Antiplasmodial Activity of seed oil of *Moringa oleifera* (Lam.)

ABSTRACT

The study was carried out to determine the antiplasmodial activity of seed oil of *Moringa oleifera* extracted using n-hexane solvent. Twelve albino mice of body weight between 18-22g were randomized into 3 groups (250, 500 and 1000mg/kg respectively) of four mice each for acute toxicity test. Thirty-five mice were also randomized into five groups of seven mice each (groups A, B, C, D and E) for antiplasmodial activity. Group A - negative control (infected and untreated), group B - positive control (infected and treated with chloroquine diphosphate), group C (800mg/kg), group D (400mg/kg) and group E (200mg/kg) seed oil of *M. oleifera.* All the groups were infected with *P. berghei* and left untreated until after five days. Group C, D and E were treated with 0.2 mL of 800, 400 and 200 mg/kg body weight of seed oil of *M. oleifera* respectively. Group B (positive control) were treated with 0.2 mL of 10 mg/kg body weight of chloroquine. Group A (negative control) were administered with 0.2 mL of normal saline. All treatment were 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 *M. oleifera* was safe and nontoxic to all mice. There was gradual reduction in PCV values daily, however the higher suppression of malaria can translate to higher PCV in treated animals. Group A (infected and untreated group) had PCV value of 22.23±1.98% which fell short of normal range (40 - 55%) when compared with treated groups (B, C, D and E). For the group A (infected and not treated), the parasitemia load increased by 205% while in groups B, C, D and E the parasitemia load had decreased or inhibited by 100%, 97.02%, 90.48% and 67.65% respectively after fourteen days of study. 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 were recorded during and after treatment with the seed oil. Thus, the seed oil of *M. oleifera* could be suitable for the treatment of human malaria.

Key words: Antiplasmodial; *Moringa oleifera*; seed oil

1.0 Introduction

Malaria is still among the top 10 causes of mortality despite 48% reduction of incidence rate and 44% of mortality between 2010 and 2016. It is the most dangerous infection in the world and contributed to major socioeconomic problems which lead to global instability and poverty (Muhammed *et al.,* 2018). It is a disease that causes high fever, chills and muscle pain. Caused by a protozoan parasite of the genus *Plasmodium* and transmitted by the bite of an infected female *Anopheles* mosquito, and according to Muhammed *et al.,* 2019, five species of *Plasmodium* (*Plasmodium falciparum*, *P.vivax, P. malariae, P. ovale* and *P. knowlesi*.) have been implicated in humans). Ogundolie *et al.* (2017) stated that *P. falciparum* is the most dominant and pathogenic species, responsible for almost all mortality caused by malaria in tropical and sub-tropical countries, where the temperature and rainfall are optimum for the development of vectors and parasites. The fifth species, *P. knowlesi*, which is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques that has recently been implicated to be a major cause of human malaria in Malaysia.

P. berghei is a practical model organism in the laboratory for the study of human malaria aimed at developing a new management measure for the control and prevention of malaria. This parasite (*P.berghei*) is transmitted to rodents, by the bites of an infected mosquito (*Anopheles dureni*) (Ogundolie *et al.,* 2017).The current efforts to reduce the global burden of malaria are threatened by the rapid emergence and spread of *P. falciparum* resistance to Artemisinin Combination Therapy (ACT) including artemisinin derivatives and their partner drugs which were at present recommended by World Healh Organisation (WHO) as the first-line antimalarial treatments worldwide. This has led to an increase pressure to develop alternative treatment using traditional herbal medicine which is less expensive, affordable, readily available to people living in rural areas (Ekeopara and Ugoha, 2017 and Dada and Muhammed, 2018).

According Boukandoul *et al*. (2018), *Moringa* the sole genus of the Moringaceae family, order Brassicales, comprises fast-growing plants, widely distributed along tropical and subtropical climates and consist of 13 known species. Among its 13 species, *Moringa oleifera* Lam. is the one receiving more attention worldwide, being among the most economically important tree crops, especially in developing countries. The oil of *M. oleifera* may be explored for the development of useful 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 (Kayode *et al.,* 2015). Vergara-Jimenez *et al*. (2017) 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. Hence, the present study is aimed at determining the antiplasmodial effect of seed oil of *M. oleifera* in Swiss albino mice infected with *Plasmodium berghei.*

2.0 MATERIALS AND METHODS

2.1 Seed Collection and Identification

The seeds were collected from a farmland in Bolorunduro, Ifedore LGA, Ondo State, Nigeria. The seeds wereidentified and authenticated by using the herbarium specimens and the voucher specimen number of the seed (Bio/ FUTA/ 80) was left in the herbarium of the Department of Crop Soil and Pest Management, School of Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Preparation of the Extracts:

The method of Efeovbokhan *et al.* (2015) was adopted with some modifications. The seeds were first dehulled, cleaned, sun dried and oven dried to a constant weight of 2kg. It was then crushed using a mortar and pestle, and finely pulverized using a Philip blender. The pulverized sample was extracted using the soxhlet extractor arrangement equipped with thimble. The extraction was carried out at varied times using 240 mL of hexane. The process was repeated twice to ensure that most of the oil in the seed was extracted using fresh solvent. The crude oil with solvent extracted was then collected into the pre-cleaned beaker for distillation. The seed oil was poured into the round bottom flask of the rotary evaporator arrangement to evaporate the hexane in the seed oil. The amount of oil obtained was then be weighed and stored for further analysis.

2.3 Source of Experimental Mice

Forty-seven (47) Swiss albino mice of body weight between 18-22g were obtained from the Animal House, Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The animals were housed in cages with saw dust bedding at room temperature and fed with standard diet (Grand cereal) and water ad libitum. They were acclimatized for 7 days prior to the study (Muhammed *et al.*, 2019).

2.4 Grouping of Albino mice

The method described by Olaniran *et al.* (2019), was used to group the experimental mice. A sum of twelve (12) mice were used for acute toxicity test and the mice were grouped into three while a total of thirty-five (35) mice were divided into five groups of seven mice eachfor antiplasmodial activity - Group A- not treated (negative control), group B - chloroquine treated (Positive control) and Groups C, D and E - *M. oleifera* seed oil treated.

2.5 Acute Toxicity

Acute toxicity test of the seed oil of *M. oleifera* was carried out using the method of Alo *et al*. (2018). Each mouse in group 1 to 3 was respectively administered orally with 0.2 mL of 250, 500 and 1000 mg/kg body weight of *Moringa oleifera* seed oil. The mice were observed for seven days for mortality, body weakness, hyper-activity, reduce-activity, licking paw, salivation, inactiveness and death.

2.6 Preparation of Oil Extracts Dosage

The dosages of the seed oil administered to the mice in group C were prepared by dissolving 0.8g of the seed oil in 8 mL of distilled water containing 2 mL of tween20 to obtain 800 mg/kg in sterile universal bottle. The 400 and 200mg/kg dosages were successively obtained by 1/2 dilution from 800mg/kg and thereafter 400mg/kg obtained body weight dosage and administered orally respectively as treatment dose to mice in groups D and E (Ogundolie *et al*. (2017) and Muhammed *et al.* (2019).

2.7 Collection of Parasites.

Chloroquine sensitive strain of malaria parasite (*Plasmodium berghei NK 65*) in a donor mouse was obtained from IMRAT, University of Ibadan, Oyo State, Nigeria. Parasite was kept alive by inoculating 0.2mLs of it into a healthy mouse (infected mouse) through intraperitoneal route. By cardiac puncture, 0.2mL of the parasites were withdrawn from the infected mouse and serially diluted with sterile 4.8mLs of normal saline to obtain 1x 10^7*Plasmodium berghei* infected erythrocyte. Mice in groups A, B, C, D and E were given 0.2mLs of the parasite and left for five days before treatment for the high establishment of parasitemia level (≥40% parasitemia level). Mice were visually observed for behavioral changes (decreased activities, loss of appetite) (Alo *et al.*, 2018).

2.8 Determination of Packed Cell Volume

The packed cell volume (PCV) of each mouse was measured before and after infection as well as during and post treatment of the mice. Blood was collected from the tail of each mouse in heparinized capillary tubes, up to three-quarter of the entire length. The tubes were sealed using crystal sealant and placed in a microhematocrit centrifuge with the sealed end outwards. The blood sample was centrifuged at 12,000 rpm for 5 minutes. The result was read using the microhaematocrit reader. The volume of the total blood and the volume of erythrocytes were measured, and PCV were calculated as;

PCV = Volume of erythrocytes in a given volume of blood X 100 Total blood volume (Muhammed *et al.*, 2018 and Olaniran *et al.* 2019).

2.9 Determination of Parasiteamia and Percentage Chemosuppression

Treatment of infected mice were carried out on day six to nine (four days) and both the infected and treated mice were left till day fourteen (five days after treatment) to determine post-treatment effect on the mice before sacrifice . On day five to fourteen, the parasiteamia levels of the mice were determined by collecting a drop of blood on a microscope slide from each mouse by venesection of the tail. Thin and thick blood smear was made and allowed to air dry at room temperature. It was fixed with methanol for two minutes before staining with 10% Giemsa for 15minutes. The slides were allowed to air-dried, examined and counted under a light microscope at X 100 magnification using oil-immersion. The parasiteamia was determined by counting a minimum of three fields per slide with 100 RBC per field (Muhammed *et al.*, 2018).

Parasitemia = Number of parasitised RBC x100/ Total Number of RBC examined.

$$\% \text{ chemosuppression} = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in treatment} \times 100}{\text{Parasitemia in negative control}}$$

2.10 Statistical Analysis

All data were expressed as Mean of three determinations ± SEM. One-way analysis of variance was used to analyse data. $P < 0.05$ was considered a significant difference between means (Duncan's multiple range test)

3.0 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 dosage levels used.

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 = 250mg/kg of seed oil of *Moringa oleifera*

Group 2 = 500mg/kg of seed oil of *Moringa oleifera*

Group 3 = 1000mg/kg of seed oil of *Moringa oleifera*

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 extract on body weight of mice is shown in table 2. The body weight of mice in group A (infected and untreated group), reduced from 19.87g to 18.86g in 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 increase even though the loss was not statistically significant except in group E (18.71 to 15.25). However, weight of mice in group B (choloroquine treated group) increases as the treatment progressed with days from 20.14g to 20.71g. This is not statistically significant.

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 presented as Mean ± S.E (n = 3). Means with different superscripts in the same row show significant difference ($P < 0.05$).

A = Infected and untreated group

B = Infected and treated with chloroquine group

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

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

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

The figure 1, showed the gradual reduction in percentage of pack cell volume (PCV) of mice under study before infected and after treated. The result obtained showed that group A (infected

and untreated group) had PCV value of 22.23±1.98% which fell far short from normal range (40 - 55%). 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±1.82% which was statistically not significant when compare with the normal range.

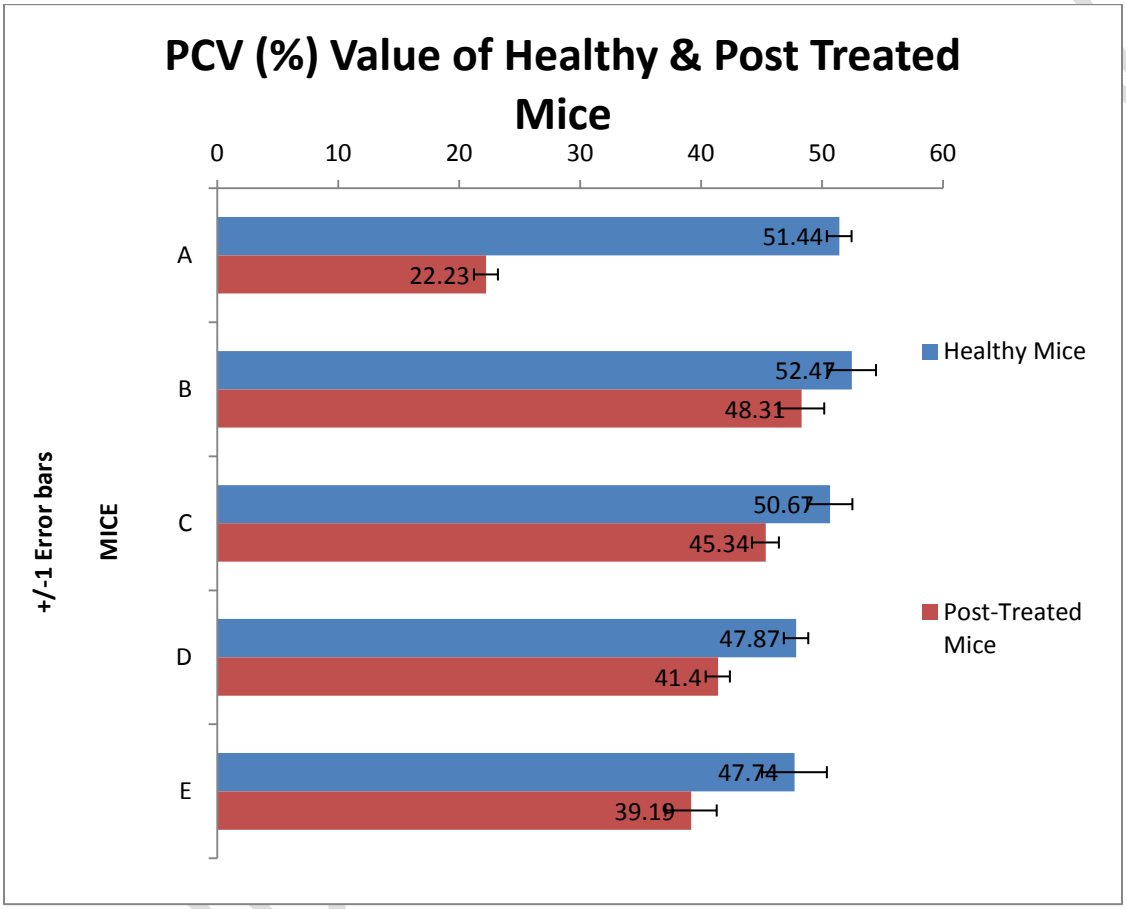

Figure 1: Pack Cell Volume (PCV) before Infected and after Treatment of Experimented Mice

Keys:

A = Infected and untreated group

B = Infected and treated with chloroquine 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.

3.3 Percentage Mean of Parasiteamia and Mortality

The outcome of the percentage mean of parasiteamia in four days of treatment and five days post-treatment was presented in table 3. Group A = infected and untreated group, B = infected and treated with chloroquine group, C = 800mg/kg of seed oil of *M. oleifera* (M.O), D = 400mg/kg of seed oil of M.O. and E = 200mg/kg of seed oil of M.O. As expected, the negative control (A) group had increased 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 parasiteamia 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 parasiteamia count is statistically significant.

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.

Table 3: Percentage Mean of Parasiteamia 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 ± 0.42e	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}

Values are mean ± S.E (n=3). Means with different superscripts in the same row show significant difference ($P < 0.05$).

A = Infected and untreated group

B = Infected and treated with chloroquine 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

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 = Infected and untreated group

B = Infected and treated with chloroquine 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

3.4 Percentage Chemosuppression of Parasitemia

Figure 2, showed the trend analysis of percentage inhibition of parasiteamia 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 (800mg/kg) and D (400mg/kg) respectively and low parasites suppression in group E (200mg/kg treated with seed oil of M.O.) of about 67%.

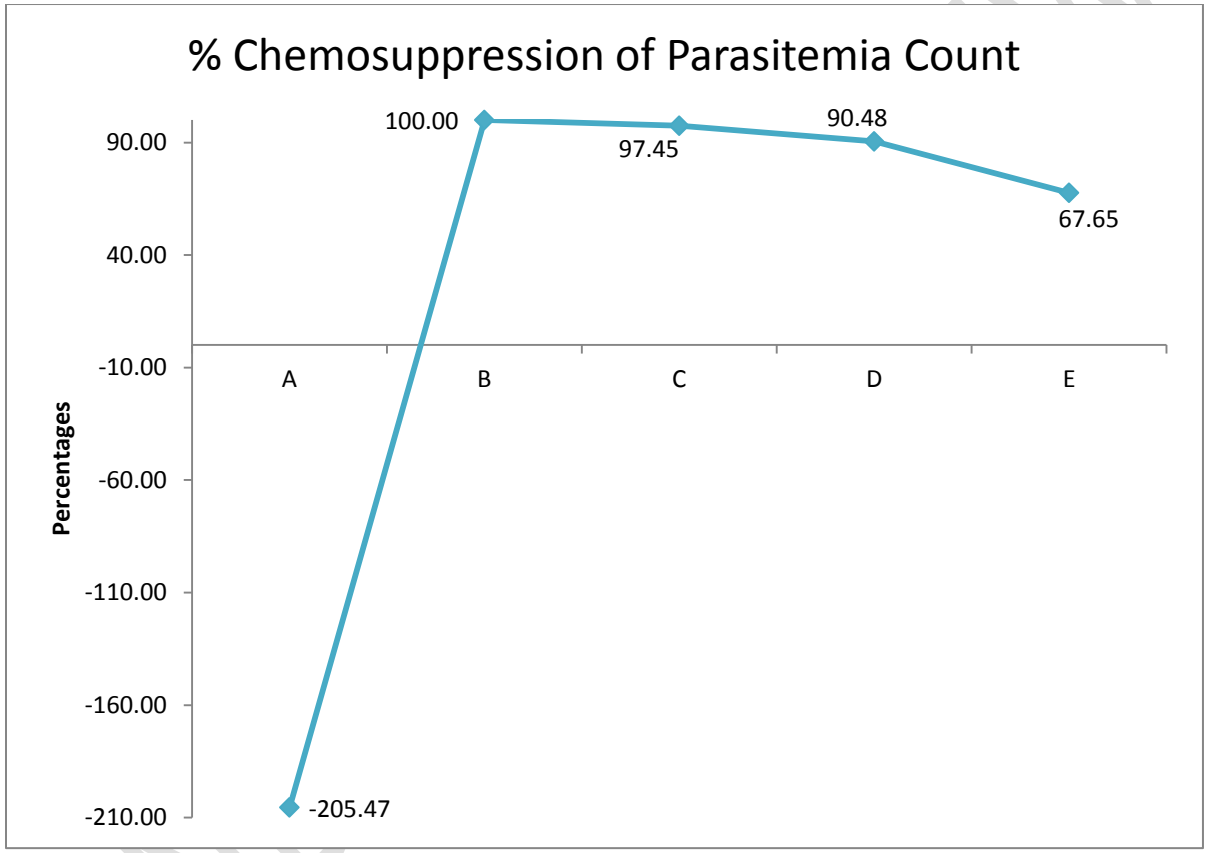

Fig. 2: Percentage Parasitemia Count Inhibition in After Treatment.

A = Infected and untreated group

B = Infected and treated with chloroquine 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

4.0 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 indicates that the *M. oleifera* oil could probably be non-toxic. This is in agreement with Hodge and Sterner Toxicity Scale, that any chemical exhibiting LD_{50} above 1000mg/kg is practically non-toxic (Da *et al.* 2016).

Decreased body weight observed in the infected treated and infected not treated mice could be due to loss of appetite, increased in the metabolic rate, reduced feed conversion efficiency and invariably a sign of malaria-infected mice. This confirmed there port of Alo *et al*. (2018), and Muhammed *et al.* (2018). The gain of body weight was however reported in positive control (group B), after treatment in seven days.

Olaniran *et al.* (2019), reported that the Packed Cell Volume (PCV) is a measure of the proportion of the erythrocyte in a given blood sample and can indicate the degeneration of a diseased state or its amelioration by pharmacological agents on the blood of an animal or man. In addition, Olaniran *et al.* (2019), described PCV as a routine test in the hospitals and health facilities to assess the state of health of an individual or animal and that the degree of infection of the erythrocytes can be assessed by the PCV level and agents that can protect from infection or kill parasites will give significantly higher levels of PCV than in untreated animals. This study corroborated Olaniran *et al.* (2019) findings. The positive control (group B) gave PCV value (48.30±1.84%) which was significantly higher ($p<0.05$) than that obtained for the animals of negative control (22.23±1.98%). However, the values obtained for the animals treated with 800mg/kg (group C) of the seed oil of *M. oleifera* were significantly higher too (45.34±0.98%) followed by those treated with 400mg/kg (41.40±1.57%) and least in 200mg/kg (39.19±1.82%). The findings from Olaniran *et al.* (2019) showed that the higher the doses of the extract the higher the percentage chemosuppression which produced high PCV values. This shows that higher suppression of malaria can translate to higher PCV in treated animals.

General decrease in pack cell volumes (PCV) observed in all groups of mice under study corroborated the findings of Dada and Muhammed (2018). However, the decrease in PCV in mice infected but treated with the chloroquine and seed oil of *M. oleifera* was not significant compared with the mice infected but untreated (group A) which contained PCV value (22.23%) that fell below normal (35-37%).

On the basis of the result obtained in this study, the antiplasmodial investigation of the seed oil of *M. oleifera* revealed a decrease in percentage parasitaemia in groups C (800mg/kg treated), D (400mg/kg treated) and E (200mg/kg treated) compared with group A (infected and

not treated) which had higher parasiteamia with time. The *M. oleifera* seed oil showed promising inhibition of parasiteamia ranging from 97% in group C, 90% in group D and 68% in group E as depicted in figure 2. However, the highest parasitemia 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 200mg/kg. Thus, the treatment of *Plasmodium* with seed oil of *M. oleifera* could be favorably compared with the chloroquine treated group of B. This finding is in line with *Orman et al.* (2015), 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.

The seed oil of *M. oleifera* showed a progressive reduction in parasiteamia with time; the highest clearance was observed on the ninth day. Considering that the oil of varied concentrations was administered once daily for four days, it is likely that extracts of *M. oleifera* seed oil has residual potency and so as more of the oil were administered, there is a cumulative antiplasmodial effect thus the reason for the progressive reduction in parasiteamia. This is a very promising feature in the potentiality of the use of seed oil of *M. oleifera* as an antimalarial drug considering the difficulty in bringing down to very low levels, parasiteamia in malaria patients. This report confirmed the study of Olasehinde *et al.* (2012). Daskum *et al.* (2019) 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 this plant a good candidate source for antimalarial formulations. The death of two to four mice observed in group C, D and E with continuous administration of the *M. oleifera* seed oil; even after parasites were significantly reduced from the mice bloodstreams suggested that the extracts may have some cumulative toxic effects.

5.0 CONCLUSION

The results of this study have revealed that the seed oil of *Moringa oleifera* possesses some bioactive chemicals capable of inhibiting *Plasmodia* activity. This oil may be suitable for the treatment of human malaria but further investigation is required to determine the pure, active components of the seed oil of the *Moringa oleifera* responsible for these activities and its effect on long-term administration is recommended for further studies.

ETHICAL APPROVAL

The whole experimental management, handling and care were approved by the Research and Ethics Committee of the Department of Microbiology, School of Sciences, The Federal University of Technology, Akure, Nigeria.

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