



## **Antiplasmodial, Antipyretic, Haematological and Histological Effects of the Leaf Extracts of *Moringa oleifera* in *Plasmodium berghei berghei* Infected Mice**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The aqueous and ethanolic extracts of *Moringa oleifera* leaf were evaluated for safety, antimalarial and antipyretic activities because it is commonly used for various types of ailments especially malaria and fever. The various concentration of these extracts (0-800mg/kg) were tested against *Plasmodium berghei berghei* infected albino mice of either sex, in a Peters' four day antiplasmodial test while antipyretic activities were evaluated in malaria- induced mice. Their possible effects on haematological parameters of PCV, WBC, neutrophil, lymphocytes count and liver enzymes were also determined as well as on the liver and kidney architecture. All the tested doses of the aqueous extract were comparable in antiplasmodial activity ( $p>0.05$ ) to each of the tested doses of the ethanolic extract which were significantly different ( $p<0.05$ ) from the negative control. Only the highest doses of both extracts gave significantly higher ( $p<0.05$ ) antiplasmodial activity than the standard drug. For the ethanolic extract, a significant antipyretic activity was not observed until at 800mg/kg in time  $T_3$  while the aqueous extract exerted no significant antipyretic activity at all doses and at all times. The ethanolic extract gave significantly higher PCV values than that of the aqueous. The administration of the aqueous extract gave significantly lower WBC than the ethanolic extract while comparable increase in lymphocyte count was noticeable at each of the doses of both extracts when compared to the negative control. Only 100mg/kg and 800mg/kg of the aqueous extract maintained a relatively high neutrophils count in this study. Also, the aqueous extract elicited higher concentration of ALT (greater than fourfold of normal) but the ethanolic extract produced the highest level of AST at the highest dose of 800mg/kg when compared to the ethanolic extract. Histological examination of the kidney showed progressive degeneration while that of the liver showed recovery when compared to negative control as a result of progressive increase in dosage of the extracts in malarial treatment. In conclusion, though both aqueous and ethanolic extracts of *M. oleifera* leaves exert chemosuppressive antiplasmodial activities in *Plasmodium berghei berghei* infected mice and exhibited antipyretic activity with some improved haematological parameters, caution should be taken in its indiscriminate use because of probable toxic effects on the kidney and the liver.

**Keywords:** Antiplasmodial; antipyrexia; histology; *Moringa oleifera*.

## 1. INTRODUCTION

The history of the relationship between man and plants is as old as creation and man has used plants for different purposes including food and medicines. In this dispensation, man has been able to classify plants into food and medicinal plants and has also discovered that solution to his health problem comes from nature [1].

Malaria is a tropical, mosquito - borne infectious disease caused by the protozoan parasites of the genus *Plasmodium* of which about four, *P. falciparum* which causes malignant tertian malaria, *P. vivax* and *P. ovale* which cause benign tertian malaria and *P. malariae* which is responsible for quartan malaria are known [2,3,4,5]. Its signs and symptoms which are recurring rigours, anaemia, toxemia and splenomegaly are caused by the presence of the erythrocytic stages of the parasite in the blood circulation while persistent infection in the liver (the extra – erythrocytic form) is the factor responsible for relapses [6,3]. In Nigeria, particularly in the south eastern part, studies on

the effects of herbal extracts on malaria parasites have been minimal whereas the malaria burden in the region is enormous [7]. Also, the incidence of antimalarial drug resistance, demands a commensurate efforts towards the discovery of novel and potent antimalarial drugs to combat it [8].

*Moringa oleifera* is a highly valued medicinal plant distributed in many countries of the tropics and sub tropics with high nutritional value and a rare combination of compounds like zeatin, quercetin, beta-sitosterol, caffeoylquinnic acid and kaempferol. Various parts of this plant and have been implicated to act as antitumor, cardiac and circulatory stimulant, antihypertensive, antioxidant, antidiabetic, hepatoprotective, antibacterial, antipyretic, antiepileptic, anti inflammatory, antiulcer, antispasmodic, diuretic, antifungal among other pharmacological properties [9]. It is also a good source of important minerals, protein, vitamins, beta carotene, amino acids and various phenolics. The multi component nature of the leaves and its common employment in the management of

many ailments can promote adverse effects on the organs of the body.

Therefore, this work is to determine antimalarial activity of the leaf extract of *Moringa oleifera* against *Plasmodium berghei berghei* and its toxicological effect on the hematological, biochemical and histological parameters in mice.

## 2. METHODOLOGY

### 2.1 Plant Collection and Extract Preparation

The leaves of *M. oleifera* were collected from Esuyare, near Ile-Ife, Osun State, Nigeria. It was identified and authenticated by Mr. I. I. Ogunlowo at the Faculty of Pharmacy Department of Pharmacognosy, Obafemi Awolowo University Herbarium. Voucher specimen FPI 2094 was deposited. Healthy leaves were selected; air dried and powdered using laboratory pestle and mortar. To each of 50g of the leaf powder was added 300mls of distilled water and ethanol respectively, thoroughly mixed, allowed to stand for 72 hours, clarified and subsequently filtered. The filtrates were concentrated *in vacuo* using rotary evaporator at the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The concentrate of the leaf extracts were prepared for administration to mice for the studies.

### 2.2 Test Animals and Parasite

Forty (40) healthy albino mice of either sex, aged 2 to 3 weeks were purchased from the animal house of the College of Health Sciences Obafemi Awolowo University Ile-Ife, Nigeria. The animals were allowed to acclimatize for one week and subsequently screened for the absence of malaria infection. The mice were divided into six groups (I-VI), of four mice each groups I and II were negative and positive control while III to VI were for test doses 100mg, 200mg, 400mg and 800mg/kg body weight respectively. *Plasmodium berghei berghei*-infected donor mouse with rising parasitaemia obtained from the Faculty of Pharmacy OAU was anaesthetized, and blood was obtained into heparinised bottle through cardiac puncture using sterile needle and syringe. The blood was diluted with normal saline such that 0.2ml of the resultant solution contained  $1.0 \times 10^7$  million infected red blood cells. Each of the thirty mice was inoculated with 0.2mls of the diluted

blood intraperitoneally. The test doses above were administered to the mice 2 hours after inoculation and daily for 3 days. Distilled water and chloroquine (10mg/kg) were given to the negative and positive control groups respectively. The temperature of the animals were taken daily before extract administration [10].

### 2.3 Preparation and Staining of the Blood Films

#### 2.3.1 Thick films

A drop of blood from the tail of the infected mice was put on a clean slide resting on a flat surface. The blood was then spread rapidly and evenly with the edge of a clean glass slide making a circular movement till the desired diameter is achieved to form a thick blood film. The films were kept horizontally and protected from dust. They were then dried for a few minutes at 37°C and stained using Giemsa stain. Microscopic examination of stained slide was done using X100 (oil immersion) objective.

#### 2.3.2 Thin films

A drop of blood was collected from the excised tail of the mouse onto a clean slide. A thin film was made by moving another slide on the blood at an angle of 45°. This was allowed to dry and fixed using few drops of methanol before placing in a slanting position to drain off and dry instantly. The stock (0.3 %) of Giemsa stain was diluted to 10 parts with water to give a 0.03 % stain that was applied on the smear and thereafter allowed to stand for 30 minutes. The stain was drained off the slide, rinsed in water and allowed to air-dry [11].

#### 2.3.3 Staining and photomicrography of the tissue

Sections were immersed in the filtered Harris Hematoxylin for 1 minute then repeatedly rinsed with tap water until the water is clear. The sections were immersed in eosin stain for 1-2 minutes then rinsed again with tap water until the water is clear. The stained sections were dehydrated in ascending concentrations (50%, 70%, 80%, 95%, 100%) of alcohol solutions after which they were cleared with xylene and examined by light microscopy and photomicrographs taken.

### 2.3.4 Estimation of parasitaemia level

Portions of the thick film where the white cells and the parasites were evenly distributed and well stained were selected and viewed using the oil immersion objective. The numbers of parasites corresponding to 200 white blood cells (WBC) in each portion were counted and recorded. This was repeated for ten portions of the film after which the averages of three counts were taken. The number of parasites per  $\mu\text{l}$  of blood was calculated from the formula:

$$\frac{\text{(WBC count of the mice blood sample} \times \text{Parasites counted against 200 WBC)}}{200}$$

The percentage of chemosuppression was calculated by using the following formula:

$$\% \text{ Suppression} = \left[ \frac{100 (\text{Parasitaemia in negative control} - \text{Parasitaemia in test group})}{\text{Parasitaemia in the negative control}} \right] [10]$$

## 2.4 Determination of the Hematological Parameters of the Infected Mice

### 2.4.1 White blood cells

0.38 litre of Turk's solution, as diluting fluid was measured and dispensed into a small tube, and properly mixed with 0.02 ml of EDTA - anticoagulated blood from mice. The counting chamber was assembled and one of its grids carefully filled with the diluted blood, using a capillary tube held at an angle of about  $45^\circ$ . The chamber was left undisturbed for 2 minutes to allow the white blood cells to settle. The underside of the chamber was wiped dried, placed on the microscope stage and viewed using the X10 objective with the condenser iris sufficiently closed to give good contrast; the cells were focused until they appear as small black dots. The cells in the four large corner squares of the chamber including those lying on the lines of two sides of each large square were counted and recorded.

### 2.4.2 Differential white cell count

A thin blood film was prepared and stained with Lishman's stain and a drop of immersion oil was placed on its the lower third portion and covered with a clean cover glass. The film was examined microscopically using X10 objective with the condenser iris sufficiently adjusted to see the cells clearly and to view the part of the film where

the red cells are just beginning to overlap and the x40 objective was used to focus the blood film. The different white cells were counted as seen in each field using an automatic differential cell counter.

### 2.4.3 Estimation of packed cell volume (PCV)

Duplicate samples of blood were drawn from the tail each of the animals using the micro haematocrit tube; duplicate determinations were carried out on the relative volume of the blood occupied by erythrocytes in micro haematocrit tube by using the formula:

$$\text{PCV} = \left[ \frac{100 (\text{Length of red cell column in mm})}{\text{Length of total column (mm)}} \right]$$

## 2.5 Determination of the Serum Biochemical Parameters of the Infected Mice

### 2.5.1 Estimation of alanine aminotransferase (ALT)

To each of test tubes representing animals administered with doses 0, 100, 200, 400, 800mg/kg for the aqueous extract and chloroquine, was pipetted 0.5ml of the substrate buffer containing 200mmol/ liter of L-alanine, 2.0mmol/l of  $\alpha$ -oxoglutarate and Phosphate buffer 100mmol/l at pH 7.4 and transferred into water bath set at  $37^\circ\text{C}$ , after which 0.1ml (100  $\mu\text{l}$ ) of the respective animals serum was added after 5 minutes, mixed and incubated at  $37^\circ\text{C}$  for *exactly* 30 minutes. Just before 30 minutes was due, 0.1 ml distilled water was pipetted into a sample tube labeled B representing the reagent blank. All were removed from the water bath and placed on the bench.

0.5 ml DNPH Colour reagent (Reagent 2) was immediately added to each tube, mixed well and left at room temperature ( $20\text{--}28^\circ\text{C}$ ) for 20 minutes followed by 5 ml of 0.4N sodium hydroxide, mixed well and the mixture also left at room temperature for 20 minutes.

The absorbance of the standard, and test samples were afterwards read in a spectrophotometer set at wavelength 546 nm. The instrument was tarred with the reagent blank solution in tube B. The colours of the solutions are stable for up to 1 hour. The ALT activity in IU/l in the test samples was read from the calibration graph, making sure that the reading of the standard [12,13].

### 2.5.2 Estimation of aspartate amino-transferase (AST)

The same procedure above used in estimating ALT in the experimental animals administered with doses 0, 100, 200, 400, 800mg/kg for the aqueous extract and chloroquine, was used except that 200mmol/ liter L-alanine in ALT was replaced with 100mmol/ liter L-aspartate in AST as the substrate buffer (Reitman and Frankel, 1957; [12] The AST activity in IU/l in the test samples was read from the calibration graph, making sure that the reading of the standard which corresponds to ALT 47 IU/l agrees with the calibration curve [12,13].

### 2.5.3 Histology of mice tissues

Pairs of the test and uninfected mice were selected randomly and sacrificed on post infection day four. Then samples of liver and kidney were harvested and preserved in 10% formalin and processed for light microscopy through a process of fixation, dehydration clearing and embedding in paraffin wax and thereafter sectioned. Sections were stained with Haematoxylin and Eosin [11].

### 2.6 Statistical Analysis

The analysis was done by one way ANOVA using Graph pad Instat with Student Newman Keul as *post hoc* test. All reported p values were two-sided and a p value of less than or equal to 0.05 was considered to be statistically significant.

### 2.7 Animal Ethical Issue

Letter of permission to use the animals was duly signed by the Director, Multidisciplinary Laboratory who is charge of Animal house before embarking on the study.

## 3. RESULTS AND DISCUSSION

The use of plant and plant-based products in amelioration of diseases; including malaria is rampant in developing countries because of cost and resistance to orthodox drugs. Particularly, people in this region are in search of medicinal plants that will be useful in treating many ailments that afflict them at the same time. The leaves *M. oleifera* has been claimed to be effective in managing a lot of diseases including malaria, therefore the effect of its ethanol and aqueous extract on the plasmodial parasite and

malarial induced pyrexia is assessed in this study. Also the indiscriminate use of this plant necessitates toxicological evaluations of its effects on relevant organs of the body including blood, liver and kidney in order to assess the safety profile under the normal dosage regimen and its medical applications. Therefore the effects of *M. oleifera* extracts were assessed on some blood parameters like PCV, Full and differential blood count and also on liver and kidney of mice. This is reasonable based on the physiological, anatomical and biochemical similarities of mice to man. Malaria is always accompanied by fever and chills and pains, so the effects of both extracts were also assessed on malaria-induced pyrexia. The result of the antiplasmodial effects of both extracts was given in Table 1 while their effects on the blood parameters of the treated compared to untreated mice were in Table 2. Figs. 1 and 2 however summarize their effect on malarial – induced pyrexia and the liver enzymes respectively. Plate 1 and 2 depicts the photomicrographs of the effects of the extracts on kidney and liver.

### 4.1 Antiplasmodial Activity

The graded doses of the aqueous and ethanolic extract including the positive control drug, chloroquine gave significantly different ( $p < 0.05$ ) percentage parasitaemia from the negative control table 1. Both extracts were comparable ( $p > 0.05$ ) at all doses implying that dose for dose they have similar activities and that there is no obvious advantage in choosing either of the extracts. All the tested doses of the aqueous extract were comparable ( $p > 0.05$ ) to each of the tested doses of the ethanolic extract. The 100mg/kg of the aqueous extract was comparable to the 100mg/kg dose of the ethanolic extract and gave significantly lower activity than all the tested ethanolic extracts. All the doses tested except the highest doses of both extracts were comparable in activity to the standard drug. The aqueous was a better chemosuppressive agent (94%) compared to the ethanolic. This may indicate a better antiplasmodial activity of the aqueous than the ethanolic extract.

### 4.2 Malaria- induced Antipyretic Activity

In man, pyrexia is a useful indication of malaria infection when coupled with other diagnostic symptoms. However in mice, a rise in temperature depicts the abating of malarial and vice versa. (Hansen and Pappas, 1977)

Therefore, malaria-induced antipyretic activity was determined by a concomitant measurement of the temperature in the antiplasmodial test. For the ethanolic extract, the starting temperature,  $T_0$ , was comparable ( $p>0.05$ ) for all the doses while at  $T_1$ , all the other doses were comparable to the temperature of the negative control except the 800mg/kg which was also significantly different at  $T_1$ ,  $T_2$  and  $T_3$  at the same dose This implies that for this extract a significant antipyretic activity was not observed until 800mg/kg. Like the ethanolic extract, chloroquine gave a consistently high temperature reading indicative of antipyretic activity. For the aqueous extract, there was no significant antipyretic activity at all doses and at all times. Only chloroquine exerted antipyretic activity at  $T_3$  when compared to  $T_0$  and  $T_1$ .

#### 4.3 Packed Cell Volume

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. It is also a routine test in the hospitals and health facilities to assess the level of blood and hence the state of health of an individual or animal. Though a mosquito-borne disease, malaria is also a blood-borne disease in that the parasite attacks it at the erythrocytic phase leading to a reduction of blood cells through haemolysis and consequent fall in the PCV. 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. The positive control gave

PCV value which was significantly higher ( $p<0.05$ ) than that obtained for the animals treated with the aqueous extract and those of the negative control which was comparable ( $p>0.05$ ). However, the values obtained for the animals treated with higher doses of ethanolic extract were significantly higher ( $P =.05$ ) than that of 100mg/kg ( $24.75\pm 3.82\%$ ) and were comparable ( $p>0.05$ ) to each other and that of chloroquine. The higher doses of the ethanolic extract which gave higher % chemosuppression produced high PCV values. This shows that higher suppression of malaria can translate to higher PCV in treated animals.

#### 4.4 White Blood Cell Count

Leucopenia was frequently seen in malarial-infected humans [14] [15]. WBC was higher in untreated animals than those that received aqueous extract (Table 2). This is consistent with a raised WBC in any infective state. Leucopenia was defined as total WBC count less than  $4 \times 10^3$  / $\mu$ L (16). Peripheral leucocytosis of more than 12000/l is indicative of a poor prognosis of malaria (8). WBC in the animals treated with the aqueous extract were significantly lower ( $3.2-3.7 \times 10^3$ ) than in the untreated ( $5.9 \times 10^3$ ) while with the ethanolic extract, the value was dose-dependent ( $14.0 -3.2 \times 10^3$ ) up to 400mg/kg but comparable ( $5.78 \times 10^3$ ) to that of the positive control at 800mg/kg, the highest dose. It could be deduced that aqueous extract was more effective than the ethanolic in affecting this haematological parameter in mice. The significant reduction of WBC is indicative of a good prognosis of the malarial infection in the treated animals with the extract.

**Table 1. Mean parasitaemia levels and percentage chemosuppression of *Plasmodium berghei berghei* in mice treated with water and ethanol extracts of *M. oleifera* leaves, chloroquine, and distilled water (control)**

Dose (mg/kg)	Water parasitaemia $\times 10^4$ ( $\mu$ l/ml)	% suppression	Ethanol Parasitaemia $\times 10^4$ ( $\mu$ l/ml)	% Suppression
0mg	5.78 $\pm$ 0.23	0.00	5.78 $\pm$ 2.33	0.00
100	2.40 $\pm$ 0.08	58.50	2.14 $\pm$ 0.67	62.98
200	1.57 $\pm$ 0.02	72.83	0.81 $\pm$ 0.23	85.98
400	0.65 $\pm$ 0.02	88.75	0.71 $\pm$ 0.18	87.72
800	0.03 $\pm$ 0.01	99.48	0.13 $\pm$ 0.06	97.75
CQ(10)	1.80 $\pm$ 0.98	68.86	1.80 $\pm$ 0.98	68.86
ED <sub>50</sub>		216.80		253.46
ED <sub>90</sub>		475.58		426.60

Keys: ED<sub>50</sub> = dose mg/kg that will reduce parasitaemia by 50%; ED<sub>90</sub> = dose in mg/kg that will reduce parasitaemia by 90%. CQ = Positive Control

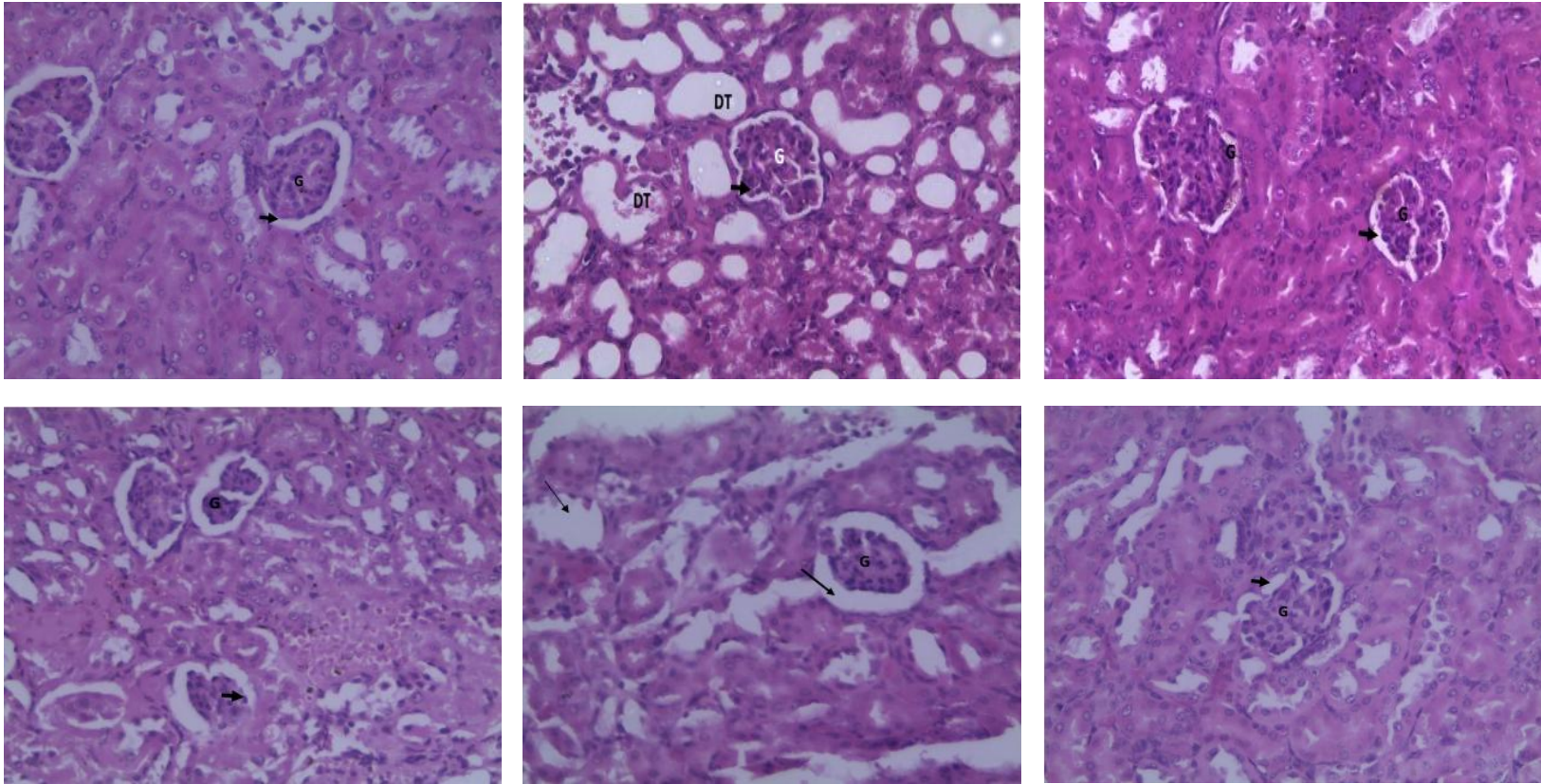


Plate 1a, b, c, d, e, f: Photomicrograph of the kidney of the mice treated with distilled water (Negative control), chloroquine (10mg/kg), 100, 200, 400, and 800mg. (H&E).X160.Comment: (a) The glomerulus is normal but renal tubule with cortex appears dilated as indicated with the arrow. (b) the glomerulus is normal as indicated with the arrow. (c) The glomeruli appear normal as indicated with the arrow. (d) Most of the glomeruli are mildly distorted; there is also mild interstitial nephritis as indicated with the arrow. (e) Glomeruli are segmental i.e. mild distorted architecture. There are also areas of interstitial nephritis as indicated with the arrow. (f) Most of the glomeruli are degenerated with dilated Bowman space and some interstitial nephritis as indicated with the arrow

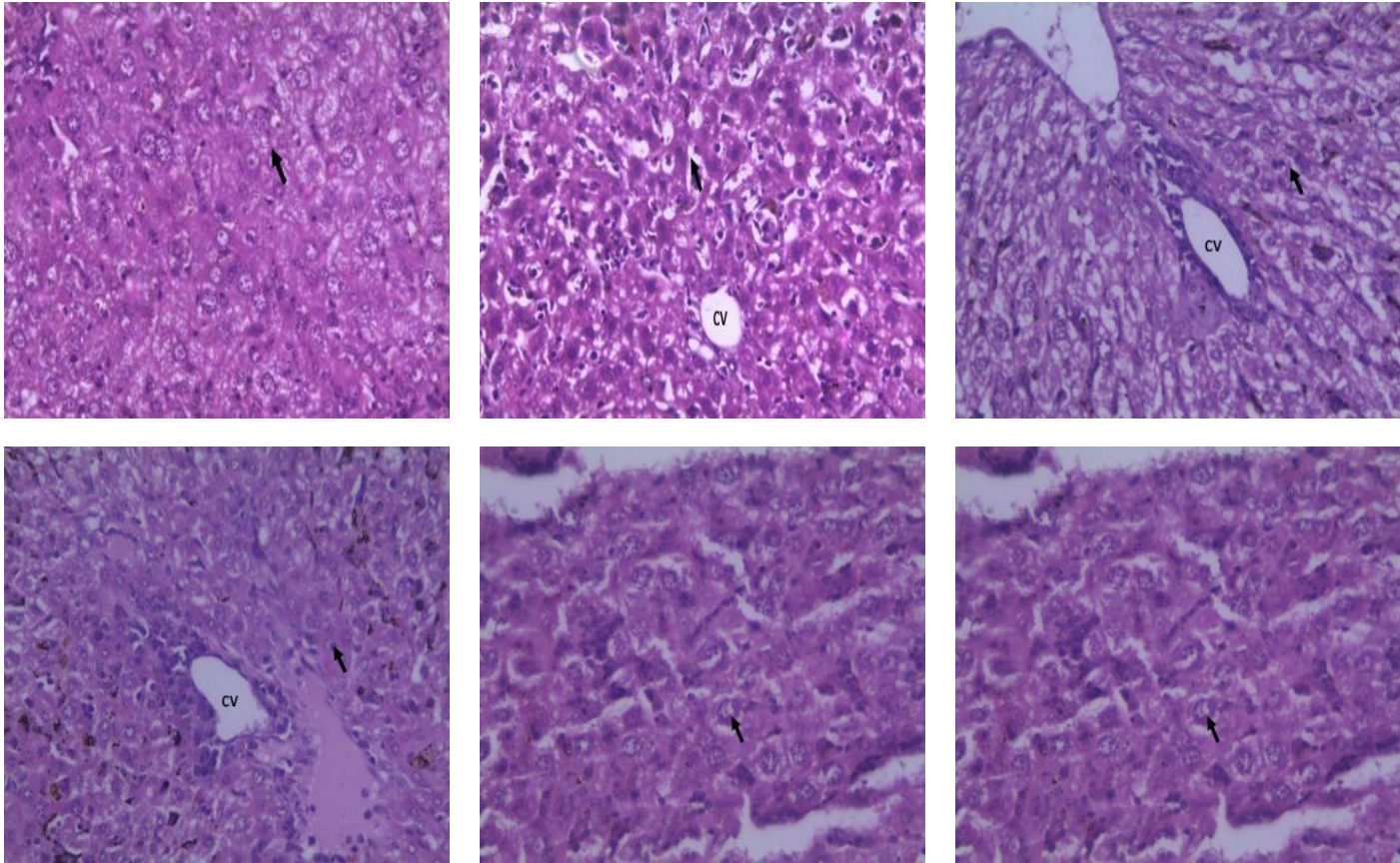


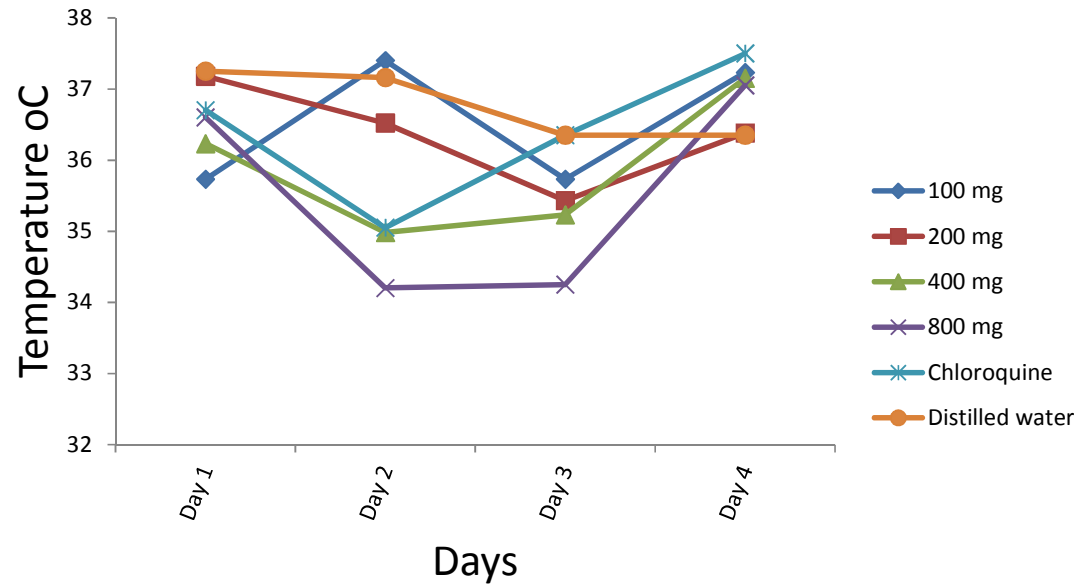
Plate 2: a, b, c, d, e, f. Photomicrograph of the liver of the mice treated with distilled water (Negative control), Chloroquine, 100, 200, 400, 800mg/kg (H&E).x160. Comment: - (a) the hepatocytes are too large compared to positive control and the liver architecture is not well defined as indicated with the arrow. (b) Hepatocyte are normal and are arranged in plate around the central vein as indicated with the arrow. (c) Hepatocyte showing sign of recovery as indicated with the arrow. (d) Normal. (e) Normal (f) hepatocytes are not remarkable (too large) but the liver architecture is normal



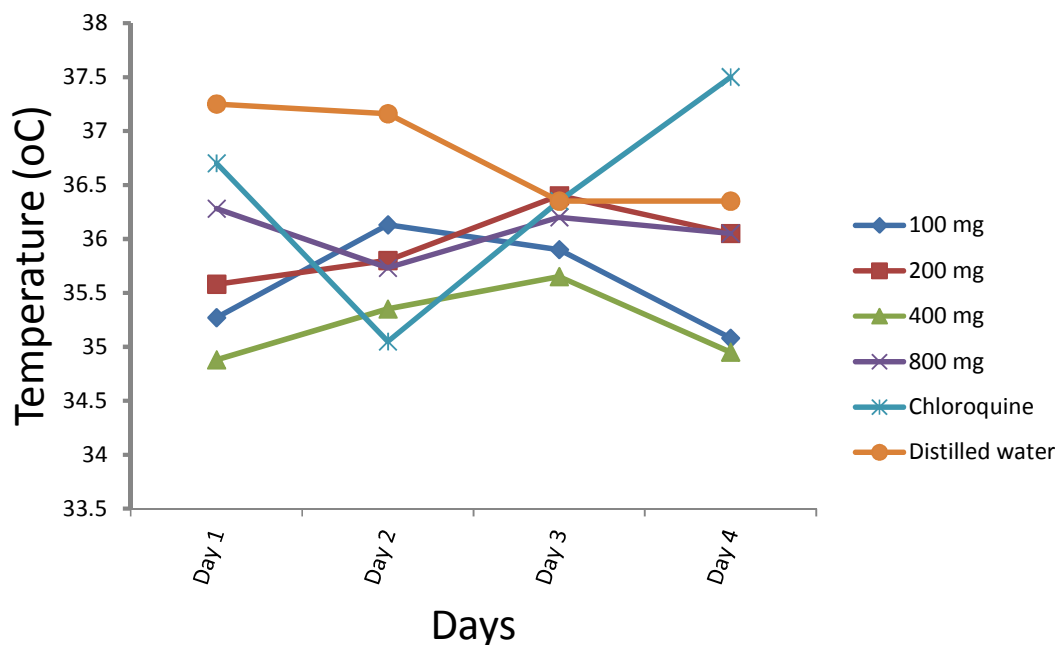
**Table 2. Profile of the packed cell volume, White Blood cell, Neutrophils and Lymphocyte counts in *Plasmodium berghei berghei* mice treated with the aqueous and ethanol extract of *M. oleifera***

Dose (mg)	PCV		WBCx10 <sup>3</sup>		NEUT		LYMPH	
	aqueous	ethanol	aqueous	ethanol	aqueous	ethanol	aqueous	ethanol
0mg	30.80±3.04	30.80±3.04	5.95±0.67	5.95±0.67	37.60±7.7	37.60±7.70	59.30±7.76	59.30±7.76
100mg	32.30±0.48	24.75±3.82	3.33±0.49	14.00±9.53	24.80±7.63	11.30±6.09	72.30±7.63	88.80±6.09
200mg	32.30±3.22	43.25±1.44	3.15±0.26	6.15±0.56	15.80±2.17	10.80±1.55	83.80±2.25	88.30±1.49
400mg	32.80±1.32	39.00±2.80	3.73±0.92	3.15±0.06	13.80±1.70	12.80±2.02	87.50±1.85	88.80±2.02
800mg	33.00±1.22	35.75±4.66	3.68±0.98	5.60±0.80	22.30±1.75	8.50±2.22	74.30±1.70	91.50±2.22
10mg	49.50±4.94	49.50±4.94	5.78±0.50	5.78±0.50	30.50±0.65	30.50±0.65	67.25±1.38	67.25±1.38

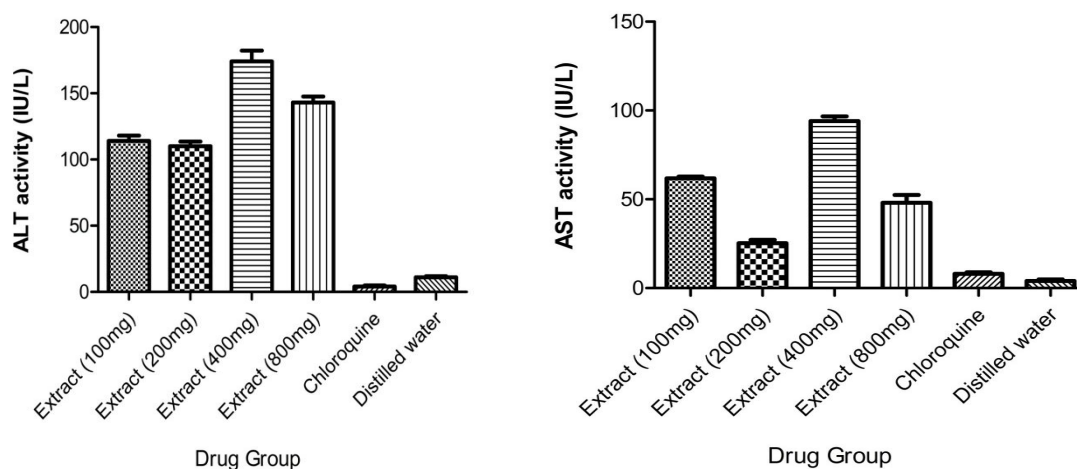
Keys: PCV= Packed cell volume, WBC= White blood cell count, NEUT= Neutrophils count, LYMP= Lymphocyte count



**Fig. 1. Effect of the aqueous extract of *M. oleifera* leaves, chloroquine and distilled water on the temperature of *Plasmodium berghei berghei* infected mice**



**Fig. 2. Effect of the ethanolic extract of *M. oleifera* leaves, chloroquine and distilled water on the temperature of *Plasmodium berghei berghei* infected mice**



**Fig. 3. Profile of the toxicological effect of aqueous leaf extracts of *M. oleifera* leaves, chloroquine and control by estimation of Alanine Amino transferase (ALT) and Aspartate aminotransferase (AST) concentration in mice**

#### 4.5 Neutrophils Counts

Neutrophil (also known as neutrophils or occasionally neutrocytes) are the most abundant type of granulocytes and the most abundant (40% to 75%) type of white blood cells in most mammals. They form an essential part of the innate immune system. Its functionality varies

in different animals. They can be suppressed in malarial infection. Neutrophils counts with negative control were significantly higher than each for the doses of both extracts and positive controls whereas lower neutrophils counts are really predictive of malarial infections. All the doses of ethanolic and 200 with 400mg/ kg of the aqueous gave comparably low neutrophils count

indicating that only 100mg/kg and 800mg/kg of the aqueous extract maintained a relatively high neutrophils count or improve this haematological parameter in infected mice. Drugs have been known to induce neutropenia in man [13].

#### 4.6 Lymphocyte Counts

Lymphocytes are a type of white blood cells that are responsible for initiating an immune response when a foreign matter enters the body. These cells are primarily in the nostrils, lymph nodes and the spleen but they also circulate in the blood. Parasitic infections elicit the reaction to produce lymphocytes and it is one of the haematological parameters that change in malarial infections along with neutrophils, WBC and RBC and so can be used in the supportive diagnosis of malaria in case of low level of parasite number in man [16] or to assess the abating or absence of malarial infections. Increased lymphocyte count was noticeable at all doses for both aqueous and ethanolic extracts of *M. oleifera* tested in this study when compared to the negative control. All the doses of the ethanolic extracts tested and the 200 and 800mg/kg of aqueous extracts gave significantly higher values than the negative and the positive control indicating that malarial infected animals evoked the production of significant amounts of lymphocytes than the negative and the positive control. An increase in lymphocyte count is usually associated with malaria being a parasitic infection [16]. This may be due to reactive (atypical) lymphocytes present in malaria [13]. This could also mean resistance of the parasite to chloroquine because negative and the positive control were comparable to each other. It could also be observed that both extracts produced comparable values of lymphocytes at each of their doses indicating their effectiveness in combating malarial infection at each of these doses. In summary, the results indicate there was a significant increase in haematological indices, suggesting that *Moringa oleifera* is useful in immune activation in these mice.

#### 4.7 Serum Biochemical Parameters of the Infected Mice

##### 4.7.1 Alanine aminotransferase (ALT)

It is found in plasma and various body tissues but is most commonly found in the liver. Its ratio with AST is commonly measured clinically as biomarkers of liver health, ALT is part of a

diagnostic evaluation of hepatocellular injury. For experimental studies the range is 10- 40 IU/L. It is of diurnal variation. Therefore the level of these enzymes could be used to predict damage to the liver and can be coupled with the histology for proper assessment [17,18,19]. The values of ALT obtained as a result of administering different concentrations of the ethanolic and aqueous extracts of *M. oleifera* leaves are depicted in Figs. 2 and 3 respectively. It was observed that negative and positive control elicited very low values of this liver enzyme (between 4 and 11IU/L in both cases). The highest values were obtained on the administration of 800 and 400mg/kg respectively for ethanolic and aqueous extracts respectively. The lowest dose (100mg/kg) of the ethanolic extract elicited very low concentration of the enzyme compared to the aqueous extract. Generally, the aqueous extract of *M. oleifera* elicited higher concentration (greater than fourfold of normal) of this enzyme when compared to the ethanolic extract. This may call for caution in the indiscriminate use of the extract of this plant especially the aqueous extract.

##### 4.7.2 Alanine aspartate transferase (AST)

Alanine aspartate transferase (AST) was also assayed in order to ascertain hepatic preservation after drug administration. With ethanolic extract, the lowest dose (100mg/kg), gave significantly lower 3.0 IU/L than the aqueous 89 IU/L; the highest dose gave 180 and 143 IU/L. Only 200mg/kg dose gave comparable (110 IU/L) AST level for both extracts. The relatively high level of these enzymes secreted by the liver on the administration of both extracts calls for great caution in their indiscriminate administration. The increased ALT and AST obtained in this study was corroborated by [20].

#### 4.8 Histology of Kidney and Liver

The histological effect of the extracts on the liver and kidney were also determined being organs of metabolism and excretion respectively. With the kidney, the glomerulus is normal but renal tubule with cortex appears dilated with the administration of distilled water; chloroquine indicated that the glomeruli appear normal while with 100mg/kg, most of the glomeruli were mildly distorted; with mild interstitial nephritis. Also, 200mg/kg presented a segmental mild distorted architecture of the glomeruli and 400mg/kg gave

areas of interstitial nephritis while the highest dose presented that most of the glomeruli are degenerated with dilated Bowman space and some interstitial nephritis which is indicative of an affected kidney. The histological samples from the kidney showed dilated tubule and also enlarged glomeruli [21]. Administered with distilled water, the hepatocytes were observed to be too large and the liver architecture is not well defined (Plate 2) compared to the positive control where the hepatocyte are normal and are arranged in plate around the central vein. At 100mg/kg, hepatocyte showed sign of recovery while it became normal at 200 and 400mg/kg, they presented as non-remarkable too large with normal liver architecture at 800mg/kg. Hepatocyte necrosis and portal tract inflammation have been reported as a result of the effects of aqueous extract of the leaves of *M. oleifera* in mice [21,22].

## 5. CONCLUSION AND RECOMMENDATION

Though *Moringa oleifera* leaf ethanolic and aqueous extracts have chemosuppressive suppressive effects on *Plasmodium berghei berghei* and exhibited antipyretic activity, the toxicological effect on the kidney and the liver calls for caution in its indiscriminate and large scale consumption. Hence more toxicological evaluation should be carried out to ascertain effects on other organs of the body like the bone. Also, the effect of the extracts of this plant on human species of *Plasmodium falciparum* should be ascertained.

## CONSENT

The consent /permission of the management of the Animal house, multidisciplinary laboratory, Obafemi Awolowo University was obtained.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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