Advanced Journal of Graduate Research
ISSN:2456-7108
Volume 11, Issue 1, pp. 71-79, January 2022
DOI: https://doi.org/10.21467/ajgr.11.1.71-79

GRADUATE RESEARCH ARTICLE

Histopathological Effects of Seed Oil of *Moringa oleifera* Lam. on Albino Mice Infected with *Plasmodium berghei* (NK65)

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Article History

Received: 31 August 2021
Revised: 17 November 2021
Accepted: 08 December 2021
Published: 23 December 2021

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Academic Year: 2019-2020
Course Level: Master
Course Name: M.Sc.
Course year: Final Year

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ABSTRACT

The study assessed the histopathological effects of seed oil of Moringa oleifera on albino mice infected with Plasmodium berghei. This work included a good idea in the treatment of a causing agent of malaria with Moringa seed oil as bio-natural treatment. Thirty-five mice were divided equally and grouped into five. The mice were acclimatised for seven days and thereafter infected with 0.2 mL Plasmodium berghei (NK65) parasite. The parasites were allowed to establish in the mice for five days before commencement of treatment. Group A - negative control (untreated), group B - positive control (10mg/kg chloroquine treated), group C, D and E were respectively treated with 800, 400, 200 mg/kg seed oil of Moringa oleifera. By oral administration of 0.2 mL of treatment dose, treatment was carried out in four consecutive days and the mice were sacrificed five days thereafter. The liver and kidney extracted from the mice were processed for histological studies. Findings revealed group A had the least packed cell volume (PCV) of 22.23±1.98% and group B had the most PCV of 48.31±1.55% after treatment. The PCV in groups C, D and E were 45.34±1.11%, 41.40±1.00% and 39.19±1.82% respectively after treatment. Coagulative necrosis and inflammation characterised the liver and kidney of mice in groups C and D. Lesions were observed in all the liver of mice treated with the seed oil of M. oleifera and chloroquine. Overall, it can be inferred that the higher the PCV of mice after treatment, the higher the performance of chemotherapeutic agents against parasitaemia. Thus, at 800, 400 and 200 mg/kg dosage, the seed oil of Moringa oleifera could possibly treat malaria. However, administration of a higher dose of the oil and chloroquine should be with caution as both drugs may pose adverse effects on the kidney and liver.

Keywords: Histopathological, Moringa oleifera, Seed oil

1 Introduction

Malaria is among the top 10 causes of mortality in the world [1]. It is one of the most dangerous infections which result in socioeconomic problems and poverty [1]. Malaria is caused by a protozoan parasite of the genus *Plasmodium*. Five species of *Plasmodia* have been implicated in humans and they include *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi* [2]. *Plasmodium* is transmitted by the bite of an infected female *Anopheles* mosquito. High fever, chills and muscle pain are clinical presentations of malaria infection [2].

Moringa is the sole genus of the family Moringaceae [3]. It comprises fast-growing plants widely distributed along with tropical and subtropical climates [3]. Among the 13 known species, *Moringa oleifera* Lam. has continued to receive more attention worldwide owning to its economic importance, multipurpose uses and medicinal purposes [3], [4] and [5]. The *M. oleifera* seeds are rich in oil and the oil represents 36.7% of the seed weight [4]. The oil which is the main component of the seed contains oleic acid similar to olive oil fatty acid and has a great potential to become a commercial source of edible oil for the food industry [4]. The seed oil may also be explored for the development of useful plant-based pharmaceuticals, food preservatives, antioxidant agents and additives [5].

Although the hepatoprotective effects of the seed extract of *M. oleifera* have been documented, its safety or toxicity has been a subject of controversy [6]. The review [7] on acute toxicity studies in rats showed that no mortality was observed at 2000 mg/kg dose of the *M. oleifera* extract. Even more, Zade *et al.* [8] reported that the seed extract of *M. oleifera* would not be toxic even at 5000 mg/kg in albino mice. Meanwhile, findings [9] established that the seed oil of *M. oleifera* could inhibit and treat the *Plasmodium berghei;* the causative agent of malaria in mice. However, little death recorded in the course of treatment [9] called for the basis of this finding. The study assessed the histopathological effects of seed oil of *Moringa oleifera* on mice infected with *Plasmodium berghei.*

2 Materials and Methods

2.1 Seed Collection and Identification

The seeds of *Moringa oleifera* were collected from 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 seeds were dehulled; sun dried to a constant weight of 2kg; crushed and finely pulverised using a Philip blender (model HR2102). The pulverised seeds were subjected to 240 mL of n-hexane in soxhlet apparatus to extract the *M. oleifera* oil [10]. The amount of the oil obtained was determined and stored in a glass bottle for use.

2.3 Source of Experimental Mice

Forty-seven albino mice of body weight 18-22 g were obtained from the Animal House, Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The mice were housed in dust bedding cages at room temperature. Standard diets (grand cereal) and water ad libitum were provided for the mice in each cage and the mice acclimatised for 7 days before experiments began [2].

2.4 Grouping of Albino Mice

Twelve albino mice were randomised into three groups (1, 2 and 3) of four mice for acute toxicity test [1] and [12]. A total of thirty-five (35) mice were divided equally into five groups of seven mice for antiplasmodial activity and histopathological studies: Group A - negative control (not-treated), B - positive control (10 mg/kg chloroquine treated), group C (800 mg/kg), group D (400 mg/kg) and group E (200 mg/kg) of seed oil of *M. oleifera* [11].

2.5 Acute Toxicity

Before treatment, mouse in each group was orally administered with a single dose of 0.2 mL of prepared seed oil of *M. oleifera* dosage; 250, 500 and 800 mg/kg were respectively administered to the mice in group 1, 2 and 3 and thereafter observed for three days [12]. To prepare 1000 mg/kg dosage, 1.0 g of the seed oil was weighed and dissolved in a sterile universal bottle containing 8 mL of distilled water and 2 mL of

tween20. Two-fold serial dilution was carried out on 1000 mg/kg to obtain 500 mg/kg dosage and also on 500 mg/kg to obtain 250 mg/kg dosage.

2.6 Seed Oil Dosage Preparation

Exactly 0.8 g of the seed oil of *M. oleifera* was weighed and dissolved in a sterile universal bottle containing 8 mL of distilled water and 2 mL of tween20 to make 800 mg/kg. Two-fold serial dilution was carried out on 800 mg/kg to obtain 400 mg/kg and also on 400 mg/kg to obtain 200 mg/kg dosage [13]. From the seed oil dosages, 0.2 mL of 800, 400 and 200 mg/kg of the seed oil of *M. oleifera* were administered to each mouse in group C, D and E, respectively.

2.7 Collection of Parasites

From an infected donor mouse at IMRAT, University of Ibadan, Oyo State, Nigeria, *Plasmodium berghei NK* 65 were collected by cardiac puncture. The withdrawn 0.2 mL of infected erythrocytes diluted with sterile 4.8 mL of normal saline to obtain 1 x 10^7 *P. berhei* stock [12]. Administration of 0.2 mL of *P. berhei* stock was carried out in each mouse of group A, B, C, D and E. The mice were left for five days to obtain high loads of parasitaemia (≥ 40%).

2.8 Determination of Body Weight and Packed Cell Volume

Before exposing the mice to infection, the body weight and packed cell volume (PCV) of each mouse were carried out. Also, during and after treatment, the body weight and the PCV of the experimented mice were as well determined. The blood obtained from the tail of each mouse was collected into heparinized capillary tubes, and filled up to ¾ of the entire tube and sealed. The sealed tubes were placed in a micro haematocrit centrifuge and operated at 12,000 revolutions per minutes for 5 minutes [11]. The centrifuged tubes were placed in micro haemataocrit reader to determine the PCV.

2.9 Histopathology Study

The organs such as livers and kidneys extracted from each group after treatment were stored in 10% buffered formalin [14]. The tissues were fixed, processed and embedded in paraffin wax. Sections of 5 µ thickness were cut with a rotary microtome and the slides obtained were then stained with hematoxylin and eosin and examined under light microscope. The microscopic features and photographs of organs of seed oil treated mice were compared to those of the control groups.

2.10 Statistical Analysis

All data were presented as a mean of three determination ± standard error means using one-way analysis of variance. $P < 0.05$ was considered a significant difference between means.

3 Results

3.1 Toxicity Effect of Seed Oil of *Moringa oleifera*

The result of acute toxicity shown in Table 1 revealed no signs of toxicity. Signs such as paw licking, sleeping, reduced activity and respiratory distress nor death were not observed in mice at all dosages level 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, 2 and 3 = 250, 500 and 1000 mg/kg body weight of seed oil of *M. oleifera* respectively.

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

Table 2 revealed that the mice body weight in group A (negative control) reduced from 19.87±0.15 to 18.86±0.74 g after treatment. In the same vein, weight loss was also observed in mice treated with seed oil of *M. oleifera* as the days progressed; Group C (18.86±0.51 to 17.20±0.81), group D (20.14±1.08 to 19.33±1.14) and group E (18.71±0.36 to 16.00±0.82). However, mice in group B (positive control) had a slight increase in body weight after treatment (20.14±0.59 to 20.71±0.47). In all, the decrease or increase in body weight was not statistically significant (P > 0.05).

Table 2: *Effects of seed oil of M. oleifera on mice body weight.*

Mice mean weight per day in grams					
DAY	**A**	**B**	**C**	**D**	**E**
1	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}
14	18.86±0.74^{b}	20.71±0.47^{a}	17.20±0.81^{d}	19.33±1.14^{c}	16.00±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).

Legend: A = Untreated mice
B = Chloroquine (10mg/kg) treated mice
C, D and E = 800mg/kg, 400mg/kg, 200mg/kg of seed oil of *M. oleifera* treated mice respectively.
1 = infected day, 6-9 = treatment days and 14 = post treatment day.

Mice in group A to E had a gradual reduction in packed cell volume (PCV) (%) as shown in figure 1. However, mice in group A had an abnormal shortage of PCV when compared with other groups. Group A had 22.23±1.98%, B had 48.31±1.55%, C had 45.34±1.11%, D had 41.40±1.00% and E had 39.19±1.82% PCV after treatment. When compared with the normal, the PCV were not statistically significant except in group E (p<0.05).

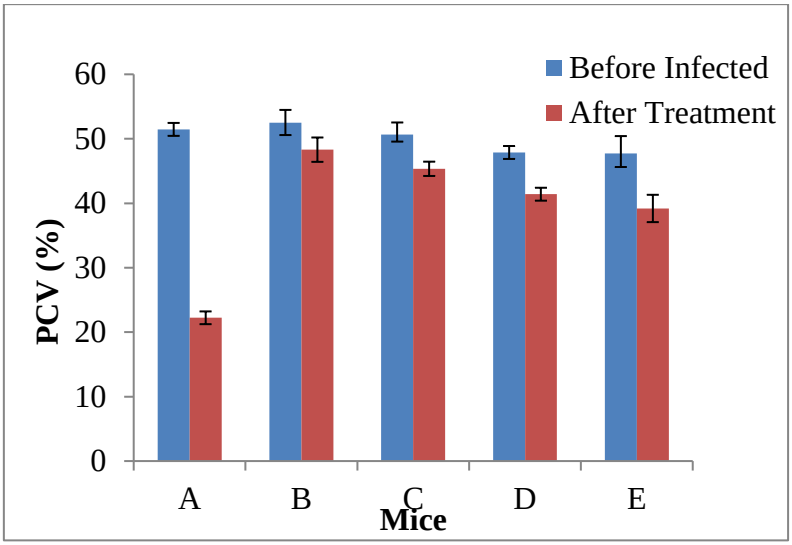

Figure 1: *Packed cell volume (%) Measured before infection and after treatment in experimented mice.*

Legend:
A = Untreated mice
B = Chloroquine (10mg/kg) treated mice
C, D and E = 800mg/kg, 400mg/kg, 200mg/kg of seed oil of *M. oleifera* treated mice respectively.

3.3 Histopathological Effects of Seed Oil of *M. oleifera* on Organs

The kidney and liver organs of mice in each group were extracted and examined for diseased, amelioration and damage. The kidney in figures 2 – 6 revealed that groups A, B and E had no observable lesion. Group C had moderate atrophy of tubular epithelium, luminal ectasia and a few inflammatory cells in the interstitium, and group D had multiple foci of tubular atrophy, coagulative necrosis and inflammation.

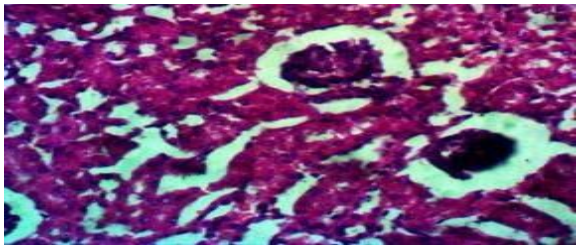

Figure 2: *Kidney in group A showed no observable lesion. HE x400*

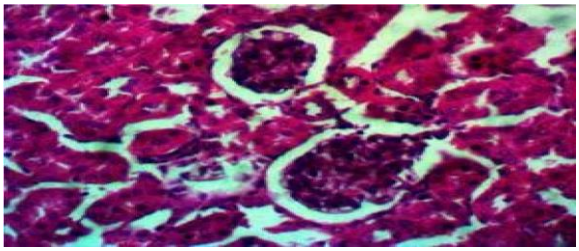

Figure 3: *Kidney in group B showed no observable lesion. HE x400*

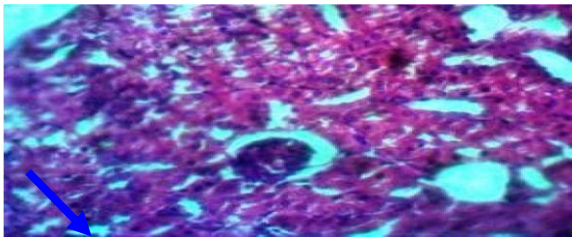

Figure 4: *Kidney in group C depicted moderate atrophy of tubular epithelium, luminal ectasia and a few inflammatory cells in the interstitium (arrow). HE x400*

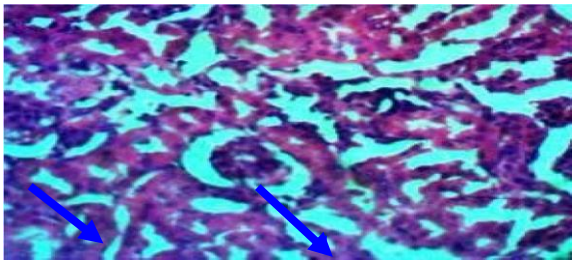

Figure 5: *Kidney in group D had multiple foci of tubular atrophy, coagulative necrosis and inflammation (arrow). HE x400*

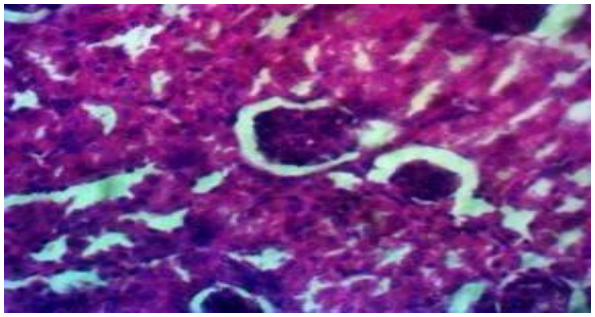

Figure 6: *Kidney in group E showed no observable lesion. HE x 400*

Figure 2 - 6: *Histology of Kidney of Mice in each Group of Experimented Mice.*

Legend:
Group A = Untreated mice
Group B = Chloroquine (10mg/kg) treated mice
Group C, D and E = 800mg/kg, 400mg/kg, 200mg/kg of seed oil of *M. oleifera* treated mice respectively.

The liver histology (figures 7 -11) results obtained for mice in each group showed that group A had no observable lesion; group B had multiple hepatocellular coagulative necrosis and inflammation; group C and D had multiple foci of hepatocellular swelling, coagulative necrosis and inflammation and group E had

centrilobular hepatocellular degeneration, coagulative necrosis and inflammation. Thus, except for liver of mice in negative control, group A, all other treated groups (B to E) had necrosis and inflammation.

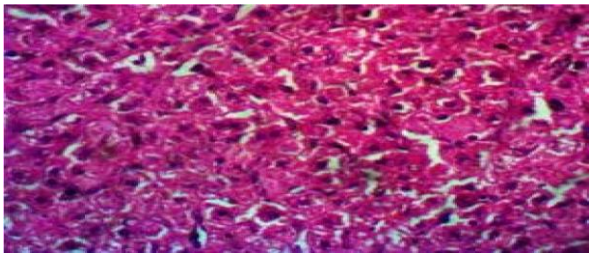

Figure 7: *Liver in group A showed no observable lesion. HE x400*

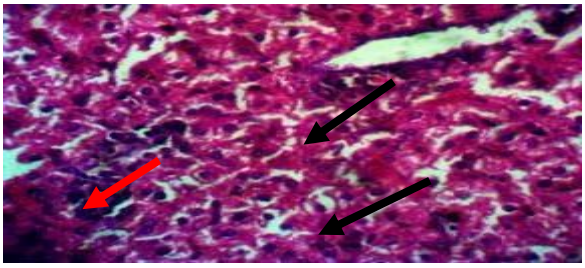

Figure 8: *Liver in group B had multiple hepatocellular coagulative necrosis (black arrow) and inflammation (red arrow). HE x400*

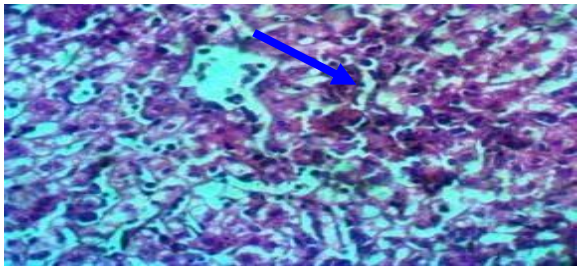

Figure 9: *Liver in group C showed multiple foci of hepatocellular swelling, coagulative necrosis and inflammation (arrow). HE x400*

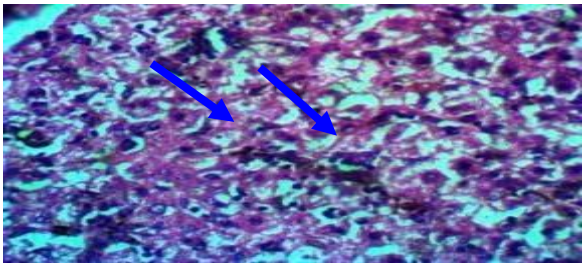

Figure 10: *Liver in group D had multiple foci of hepatocellular swelling, coagulative necrosis and inflammation (arrow). HE x400*

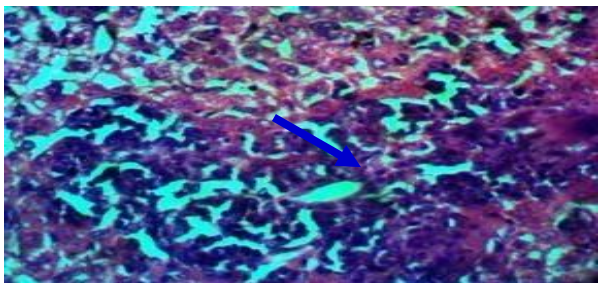

Figure 11: *Liver in group E showed centrilobular hepatocellular degeneration, coagulative necrosis and inflammation. HE x400*

Figure 7-11: *Histology of Liver of Mice in each Group of Experimented Mice.*

Legend:
Group A = Untreated mice
Group B = Chloroquine (10mg/kg) treated mice
Group C, D and E = 800mg/kg, 400mg/kg, 200mg/kg of seed oil of *M. oleifera* treated mice respectively.

4 Discussion

The acute toxicity test carried out on the mice showed neither mortality nor signs of toxicity on all the experimental mice. These findings are in agreement with Da *et al.* [14] who reported that any chemical exhibiting LD_{50} above 1000 mg/kg is practically non-toxic. Even more, Zade *et al.* [8] reported that extract from seed of *M. oleifera* would be non-toxic even at 5000 mg/kg body weight. These data revealed the seed oil of *M. oleifera* to be non-toxic, albeit its oral LD_{50} value may be categorized as slightly toxic [6].

The observed weight loss in all groups except in positive control group after treatment could be due to loss of appetite, increased in the metabolic rate, reduced feed conversion efficiency and a sign of malaria-infected mice. This study established the report of *Alo et al.* [12] and Muhammed *et al.* [1]. The body weight gained and the most packed cell volume (PCV) value (48.30±1.84%) reported in positive control (group B) mice after treatment could probably be a sign of total recovery from the illness. This outcome advanced Olaniran et al. [11] who documented that the higher the PCV values obtained in treated animals, the more effective the agents of chemo-suppression are. Also, the agents that protect body from infection or kill parasites will significantly improve the PCV level of infected and treated animals than the untreated ones [11]. Thus, 45.34±1.11, 41.40±1.00, 39.19±2.12 PCV value in group C, D and E respectively against 22.23±2.12 in group A (untreated mice) is a testament that seed oil of *M. oleifera* is capable of inhibiting the causative agent of malaria, *Plasmodium*. The various phyto-constituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes identified in *Moringa* contribute to numerous pharmacological uses [15].

The histopathological study of kidney and liver of mice extracted after treatment revealed that at 800 and 400mg/kg, the seed oil of *M. oleifera* could result in coagulative necrosis and inflammation (as observed in the kidney and liver of mice in group C and D). However, administration of 200mg/kg of the seed oil of *M. oleifera* was relatively safe to the mice in group E as there were no lesions except in the liver. Thus, the hepatocellular swelling, coagulative necrosis and inflammation which characterised the liver of mice in groups B, C, D and E could potent a danger not only in the use of seed oil of *M. oleifera* but also chloroquine. The study advanced Chivapat et al. [6] and Olaniran et al. [11] who reported that continuous administration of the extract of *M. oleifera* could result in cumulative toxicity. In addition, Saleem et al. [7] stated that the single use of *Moringa* extract is safe up to a dose of 2,000 mg/kg but long term repeated use of the extract could be injurious to the liver as evidenced by steatosis and elevation of liver function tests. The high antioxidant activity of *Moringa* is mainly due to its high content of flavonoids which are present in the flavanol and glycoside form [15]. Salem et al. [7] findings revealed that *M. oleifera* contains glycosides in which sugars are linked to a toxin, called aglycone. The glycoside and or the aglycone may be toxic [7]. The toxic properties may be due to the hydrocyanic acid released from the glycosides by enzyme complex activity. Furthermore, hydrogen cyanide [6] inhibits cytochrome oxidase which may halt electron transport, oxidative phosphorylation and aerobic glucose metabolism. Thus, mortality of the mice is possible through tissue anoxia [6] caused by administration of the seed oil of *M. oleifera*. In contrast, review [7] documented that the long term use of the extract was mostly devoid of major system toxicities. Meanwhile, the lesions not observed in the liver and kidney of mice in group A (untreated mice) could mean that the malaria infection was not severe and could not result in death. This finding corroborated Milner [16] who posited that the severe (complicated) malaria is characterised by serious organ failures or abnormalities in the patient's blood or metabolism.

5 Conclusion

At 800, 400 and 200mg/kg body weight dosage, the seed oil of *M. oleifera* could possibly treat malaria. Albeit, cautions should be taken when the *M. oleifera* seed oil is administered at a dose higher than 200 mg/kg as the seed oil of *M. oleifera* could be considered to be slightly toxic. However, in this study, the toxicity effects of drugs on the liver were not only peculiar to the mice administered with the seed oil of *M. oleifera* but also those with chloroquine. Therefore, it can be hard to conclude that the toxicity effects of seed oil of *M. oleifera* could result in death. It is recommended that further pharmacognosy tests should be carried out on seed oil of *M. oleifera* to determine its safety.

6 Declarations

6.1 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.

6.2 Availability of Data and Materials

The datasets supporting the conclusion of this article are included within the article. Additional data if required are available upon request.

6.3 Acknowledgements

The authors thank Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria for providing facilities to carry out the analysis.

6.4 Competing Interests

The authors declared that they have no competing interests.

7 How to Cite this Article:

S. K. Abdulahi, E. O. Dada, and R. O. Adebayo, "Histopathological Effects of Seed Oil of Moringa oleifera Lam. on Albino Mice Infected with Plasmodium berghei (NK65)", *Adv. J. Grad. Res.*, vol. 11, no. 1, pp. 71–79, Dec. 2021.

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