prophylactic Potential: The prophylactic activity was conducted according to the method adopted from another study [19]. Thirty five mice of both sexes were divided randomly into seven group of five animal each (Groups A-G) and then treated for three consecutive days (D_0-D_2). Three groups (Groups A-C) were treated orally with Artemether/Lumefantrine (20/120 mg/kg body weight), Artesunate/Mefloquine (300/375 mg/kg body weight) and Chloroquine (10mg/kg) respectively, while Group G was treated orally with 0.2ml of sterile distilled water. On the fourth day (D_3), the mice were infected with 0.2ml of $1x10^6$ *Plasmodium berghei*-parasitized erythrocyte intraperitoneally. Seventy two hours (72) hours later (D_6), thin blood films were made from the tail of the mice using an experimental microscopic slides and the mean parasitemia in each group were assessed. After seven days (D_{13}), the blood smears were again collected to determine the parasitemia level of each mouse at this day.

G. Estimation of Parasitaemia Level

Thin blood films were made each mouse from the various groups. The blood was obtained from the tail region of each mouse. The films were allowed to air dry. The dried smears were fixed with 100% methanol for 2 minutes, and then stained with 10% Giemsa stain. The stained slides were allowed to stand for 10-15 minutes before washing off with distilled water, and allowed to air dry for 20 minutes. Each of the stained slides was examined using x10 magnification.

Percentage Parasitaemia and percentage suppression were calculated using the formulas below (Deressa *et al.*, 2010):

$$\%\ \text{Parasitemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes}} \times 100$$

$$\%\ \text{Suppression} = \frac{\%\text{Parasitaemia in control group} - \%\text{Parasitaemia in study group}}{\%\ \text{Parasitaemia in control group}} \times 100$$

H. Data Analysis

The data obtained from the study were analysed using Statistical Package for Social Scientists (SPSS) version 23. Results were expressed as mean ± Standard deviation. One- Way Analysis of Variance followed by Tukey's post hoc analysis was used to compare results between the treated and untreated groups. Statistical differences in results occurred at $p<0.05$.

RESULTS

1. Phytochemical Analysis

Phytochemical investigation of *Moringa oleifera* seeds revealed the presence of Carbohydrates, Saponins, Alkaloids, Cardiac glycosides, Flavonoids, Terpenoids and Terpenes as shown below (Table 1).

Table 1: Phytochemical composition of *Moringa oleifera* seeds

Phytochemicals	Remarks
Triterpenes	Present
Phlobatannins	Absent
Triterpenoids	Present
Alkaloids	Present
Carbohydrate	Present
Carotenoids	Absent
Saponins	Present
Cardiac glycosides	Present
Tannins	Absent
Flavonoids	Present
Anthraquinones	Absent
Fixed oil	Present
Cyanogenic glycoside	Absent

2. Acute Toxicity Test

The result from this experiment revealed that ethanol extract of *Moringa oleifera* seeds was non-toxic even at 1000 mg/kg body weight (Table 2). At each dose administered, no mortality was recorded in any of the groups of mice and no significant changes in signs of toxicity, such as diarrhoea, motor activity, convulsion and coma in the mice was observed. It can be suggested that the average LD_{50} ethanol extract of *Moringa oleifera* seeds may be greater than 1000 mg/kg body weight.

Table 2: LD_{50} calculation by modified Arithmetic method of Karber for ethanol extract of *Moringa oleifera* seeds

Group	Dose (mg/kg)	Dose Difference	No. of Mice	No. Death	Mean Death	Dose diff X Mean death
A	150	0	5	0/5	-	-
B	500	350	5	0/5	-	-
C	1000	500	5	0/5	-	-

$$LD_{50} = \text{Lowest dose that killed 100\%} - \frac{\sum \text{dose diff X mean death}}{\text{number of mice in each group}}$$

3. Curative Potential

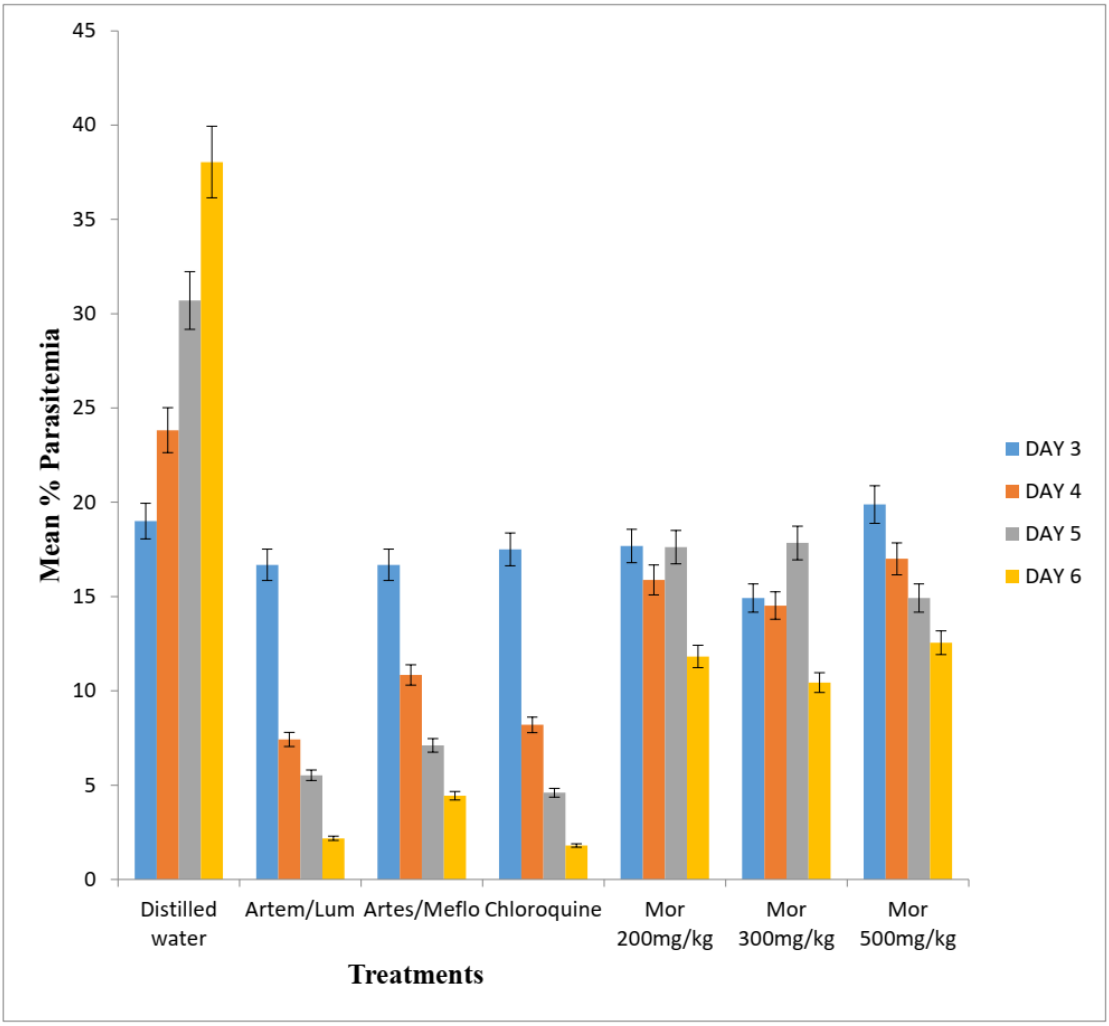

Figure 1: Effect of ethanol extract of *Moringa oleifera* seeds and standard antimalarial drugs on established *Plasmodium berghei* infection in Swiss albino mice

Table 3: Mean ±SEM of parasitemia and %chemosupression in *Plasmodium berghei* inoculated Swiss albino mice when treated with ethanolic extract of *Moringa oleifera Lam* seed and standard antimalarial drugs (curative test)

Treatments	Mean Parasitemia Day 3	Mean Parasitemia Day 6	% Chemosupression	p-value
Distilled water	19.00±2.05	38.04±1.50	0	-
Artem/Lum	16.68±4.87	2.18±0.42	94.27	<0.05
Artes/Meflo	16.68±5.30	4.44±0.73	88.33	<0.05
Chloroquine	17.50±3.11	1.80±0.29	95.27	<0.05
Moringa 200mg/kg	17.68±2.00	11.82±2.57	68.93	<0.05
Moringa 300mg/kg	14.92±1.51	10.44±1.75	72.56	<0.05
Moringa 500mg/kg	19.88±1.40	12.55±0.84	67.01	<0.05

4. Prophylactic Potential

In this study, it was a non-dose significant (P<0.05) reduction of parasitemia in *Plasmodium berghei* infected mice for ethanol extract of *Moringa oleifera Lam* seed was observed. The highest chemosupression was observed in the dose 300 mg/kg (79.18%), whereas the highest experimental dose (500 mg/kg) exhibited a low antiplasmodial effect when compared to the negative control as shown in Table 3. The standard drugs, A/L, A/M and CQ produced a much higher chemosupression of 96.72%, 96.21% and 97.16% respectively (Fig 2).

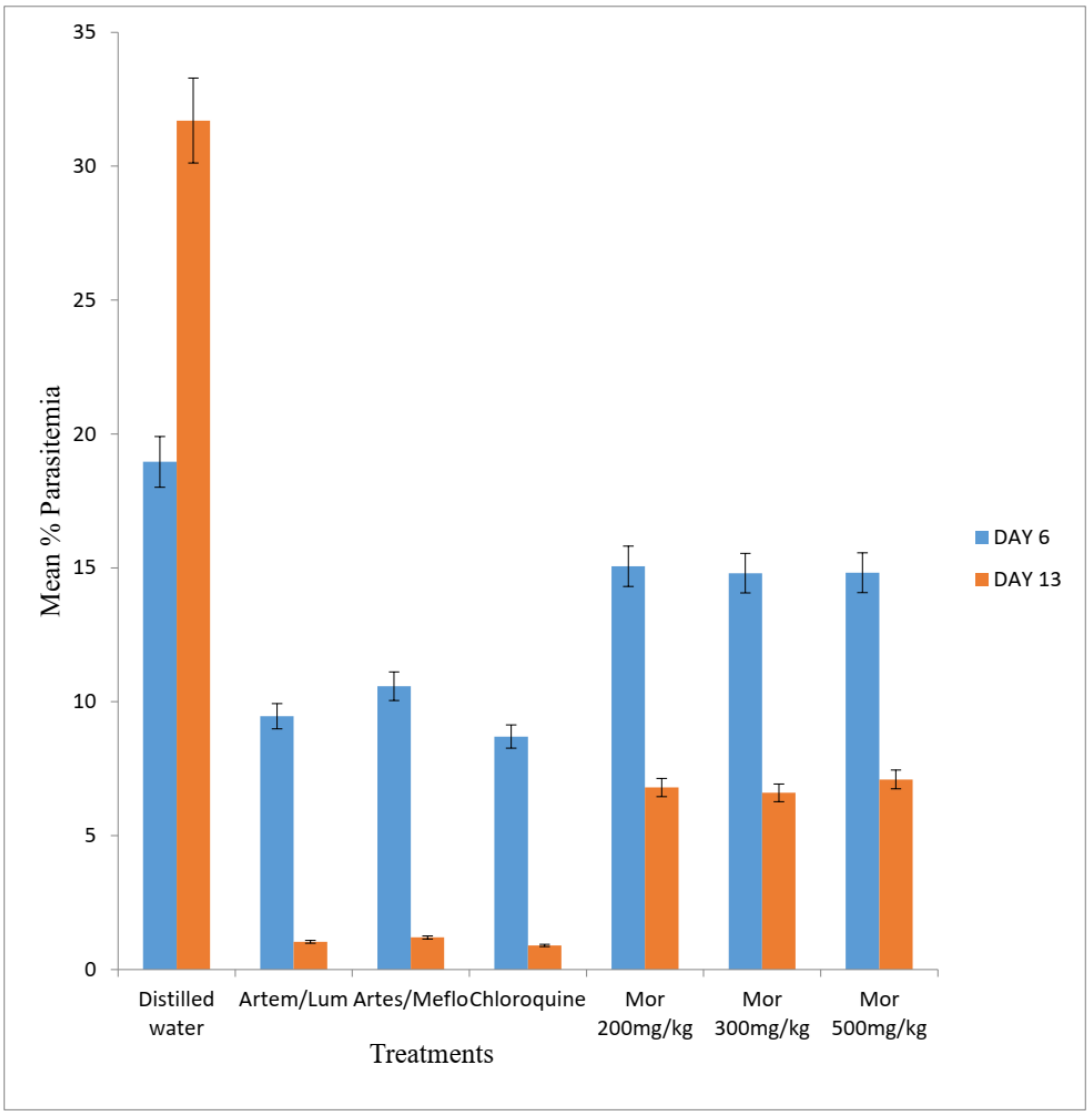

Figure 2: Prophylactic activity of ethanol seed extract of *Moringa oleifera Lam* and standard antimalarial drugs against *P. berghei berghei* infection in Swiss albino mice

Table 4: Mean percentage parasitemia and chemosupression in *Plasmodium berghei* infected Swiss albino mice treated with ethanolic extract of *Moringa oleifera Lam* seed and standard antimalarial drugs in the Prophylactic (Repository) test

Treatments	Mean Parasitemia Day 6	Mean Parasitemia Day 13	% Chemosupression	p-value
Distilled water	18.96±5.61	31.70±10.63	**0**	
Artem/Lum	9.46±2.23	1.04±0.31	96.72	<0.05
Artes/Meflo	10.58±0.60	1.20±0.49	96.21	<0.05
Chloroquine	8.70±3.20	0.90±0.39	97.16	<0.05
Moringa 200mg/kg	15.06±3.42	6.80±2.42	78.55	<0.05
Moringa 300mg/kg	14.80±3.54	6.60±0.72	79.18	<0.05
Moringa 500mg/kg	14.82±3.68	7.10±1.53	77.60	<0.05

DISCUSSION

This study evaluated the antiplasmodial potential of ethanolic extract of Moringa seeds in mice. The phytochemical study of the extract showed the presence triterpenes, triterpenoids, alkaloids, carbohydrates, saponins, cardiac glycosides, flavonoids and fixed oils, some of which have been shown to prossess antiplasmodial effects [20]. This result of the phytochemical evaluation of *Moringa oleifera* seeds agrees with the findings of other researchers [21]. The medicinal effects of herbal products have been attributed to the presence of phytochemicals in them. Flavonoids which was found to be present in *Moringa oleifera* seeds could be responsible for most antioxidant and anti-radical effects of *Moringa oleifera* that produced the antiplasmodial activity [22]. The acute toxicity study showed that the extract was safe and non-toxic up to 1000mg/kg.

There were significant reductions in the % parasitaemia in the treated mice, compared to the control group. The 200mg/kg and 300mg/kg showed non-dose-dependent redctions while the 500mg/kg showed dose-dependent reduction. However, the standard drugs gave better effect than the Moringa seeds extracts. The chemosuppression effects of the standard drugs were better than the Moringa seed extracts. The results of the curative study have showed the antiplasmodial activity of ethanol extract of *Moringa oleifera* seeds against *Plasmodium berghei* malaria parasite. This finding agrees with the work of another researcher [23].

The results from this study also indicate that the extract used for the study has potential prophylactic antiplasmodial effects. However, these effects are less comparable to those of the standard drugs. This is an acceptable therapeutic potential according to the categorization of *in vivo* antisplasmodial effects with chemosupression effect of up to 50% [24].

The result of this study suggests that the Moringa extract has more of plasmodistatic effect than plamodicidal as the parasites were not completely cleared by the seventh day and also a higher significant increase ($P \leq 0.05$) in the erythrocyte per field was observed in the CQ, A/L ad A/M treated groups when compared with the negative control which implies that the reduction in mean % parasitemia caused a rise in the red blood cell count of the infected mice.

CONCLUSION

The findings from this study shows that the ethanol extract of Moringa seed is safe and non-toxic up to the dose used in this study. The study also shows that the extract has antiplasmodial potential. However, the antiplasmodial effects of the extract is less comparable to those of the standard drugs used for this study. It is recommended that Moringa seed extract be considered for use as a potential agent for the management of malaria.

REFERENCES

1. Greenwood, B., & Mutabingwa, T. (2002). Malaria in 2002. *Nature*, *415*(6872), 670-672.
2. World Health Organization. (2011). World Malaria Report. *https://www.who.int › malaria.* Geneva.
3. Antony, H. A., & Parija, S. C. (2016). Antimalarial drug resistance: An overview. *Tropical parasitology*, *6*(1), 30-41.
4. Sinclair, D., Zani, B., Donegan, S., Olliaro, P., & Garner, P. (2009). Artemisinin-based combination therapy for treating uncomplicated malaria. *The Cochrane Database of Systematic Reviews*, (3), CD007483.
5. Silva, J. R. D. A., Ramos, A. D. S., Machado, M., de Moura, D. F., Neto, Z., Canto-Cavalheiro, M. M., ... & Lopes, D. (2011). A review of antimalarial plants used in traditional medicine in communities in Portuguese-speaking countries: Brazil, Mozambique, Cape Verde, Guinea-Bissau, São Tomé and Príncipe and Angola. *Memorias do Instituto Oswaldo Cruz*, *106*, 142-158. doi: 10.1590/s0074-02762011000900019
6. Sullivan, D. J., Kaludov, N., & Martinov, M. N. (2011). Discovery of potent, novel, non-toxic anti-malarial compounds via quantum modelling, virtual screening and in vitro experimental validation. *Malaria Journal*, *10*(1), 1-9. doi: 10.1186/1475-2875-10-274
7. Abdulkadir, A. R., Hasan, M. M., & Jahan, M. S. (2018). Antimalarial, antioxidant, antimicrobial properties of Moringa Oliefera Lam: A

review. *Australian Journal of Crop Science*, *12*(6), 905-908. doi:10.21475/ajcs.18.12.06.PNE920
8. Mishra, S. P., Singh, P., & Singh, S. (2012). Processing of Moringa oleifera leaves for human consumption. *Bulletin of Environment, Pharmacology and life sciences*, *2*(1), 28-31.
9. Stohs, S. J., & Hartman, M. J. (2015). Review of the safety and efficacy of Moringa oleifera. *Phytotherapy Research*, *29*(6), 796-804.
10. Park, E. J., Cheenpracha, S., Chang, L. C., Kondratyuk, T. P., & Pezzuto, J. M. (2011). Inhibition of lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase expression by 4-[(2′-O-acetyl-α-L-rhamnosyloxy) benzyl] isothiocyanate from Moringa oleifera. *Nutrition and cancer*, *63*(6), 971-982.
11. Kirisattayakul, W., Wattanathorn, J., Tong-Un, T., Muchimapura, S., Wannanon, P., & Jittiwat, J. (2013). Cerebroprotective effect of Moringa oleifera against focal ischemic stroke induced by middle cerebral artery occlusion. *Oxidative medicine and cellular longevity*, *2013*, 951415.
12. Fatima, T., Sajid, M. S., Jawad-ul-Hassan, M., Siddique, R. M., & Iqbal, Z. (2014). Phytomedicinal value of Moringa oleifera with special reference to antiparasitics. *Pakistan Journal of Agricultural Sciences*, *51*(1), 251-262.
13. Olasehinde, G. I., Ayanda, O. I., Ajayi, A. A., & Nwabueze, A. P. (2012). Invivo antiplasmodial activity of crude n-hexane and ethanolic extracts of Moringa oleifera (Lam.) seeds on Plasmodium berghei. *Intl. J. Med. Plants Res*, *1*, 50-54.
14. Anwar, F., & Bhanger, M. I. (2003). Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. *Journal of Agricultural and food Chemistry*, *51*(22), 6558-6563.
15. Aliu, Y. O., & Nwude, N. (1982). Veterinary Pharmacology and Toxicology experiments, 1st ed. Baraka Press and publishers Ltd Nigeria. 104.
16. Carvalho, L. H., Brandao, M. G., Santos-Filho, D., Lopes, J. L., & Krettli, A. U. (1991). Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium berghei-infected mice and in vitro against Plasmodium falciparum in culture. *Brazilian journal of medical and biological research= Revista brasileira de pesquisas medicas e biologicas*, *24*(11), 1113-1123.
17. Dawet, A., Yakubu, D. P., Wannang, N. N., & Mwansat, G. S. (2014). In vivo Antimalarial activity of stem bark of dry zone cedar Pseudocedrela kotschyi (Meliaceae) in mice. *European Journal of Medical Plants*, 4(3), 342-352.
18. Paget, G. E., & Barnes, J. M. (1964). Evaluation of Drug Activities. In Lawrence, D. R. & Bacharach, A. L. (Eds.). Pharmacometrics (pp.161). New York: Academy Press.
19. Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature reviews Drug discovery*, *3*(6), 509-520.
20. Kaur, K., Jain, M., Kaur, T., & Jain, R. (2009). Antimalarials from nature. *Bioorganic & medicinal chemistry*, *17*(9), 3229-3256.
21. Orman, E., Addo, P., Ofori, M. F., & Reimmel, K. A. (2015). Investigating the In-vivo Antiplasmodial Properties of Aqueous Extract of Moringa oleifera Lam (Moringaceae) Leaves. *British Journal of Pharmaceutical Research*, *5*(6), 419-430.
22. Sreelatha, S., & Padma, P. R. (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *Plant foods for human nutrition*, *64*(4), 303-311.
23. Nakinchat, S., & Somsak, V. (2018). A Pilot Study on Antimalarial Effects of Moringa oleifera Leaf Extract in Plasmodium berghei Infection in Mice. *Walailak Journal of Science and Technology (WJST)*, *15*(2), 151-156.
24. Deharo, E., Bourdy, G., Quenevo, C., Munoz, V., Ruiz, G., & Sauvain, M. (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology*, *77*(1), 91-98.