



Antiplasmodial Activity of Seed Oil of *Moringa oleifera* (Lam.)

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Authors' contributions

This work was carried out in collaboration between both authors. Author DEO designed the study and managed the literature searches. Author ASK performed the statistical analysis, wrote the protocol and the first draft of the manuscript and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to determine the antiplasmodial activity of seed oil of *Moringa oleifera* extracted using n-hexane solvent.

Acute toxicity test was carried out on twelve albino mice grouped into 3 according to seed oil concentrations (250, 500 and 1000 mg/kg respectively) with each group having four mice. Thirty-five mice were infected with *Plasmodium berghei* and the mice were randomized into five groups of seven mice each (groups A, B, C, D and E) for antiplasmodial activity. Group A - negative control (non-treated), group B - positive control (chloroquine treated); group C (800 mg/kg), group D (400 mg/kg) and group E (200 mg/kg) of seed oil of *Moringa oleifera*. All groups were left untreated until after five days when 0.2 mL treatment dose for each group was administered. Treatment was carried out in four days and left for another five days for post treatment effect.

The acute toxicity test showed that the seed oil of *Moringa oleifera* was safe and nontoxic to all mice. There was daily gradual reduction in PCV values; however group with the highest suppression of parasitemia had the highest PCV value after treatment. Group A as expected had the lowest PCV value of 22.23±1.98% which fell short of normal range (40 - 55%) and had its parasitemia load increased by 205% while in other groups, B, C, D and E the parasitemia had decreased by 100%, 97.02%, 90.48% and 67.65% respectively after treatment.

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Overall, the seed oil of *M. oleifera* at high concentrations showed a competitive parasite inhibition activity when compared with the result obtained in positive control group; however, few deaths recorded during and after treatment called for further investigation to determine its suitability for the treatment of malaria.

Keywords: Antiplasmodial; *Moringa oleifera*; seed oil.

1. INTRODUCTION

According to Muhammed et al. [1], among the top 10 causes of mortality despite 48% reduction of its incidence rate between 2010 and 2016 is still malaria. It was reported by the researcher that malaria is the most dangerous infection in the world and contributed to major socioeconomic problems which result in global instability and poverty. The disease is caused by a protozoan parasite of the genus *Plasmodium* and transmitted by the bite of an infected female *Anopheles* mosquito with clinical presentation such as high fever, chills and muscle pain. Muhammed et al. [2] reported five species of *Plasmodium* implicated in humans and they include *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*.

Ogundolie et al. [3] stated that in tropical and sub-tropical countries, *P. falciparum* is the most dominant and pathogenic species responsible for almost all death caused by malaria and the fifth species, *P. knowlesi*, has recently been implicated to be the most cause of human malaria in Malaysia and throughout Southeast Asia.

The resistance developed by *Plasmodium* species to Artemisinin Combination Therapy (ACT) including artemisinin derivatives and partner drugs which were currently recommended by World Health Organisation (WHO) as the first-line antimalarial treatments worldwide have endangered efforts to reduce the global burden of malaria [4].

To develop a new approach for the control and prevention of malaria Ogundolie et al. [3] stated that the study of *P. berghei* transmitted to rodents by the bites of an infected mosquito (*Anopheles durenii*) is a laboratory practical model for the study of human malaria aimed at developing an alternative medicine. An alternative treatment using traditional herbal medicine has been reported to be effective, less expensive, affordable, readily available even to people living in rural areas [5].

According to Boukandoul et al. [6], *Moringa*, the only genus of the Moringaceae family, is a fast-growing plant of 13 known species and it is generally distributed along tropical and subtropical region. Among its 13 species, *Moringa oleifera* Lam. get more attention worldwide, because of its economically important, especially in developing countries. Its oil as documented by Kayode et al. [7], are explored for the development of plant-based pharmaceuticals, food preservatives and antioxidant agents or as carriers of other additives such as flavor in processed foods and fragrance in cosmetic production. Vergara-Jimenez et al. [8] reported that the bioactive compounds such as flavonoids, chlorogenic acid, alkaloids, tannins, and isothiocyanates present in *M. oleifera* confer protection against diseases such as diabetes, atherosclerosis, non-alcoholic fatty liver disease, cardiovascular diseases/cancer and obesity in some tested model animals. Thus, this current study was carried out to determine the antiplasmodial activity of seed oil of *Moringa oleifera* in albino mice infected with *Plasmodium berghei*.

2. MATERIALS AND METHODS

2.1 Seed Collection and Identification

The seeds of *Moringa oleifera* were collected from a farmland in Bolorunduro, Ifedore LGA, Ondo State. The seeds were identified and authenticated by Dr. Fayehun Lawrence at the Department of Crop Soil and Pest (CSP) Management, School of Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Preparation of the Extracts

The method of Efevbokhan et al. [9] was adopted with some modifications. The dehulled and clean seeds were sun dried to a constant weight of 2kg and thereafter crushed and finely pulverized using a Philip blender (model HR2102). The *M. oleifera* oil was extracted from the pulverized seeds using 240 mL of n-hexane in soxhlet extractor arrangement. Rotary

evaporator was used to evaporate the n-hexane in the oil obtained and its amount was determined and stored in bottle for use.

2.3 Source of Experimental Mice

Albino mice of body weight between 18-22 g of forty-seven (47) were obtained from the Animal House, Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The saw dust bedding cages were used to house the animals at room temperature and ensure standard diet (grand cereal) and water ad libitum was provided for the animals in the each cage. The animals were left for 7 days to acclimatise before the experiments begin [2].

2.4 Grouping of Albino Mice

Olaniran et al. [10] method was adopted to group the albino mice. Twelve albino mice were grouped into 3 according to extract concentration (250, 500 and 1000 mg/kg respectively) with each group having four mice for acute toxicity test and a total of thirty-five (35) mice were divided into five groups of seven mice each for antiplasmodial activity: Group A - negative control (non-treated), B - positive control (chloroquine treated); group C (800 mg/kg), group D (400 mg/kg) and group E (200 mg/kg) of seed oil of *M. oleifera*.

2.5 Acute Toxicity

The method of Alo et al. [11] was used to carry out acute lethal test of the seed oil of *M. oleifera*. Each mouse in group 1 to 3 was respectively administered orally with a single dose of 0.2 mL of 250, 500 and 1000 mg/kg body weight of the seed oil of *M. Oleifera* and observed for three days before treatment began. Group containing 1000 mg/kg body weight dosage was prepared by dissolving 1.0g of the seed oil in a sterile universal bottle containing 8 mL of distilled water and 2 mL of tween20 to obtain 1000 mg/kg. Two-fold serial dilution was thereafter carried out to obtain 500mg/kg and 250 mg/kg group doses successively. The mice in each group were observed for body weakness, hyper-activity, reduce-activity, licking paw, salivation, inactiveness and death for 3 days.

2.6 Seed Oil Dosage Preparation

According to Ogundolie et al. [3] and Muhammed et al. [2], the seed oil dosage administered to the

mice in group C was prepared by dissolving 0.8g of the seed oil sterile universal bottle containing 8mL of distilled water and 2mL of tween20 to obtain 800 mg/kg dosage. The 400 and 200 mg/kg dosages for group D and E respectively were successively obtained by two-fold dilutions from 800 mg/kg to obtain 400 mg/kg (Group D) and thereafter two-fold dilutions from 400mg/kg to obtain 200 mg/kg (Group E) treatment dose.

2.7 Collection of Parasites

In Alo et al. [11] finding, through authenticated collection, *Plasmodium berghei* NK 65 was collected from IMRAT, University of Ibadan, Oyo State, Nigeria in a donor mouse. Parasites were withdrawn from the infected donor mouse by cardiac puncture and 0.2 mL of infected erythrocytes was diluted with sterile 4.8mL of normal saline to obtain 1×10^7 *Plasmodium berhei* stock. Each mouse in groups A, B, C, D and E were infected with 0.2mL of the parasites from the stock and each group was left for five days before commencement of treatment to obtain high loads of parasitemia ($\geq 40\%$ parasitemia level).

2.8 Determination of Packed Cell Volume

The packed cell volume (PCV) of each mouse was measured before, during and after treatment of the mice infected with *P. berhei*. Muhammed et al. [1] and Olaniran et al. [10] documented that from the tail of each mouse, collect blood into heparinized capillary tubes filled up to $\frac{3}{4}$ of the entire tube and sealed the tube using sealant and place sealed end of the tube outwards in a microhematocrit centrifuge and centrifuged at 12,000 revolutions per minutes for 5 minutes. To determine the PCV values, place the centrifuged tube into microhaematocrit reader to determine the volume of erythrocytes in a given volume of blood.

2.9 Determination of Parasitemia and Percentage Chemo suppression

Treatment of infected mice were carried out on day six to nine (four days) and all groups were left till day fourteen (five days after treatment) to determine post-treatment effect on the mice before sacrifice. According to Muhammed et al. [1] to determine the parasitemia loads for each group (A to E) from day five to fourteen, a drop of blood collected daily on a microscope slide by venesection of each mouse tail to make thin and thick films. The films on the glass slides were air-

dried at room temperature and thereafter fixed with methanol for two minutes and stained with 10% Giemsa for 15 minutes before rinse in water. The rinsed slides were air-dried, examined and counted under a light microscope at x100 magnification using oil-immersion. To measure the parasitemia loads, a minimum of three fields per slide with 100 red blood cells (RBC) per field were counted.

Parasitemia load = $\frac{\text{Count of RBC Parasitised} \times 100}{\text{Total Count of RBC Examined}}$.

% Chemosuppression = suppression =

$\frac{\text{Parasitemia before Treatment} - \text{Parasitemia after Treatment} \times 100}{\text{Parasitemia before Treatment}}$

2.10 Statistical Analysis

One-way data analysis of variance was used and all data were presented as a mean of three determination \pm standard error means.

3. RESULTS

3.1 Toxicity Effect of Seed Oil of *Moringa oleifera*

The result of acute toxicity shown in Table 1 revealed that there were no signs of toxicity such as paw licking, sleeping, reduced activity, respiratory distress observed in mice and there was no mortality at all dosages level used.

3.2 Effect of Seed Oil of *M. oleifera* on Mice Body Weight and Pack Cell Volume (PCV)

The result of the effect of *M. oleifera* seed oil on the body weight of mice is shown in Table 2. The body weight of mice in group A (negative control) reduced from 19.87 g to 18.86 g from day 1 to day 14. Similar weight losses were also observed in mice in group C, D and E (seed oil of

M. oleifera treated groups) as the number of days increased even though the loss was not statistically significant except in group E (18.71 to 15.25). However, weight of mice in group B (chloroquine treated group) increased as the treatment progressed with days from 20.14 g to 20.71 g. This is not statistically significant.

The Fig. 1, showed the gradual reduction in percentage of pack cell volume (PCV) of mice under study before infected and after treatment. The result obtained showed that group A (negative control) had PCV value of $22.23 \pm 1.98\%$ which fell far short from normal range (40 - 55%) after treatment. Other groups; B, C and D with the exception of group E had PCV values within the normal PCV range after treatment. The PCV value for Group E value was $39.19 \pm 1.82\%$ which was statistically not significant when compare with the normal range.

3.3 Percentage Mean of Parasitemia and Mortality

The outcome of the percentage mean of parasitemia in four days of treatment and five days post-treatment was presented in Table 3. Group A = negative control, B = Positive controgroup, C = 800mg/kg of seed oil of *M. oleifera* (M.O), D = 400 mg/kg of seed oil of M.O. and E = 200 mg/kg of seed oil of M.O. As expected, the negative control (A) group had increase in the parasitemia level by 205% in 9 days (from 6.09 ± 0.16^d to 18.60 ± 0.68^a) while there was total clear off of the parasites in positive control group B (9.78 ± 0.62^b to 0.00 ± 0.00^e). There was also a great reduction in parasitemia level recorded in group C (from 9.81 ± 1.59^a to 0.25 ± 0.34^c), group D (4.46 ± 0.24^e to 0.42 ± 0.29^d) and group E (7.82 ± 0.59^c to 2.53 ± 0.66^b) treated with seed oil of M.O. The increase and decrease in parasitemia count is statistically significant.

Table 1. Acute toxicity of seed oil of *Moringa oleifera*

Groups	Dosage (mg/kg)	Mortality	Mortality (%)	Signs of Toxicity
1	250	0/4	0	Nil
2	500	0/4	0	Nil
3	1000	0/4	0	Nil

Legend: Group 1 = 250 mg/kg body weight of seed oil of *M. oleifera*

Group 2 = 500 mg/kg body weight of seed oil of *M.oleifera*

Group 3 = 1000 mg/kg body weight of seed oil of *M.oleifera*

Table 2. Effects of seed oil of *M. oleifera* on mice body weight. Mice mean weight per day in grams

DAYS	A	B	C	D	E
0 - 5	19.86±0.51 ^b	20.14±0.59 ^a	18.86±0.51 ^c	20.14±1.08 ^a	18.71±0.36 ^d
6	20.29±0.47 ^b	19.43±0.78 ^d	19.57±0.90 ^c	20.71±1.41 ^a	18.14±0.74 ^e
7	20.29±0.78 ^a	19.43±0.65 ^c	19.83±1.10 ^d	19.57±1.34 ^b	17.14±0.86 ^e
8	20.43±0.78 ^a	19.43±0.72 ^c	19.50±1.20 ^d	19.57±1.49 ^b	17.00±0.90 ^e
9	20.29±1.02 ^a	19.43±0.72 ^b	19.50±1.10 ^c	19.00±1.48 ^d	16.14±0.86 ^e
10 -14	18.86±0.74 ^b	20.71±0.47 ^a	17.20±0.81 ^d	19.33±1.14 ^c	15.25±0.82 ^e

Data are represented as mean ± standard error where count (n) = 3. Different means superscripts in the same row show significant difference (P < 0.05).

A = Negative Control Group

B = Positive Control Group

C = 800 mg/kg of seed oil of *Moringa oleifera*

D = 400 mg/kg of seed oil of *Moringa oleifera*.

E = 200 mg/kg of seed oil of *Moringa oleifera*

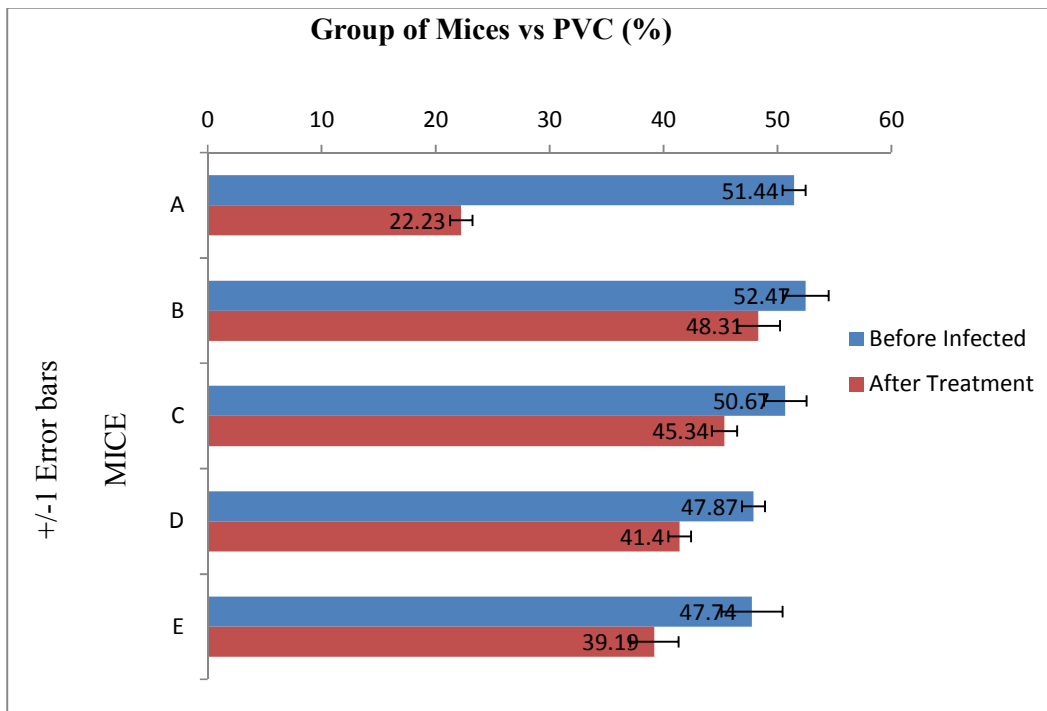


Fig. 1. Shows packed cell value expressed in % Measured before infection exposure and after treatment in experienced mice

Keys: A = Negative Control Group

B = Positive Control Group

C = 800mg/kg of seed oil of *Moringa oleifera* group

D = 400mg/kg of seed oil of *Moringa oleifera* group

E = 200mg/kg of seed oil of *Moringa oleifera* group

However, in groups treated with seed oil of *Moringa oleifera* (group C, D and E) minimal death were observed during and after treatment. Group C recorded two, D had four and E three death of mice. The percentage of death was presented in Table 4.

3.4 Percentage Chemosuppression of Parasitemia

Fig. 2, showed the trend analysis of percentage inhibition of parasitemia after treatment. Succinctly, there was no inhibition or suppression

of parasites observed in group A (infected and untreated group), a total parasite inhibition (100%) in group B, higher parasites inhibition (97% and 90%) were respectively observed in group C (800 mg/kg) and D (400 mg/kg) respectively and low parasites suppression in group E (200 mg/kg treated with seed oil of M.O.) of about 67%.

4. DISCUSSION

The acute toxicity test of the seed oil of *M. oleifera* carried out at different concentrations on the mice of average weight of 20g showed no mortality and signs of toxicity on all the experimental mice. This is in agreement with Da et al. [12] who reported that any chemical exhibiting LD₅₀ above 1000 mg/kg is practically non-toxic and with Zade et al. [13] that extract from seed of *M. oleifera* would be non-toxic even at 5000 mg/kg body weight.

The observed body weight loss in all groups except in positive control groups could be due to

loss of appetite, increased in the metabolic rate, reduced feed conversion efficiency and invariably as a sign of malaria-infected mice. This confirmed the report of Alo et al. [11] and Muhammed et al. [1]. The gain of body weight reported in positive control (group B), after treatment could probably be a sign of total recovery from the illness.

Olaniran et al. [10] defined packed cell volume (PCV) as a measure of erythrocytes proportion in a given blood sample and it is an indication of degeneration or amelioration of a diseased state in the animal or human blood. In addition, the researcher described PCV as a procedural which indicates the state of health of a human or animal and the extent of infection of the erythrocytes in health facilities. Furthermore, Olaniran et al. [10] concluded that agents that can protect body from infection or kill parasites will significantly improve the PCV level of infected animals treated than the untreated ones. This study corroborated Olaniran et al. [10] findings. The positive control (group B) gave the highest PCV value (48.30±1.84%), followed by group C

Table 3. Percentage mean of parasitemia count per days

Days	A	B	C	D	E
0 – 5	6.09 ± 0.16 ^d	9.78 ± 0.62 ^b	9.81 ± 1.59 ^a	4.46 ± 0.24 ^e	7.82 ± 0.59 ^c
6	6.64 ± 0.39 ^c	3.26 ± 0.42 ^e	7.97 ± 1.12 ^a	3.83 ± 1.21 ^d	7.78 ± 0.49 ^b
7	9.08 ± 0.24 ^a	0.53 ± 0.09 ^e	5.79 ± 0.88 ^c	1.85 ± 0.56 ^d	6.07 ± 0.45 ^b
8	11.03 ± 0.38 ^a	0.01 ± 0.01 ^e	3.60 ± 0.58 ^c	1.40 ± 0.61 ^d	4.09 ± 0.49 ^b
9	15.91 ± 0.71 ^a	0.00 ± 0.00 ^e	2.16 ± 0.49 ^c	0.89 ± 0.33 ^d	3.21 ± 0.36 ^b
10 – 14	18.60 ± 0.68 ^a	0.00 ± 0.00 ^e	0.25 ± 0.34 ^c	0.42 ± 0.29 ^d	2.53 ± 0.66 ^b

Difference ($P < 0.05$).

A = Negative Control Group

B = Positive Control Group

C = 800 mg/kg of seed oil of *Moringa oleifera* group

D = 400 mg/kg of seed oil of *Moringa oleifera* group

E = 200 mg/kg of seed oil of *Moringa oleifera* group.

Values are mean ± S.E (n=3). Means with different superscripts in the same row show significant

Table 4. Mortality rate of seven mice before, during and after treatment in days mice death rate per day in percentage

DAYS	A	B	C	D	E
0 - 5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	14.30	0	0
8	0	0	0	14.30	0
9	0	0	0	0	0
10 -14	0	0	14.30	42.90	42.90
Total Death (%)	0	0	28.60	57.20	42.90

Keys: A = Negative Control Group

B = Positive Control Group

C = 800 mg/kg of seed oil of *Moringa oleifera* group

D = 400 mg/kg of seed oil of *Moringa oleifera* group

E = 200 mg/kg of seed oil of *Moringa oleifera* group

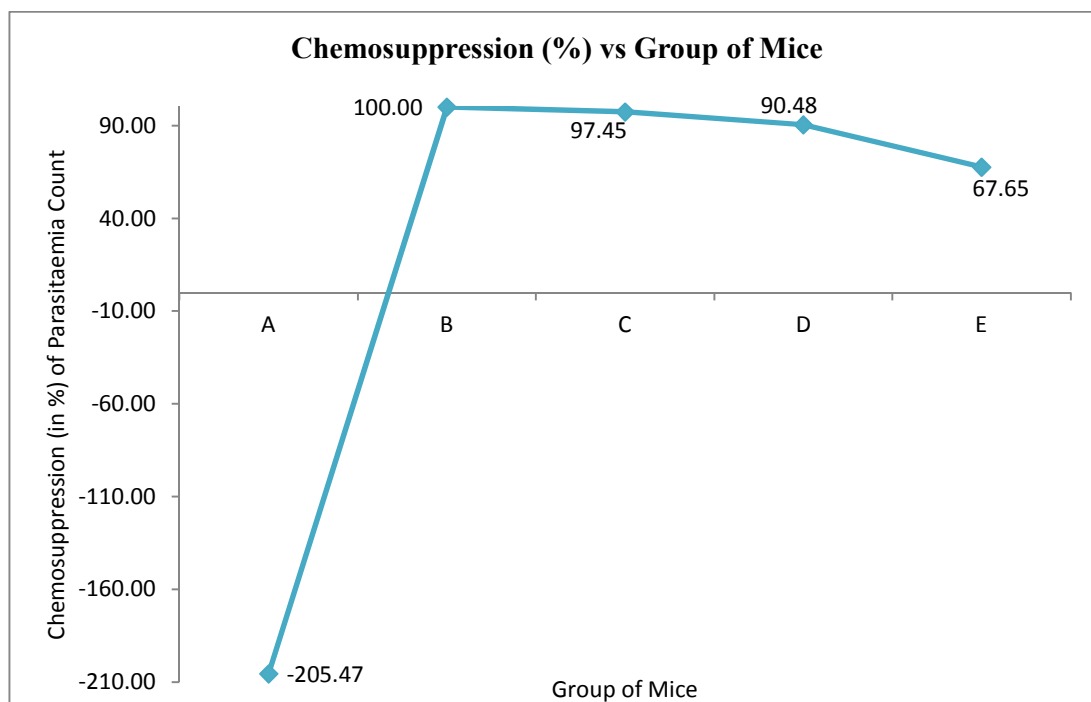


Fig. 2. Shows parasitaemia count expressed as percentage of inhibition of parasites in untreated and treated mice

A = Negative Control Group
 B = Positive Control Group
 C = 800 mg/kg of seed oil of *Moringa oleifera* group
 D = 400 mg/kg of seed oil of *Moringa oleifera* group
 E = 200 mg/kg of seed oil of *Moringa oleifera* group

(45.34±0.98%), D (41.40±1.57%) and least in group E (39.19±1.82%). The percentage means PCV values for each group approximately falls within a normal range value (40 - 55%) except of negative control, group A (22.23±1.98%). This proved that the higher the PCV values obtained in treated animals, the more effective the agents of chemosuppression.

Based on the results obtained in this research, there was significant and improved antiplasmodial activity of the seed oil of *M. oleifera* at 800 mg/kg body weight (Groups C), 400 mg/kg body weight (Group D) and 200 mg/kg body weight (Group E) when compared with group A (non-treated) with time. The *M. oleifera* seed oil showed promising inhibition of parasitaemia ranging from 97% in group C, 90% in group D and 68% in group E as depicted in Fig. 2. However, the highest parasitaemia inhibition as expected was observed in group B (chloroquine treated group) followed by group C and D and least (68%) in E with lowest concentration of 200 mg/kg. Thus, the treatment

of *Plasmodium* with seed oil of *M. oleifera* could be compared competitively with the chloroquine treated group of B. This study buttressed Orman et al. [14] research, whose finding showed that phytochemicals may be responsible for the *in-vivo* antiplasmodial activity exhibited by the extracts and that *Moringa oleifera* has been shown to be effective within the range 250– 500 mg/kg body weight.

According to Table 3, it was shown that the seed oil of varying concentrations of *M. oleifera* gave progressive decrease in parasitaemia count with time and the highest clearance of parasites were observed on the fourteenth day of the experiment after treatment had lapsed on the ninth day. It can therefore be adjudged that the seed oil of *M. oleifera* has residual potency. The dosage of the varied concentrations was administered once daily during treatment (four days) and thus there were possible accumulation of the oil in the cell leading to cumulative antiplasmodial activity. This report confirmed the study of Olasehinde et al. [15]. Daskum et al. [16] however stated that

the presence of certain phytochemicals such as phenols, tannins, alkaloid and flavonoids in crude hexane, methanol and lyophilized aqueous extracts may perhaps make the plant a good candidate source for antimalarial formulations.

The report in Table 4 of the death of two, four and three mice in group C, D and E respectively could be as a result of continuous accumulation of excessive fluid rather than the toxicological activity of the seed oil of *M. oleifera*. This finding confirmed Osman et al. [17] who reported that death occur in experimented mice due to water intoxication as fluid outside and inside the cell became imbalance which can lead cell to swell and rupture. The researcher also documented that extract of *M. oleifera* lethal dose was found to be greater than 6400mg/kg body weight.

5. CONCLUSION

At high concentration, the seed oil of *Moringa oleifera* possesses active antiplasmodial activity capable of treating malaria disease. To determine the suitability of the oil for human consumption, histopathological examination of certain organs should be carried out to detect eventual alterations as no death was recorded in the group A (negative control - the infected but non-treated) and group B (positive control – chloroquine treated) mice,.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Research and Ethics Committee of the Department of Microbiology, Federal University of Technology, Akure, Nigeria approved the entire experimental handling and management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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