

## Antiplasmodial Efficacy Of Methanolic Root And Leaf Extracts Of *Morinda lucida*

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### Abstract

Development of antimalarial drugs from medicinal plants continues to be a very appealing option. *Morindalucida*, commonly known as “Ugigo” by the Ebira people in Kogi State North-Central Nigeria, is used in the treatment of malaria, fever, amongst other ailments. Therapeutic effects of the methanolic root extract and a combination of extracts of the leaf and root parts were evaluated in *Plasmodium berghei* infection in mice. Percentage suppression of parasitaemia for the methanolic root extract was 56.30, 59.84, 67.72 and 81.80% for doses of 100, 200, 400mg/kg body weight of the extract, and 5mg/kg chloroquine respectively. The mean survival period in days were  $15.00 \pm 0.70$ ,  $18.75 \pm 0.5$ ,  $19.75 \pm 1.39$ ,  $23.25 \pm 1.38$  and  $8.75 \pm 1.25$ , for 100, 200, 400mg/kg body weight of the extract, 5mg/kg chloroquine, and the untreated control respectively. Effective dose dependent inhibitions of parasitaemia were also observed in the curative test. In the combination study, it was observed that, the antimalarial activity for leaf and root was slightly more, compared to that of each of the extracts, as seen in parasite inhibition, after 5 days of treatment (26.00, 20.00, 25.28, 21.35, 27.00, 19.50, 8.5 and 85.00), for 100, 200mg/kg leaf extract alone, 100 and 200mg/kg root extract alone, 50, 100mg/kg leaf and root extracts, 5mg/kg chloroquine and control groups respectively. It is concluded that the methanolic root and leaf extracts of *Morindalucida* are potentially useful for the development of antimalarial drug.

**Key words:** Antimalaria, Percentage suppression, *Morinda lucida*, *Plasmodium berghei*, Parasitaemia

### 1.0 Introduction

Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus *Plasmodium*. It is a complex and deadly disease, which recent estimates have shown that as many as 3.3 billion people live in areas at risk of malaria in 109 countries and territories around the world. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa (Rowe, 2006). Each year, there are approximately 350–500 million cases of malaria (CDC), killing between one and three million people, and majority of whom are young children in sub-Saharan Africa (Snow *et al.*, 2005). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. In Sub-Saharan Africa, more than 80% of the population relies on traditional medicines and healers as the primary source of health care (WHO, 2002). This is mainly because of the accessibility and affordability of consulting the healers, and their cultural sensitivity.

One of the greatest challenges facing Africa in the fight against malaria is drug resistance. Resistance to chloroquine, the cheapest and most widely used antimalarial, is common throughout Africa (particularly in southern and eastern parts of the continent) (Wellems, 2002). Resistance to sulfadoxine-pyrimethamine (SP), often seen as the first and least expensive alternative to chloroquine, is also increasing in east and southern Africa. As a result of these trends, many countries are having to change their treatment policies (Wellems, 2002). History reveals that plants have always been considered as an important source of medicine against malaria: This fact has encouraged the continuing search for new natural product-derived anti-malarial drugs. Acknowledging the efforts being made by researchers on exploring the medicinal benefits of plants, such as, *Morinda lucida*, *Morinda morindiodes*, *Alstonia boonie*, *Gossypium arboretum*, *Vernonia amygdalina* Del., (Idowu *et al.*, 2010) to mention a few, additional research is needed in order to realize the full benefits of natural plants and respond to the health needs of people, especially in developing countries like Nigeria. The plant parts of *Morinda lucida*, “Ugigo” (local name in Ebira tribe), has been

used as traditional remedy for the treatment of symptomatic malaria by the tribal population of Ebira land, Kogi State North – Central Nigeria.

Although, various works have been done on *Morinda lucida*, this study was designed to further confirm the works of other people (Antimalarial activity of extracts of the plant parts - leaf, stem bark and root (Asuzu and Chineme, 1990; Makinde and Obih, 1985; Koumaglo *et al.*, 1992), it is also reported that the stem bark infusion is used as an antimalarial (Burkill, 1997), Antimalarial effects of the petroleum ether extract and fractions of the leaf samples against *Plasmodium falciparum* using the Rabbit in vivo technique (Awe and Makinde 1998), where it was observed that the extract and some fractions inhibited the maturation of a drug sensitive strain of *Plasmodium falciparum*, active anthraquinones were isolated, the most active being damnacanthal) and also to explore other areas which have not been done, such as the combination the plant parts.

## 2.0 Materials and Methods

### 2.1 Materials

#### 2.1.1 Plant materials

The root and leaf parts of *Morinda lucida* were collected in the month of July, 2010 at idiche, in Okene, Kogi State, Nigeria. The identification and authentication was done in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, (NIPRD), Idu Abuja.

#### 2.1.2 Animals

Swiss albino mice (22 - 28g) of both sexes were obtained from the Animal facility centre of the Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria and. The animals were fed *ad libitum* with standard feed and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hrs light/darkness cycles. The animals were acclimatized for two weeks before the commencement of the study.

#### 2.1.3 Inoculum

The chloroquine-sensitive *Plasmodium berghei berghei* was obtained from the Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria. Parasitized erythrocytes were obtained from a donor- infected mouse by sacrificing in heparin- coated sample tube and made up to 20 ml with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing about  $1 \times 10^7$  parasitized erythrocytes.

### 2.2 Methods

#### 2.2.1 Preparation of Crude extracts

The Root parts of the plant were washed, chopped and air dried under shade and samples were pulverized into powder. The extraction was done according to the method described by Ogbadoyi *et al.*, 2007. In this method, 70g of the dried sample was sequentially extracted with Hexane, Ethyl acetate and Methanol in that order. The extraction with each of the solvents lasted for two hours, by reflux, after which they were filtered and the solvents evaporated using a rotary evaporator. After each extraction process, the dried marc was extracted using the next solvent. This procedure was also used to extract the leaf sample.

#### 2.2.2 In vivo Antiplasmodial test on crude methanolic root extract of *Morinda lucida*

##### 2.2.2.1 Four-day Suppressive Test

A total of twenty five mice were used for this study. Each mouse was given standard intra-peritoneal inoculums of  $1.0 \times 10^7$  *P. berghei berghei* parasites with the aid of a 1 mL disposable syringe. The animals were divided into five groups of five mice each. Different doses of the extract (100, 200, and 400 mg/kg/day) were administered orally to three groups. Chloroquine 5mg/kg/day was given as positive control to one group and 0.2 mL of normal saline to the last group, as negative control for four consecutive days (D<sub>0</sub> to D<sub>3</sub>). On the fifth day (D<sub>4</sub>), thin blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa and then microscopically examined with 100-x magnification. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected but untreated controls, with those of treated mice (Knight and Peters, 1980). Variation in weight was monitored in the course of the study. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group.

##### 2.2.2.2 Curative Test

A total of twenty mice were used for this study. On the first day (D<sub>0</sub>), standard inoculums of  $1 \times 10^7$  *P. berghei berghei* infected red blood cells were injected into the mice intraperitoneally. Seventy-two hours later, the mice were divided into five groups of four mice each. Different doses of the extract (50, 100, 200, and 400 mg/kg/day) were administered orally to three groups. Chloroquine phosphate (5mg/kg/day) was given to the positive control group and

0.2 mL of normal saline to the negative control group. The extract was given once daily for 5 days. Thin blood smears were prepared from tail of each mouse for 5 days to monitor the parasitaemia level. Variation in weight was monitored in the course of the study. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (D<sub>0</sub>-D<sub>27</sub>) (Ryley and Peters (1970); Chandel and Bagai(2010)).

### 2.2.3 *In vivo antiplasmodial test on combination of methanolic leaf and root extracts of Morinda lucida (Curative test)*

Curative test was carried out on mice to assess the antiplasmodial efficacy of combination of extracts. In this test, antiplasmodial test was carried out on the hot methanolic leaf extract of *Morinda lucida* alone, hot methanolic root extract of *Morinda lucida* alone and a combination of the hot methanolic root and leaf extracts of *Morinda lucida*, in the ratio 1:1. A total of twenty four mice were used for this study. On the first day (D<sub>0</sub>), standard inoculums of  $1 \times 10^7$  *P. berghei berghei* infected red blood cells were injected intraperitoneally. Seventy-two hours later, the mice were divided into eight groups of three mice each. Two groups were administered hot methanolic leaf extract of *Morinda lucida* alone with doses 100 and 200 mg/kg /day, another two groups were administered hot methanolic root extract of *Morinda lucida* alone with doses 100 and 200 mg/kg /day, two groups were also administered a combination of hot methanolic leaf and root extract of *Morinda lucida*, in the ratio 1:1, with doses 50 and 100 mg/kg/day. Chloroquine (5mg/kg/day) was given to the positive control group and 0.2 mL of normal saline to the negative control group. The extract was given once daily for 5 days. Thin blood smears were prepared from tail of each mouse for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 28 days (D<sub>0</sub>-D<sub>27</sub>) (Ryley and Peters(1970); Chandel and Bagai (2010)). Variation in weight was also monitored in the course of the experiment.

## 3.0 Results

### 3.1 *In vivo antiplasmodial activity of crude methanolic root extract of Morinda lucida*

#### 3.1.1 *four-day suppressive test*

The mice in all the groups were infected with *Plasmodium berghei*. Fig. 1 shows the mean parasite count of mice for suppressive test. The group treated with chloroquine showed significant ( $p < 0.05$ ) parasite suppression ( $1.84 \pm 0.15$ ), compared to all other groups. However, there was no significant difference ( $p > 0.05$ ) between the groups treated with 100 ( $4.44 \pm 0.15$ ), 200 ( $4.08 \pm 0.23$ ) and 400mg/kg ( $3.28 \pm 0.44$ ) crude methanolic root extract of *Morinda lucida*. The group which was not treated at all, but given 0.2ml normal saline, gave the highest parasite count ( $10.16 \pm 1.41$ ). Fig. 2 shows percentage (%) suppression of parasite for mice treated with crude methanolic root extract of *Morinda lucida*. The group treated with chloroquine had highest % suppression of parasitaemia (81.89%). The average % suppression for 100, 200 and 400mg/kg for crude methanolic root extract of *Morinda lucida* were 56.30, 59.84 and 67.72% respectively.

Fig. 3 shows average weight of mice before infection, and after four days of treatment for suppressive test. There was no significant difference ( $p > 0.05$ ) in weight of mice before infection and after four days of treatment in all the groups.

The mean survival period in days were calculated to be  $18.60 \pm 0.75$ ,  $20.20.75 \pm 1.40$ ,  $23.60 \pm 1.03$ ,  $24.80 \pm 1.93$  and  $12.80 \pm 0.86$ , for 100, 200, 400mg/kg body weight (b.w) of crude methanolic root extract of *Morinda lucida*, 5mg/kg b.w of chloroquine and the untreated control respectively (Fig. 4).

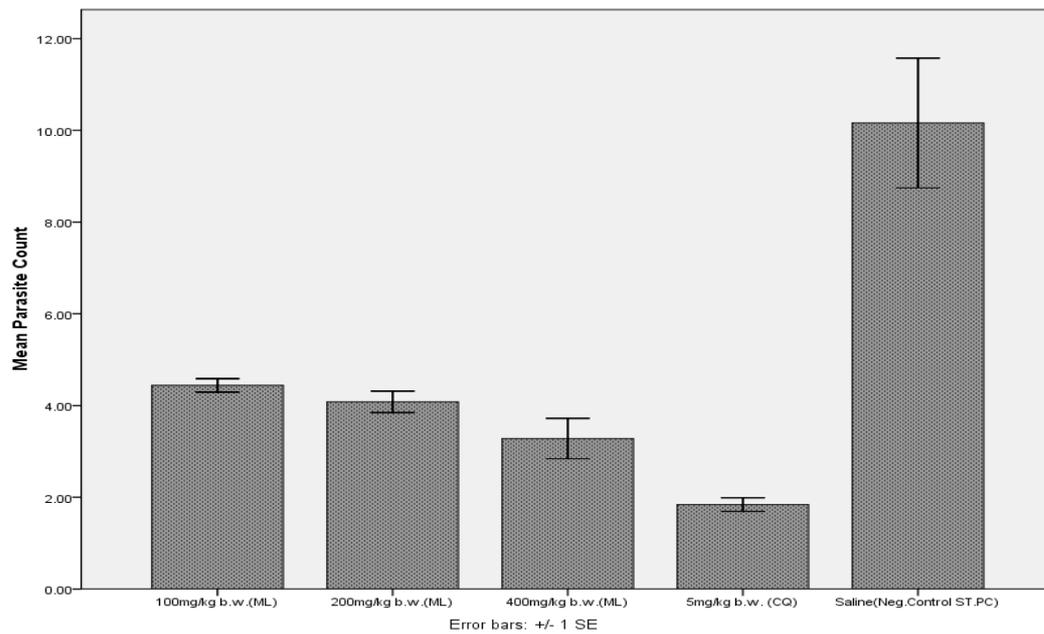


Fig. 1: Mean parasite count of mice for suppressive test

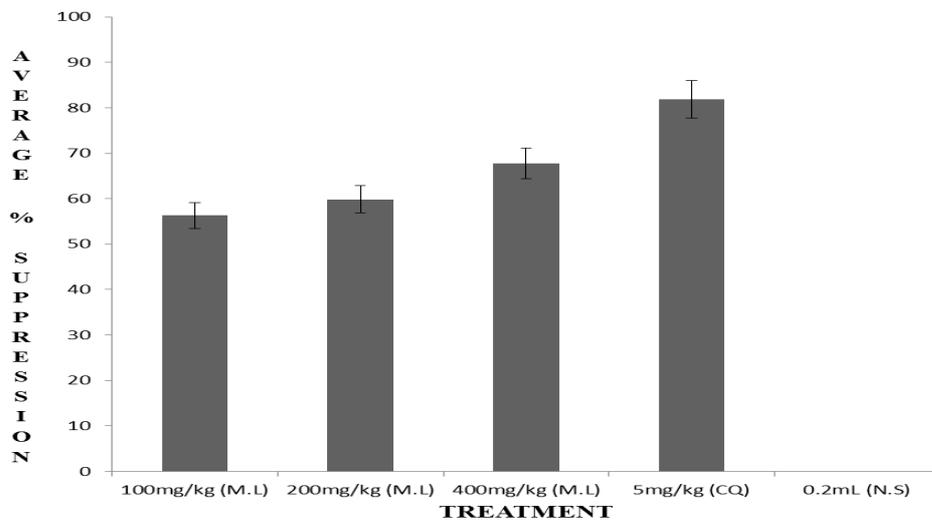


Fig. 2: Average percentage suppression of parasite by methanolic extract of *Morinda lucida* root

Key

M.L: Methanolic root extract of *Morinda lucida*, CQ: Chloroquine, N.S: Normal saline

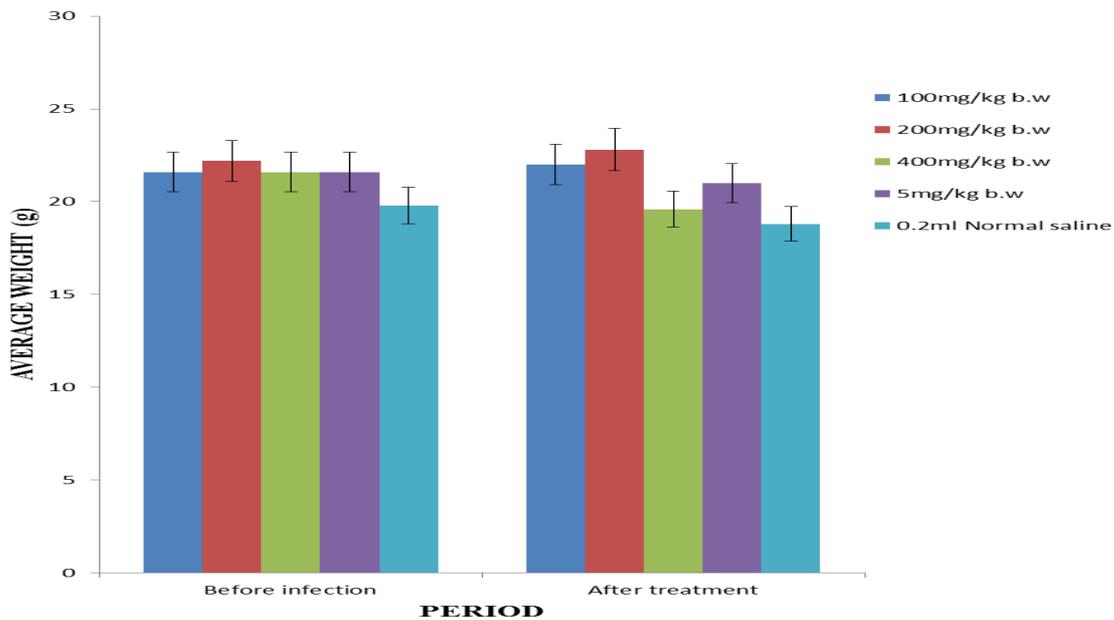


Fig. 3: Average weight of mice, before infection and after four days of treatment

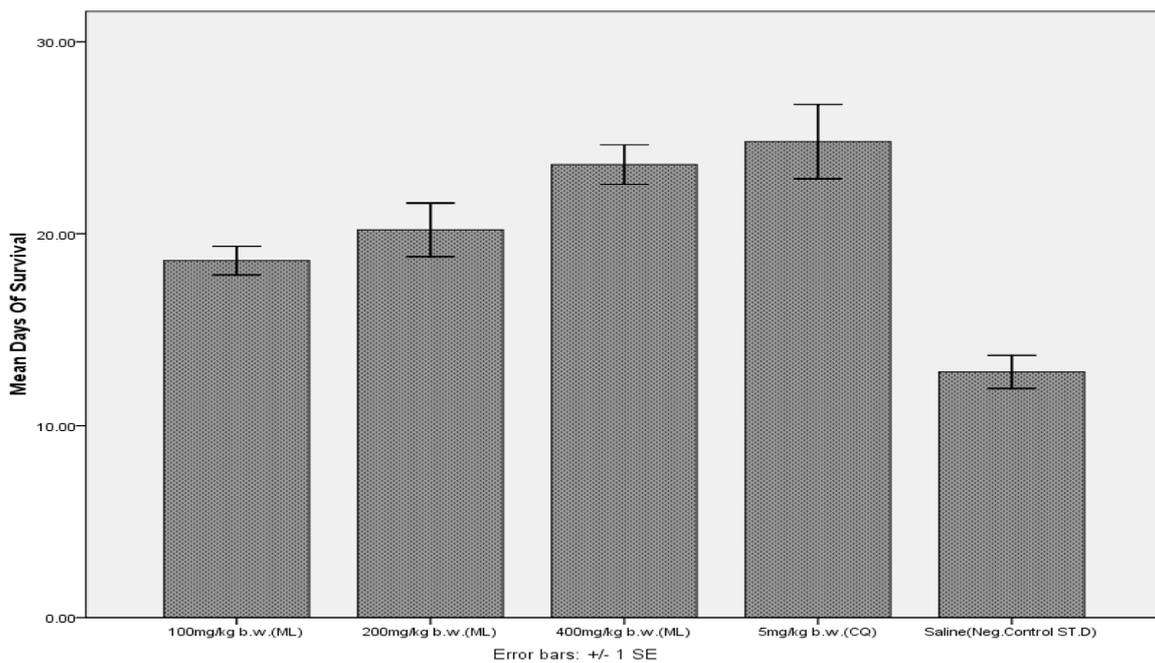


Fig. 4: Mean Days of Survival of mice for suppressive test  
 ML: Crude methanolic root extract of *Morinda lucida*, CQ: Chloroquine, ST.D: Standard

### 3.1.2 Curative test

All the groups infected with *Plasmodium berghei* and treated with the crude methanolic root extract of *Morinda lucida* and chloroquine, showed an exponential decrease in parasite count, throughout the study period, with chloroquine showing highest parasite inhibition ( $8.78 \pm 3.58$ ). However, the negative control group showed exponential increase in parasite count ( $115 \pm 5.66$ ), throughout the study period (fig. 5).

There was no significant weight change in all the experimental groups before infection, 72 hours after infection and after 5 days of treatment, however, those animals in the negative control group showed slight weight loss (fig. 6).

Fig. 7 shows the average PCV of mice before infection, 72 hours after infection and after 5 days of treatment in different test groups. It can be deduced from the chart that, there was reduction in PCV of mice in all test groups, within and after the experimental period, except for the chloroquine treated group where there was an increase in the PCV level throughout the period. However, there was a slight increase in PCV between the period before infection and 72 hours after infection, for the group treated with 400mg/kg b.w of crude methanolic root extract of *Morinda lucida*.

The mean survival period in days were calculated to be  $15.00 \pm 0.70$ ,  $18.75 \pm 0.5$ ,  $19.75 \pm 1.39$ ,  $23.25 \pm 1.38$  and  $8.75 \pm 1.25$ , for 100, 200, 400mg/kg body weight (b.w) of crude methanolic root extract of *Morinda lucida*, 5mg/kg b.w of chloroquine and the untreated control respectively

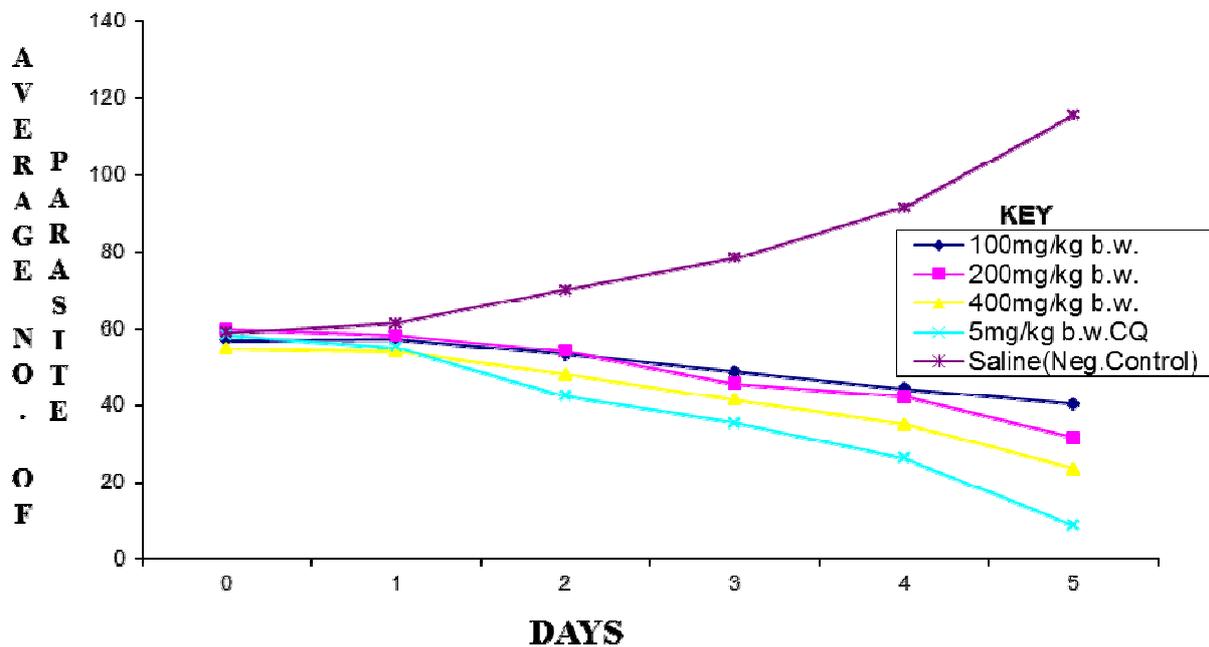
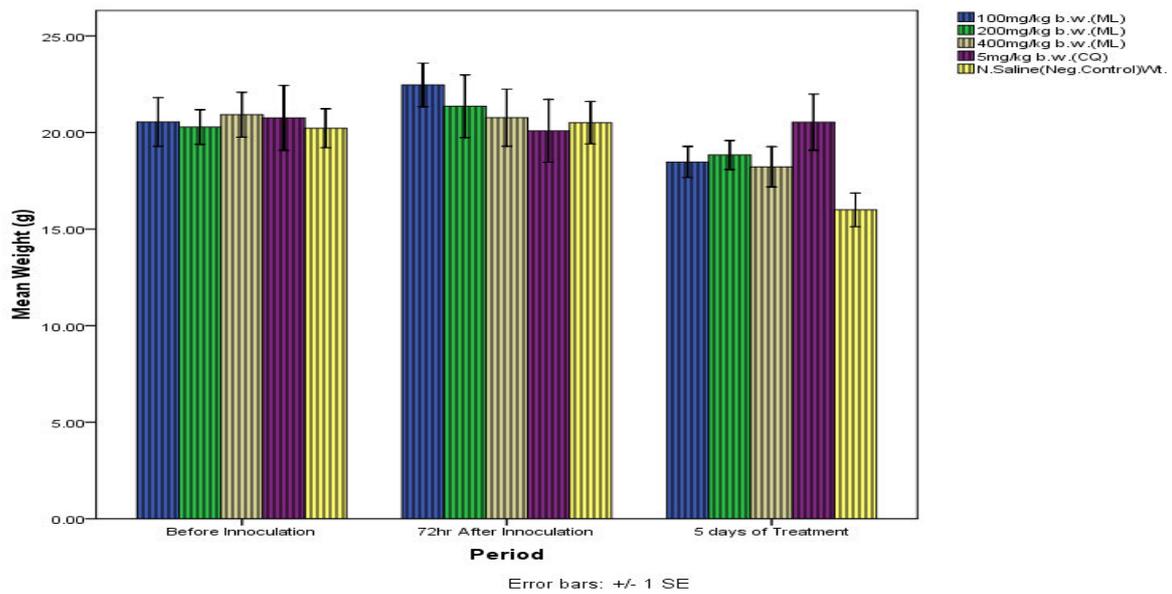
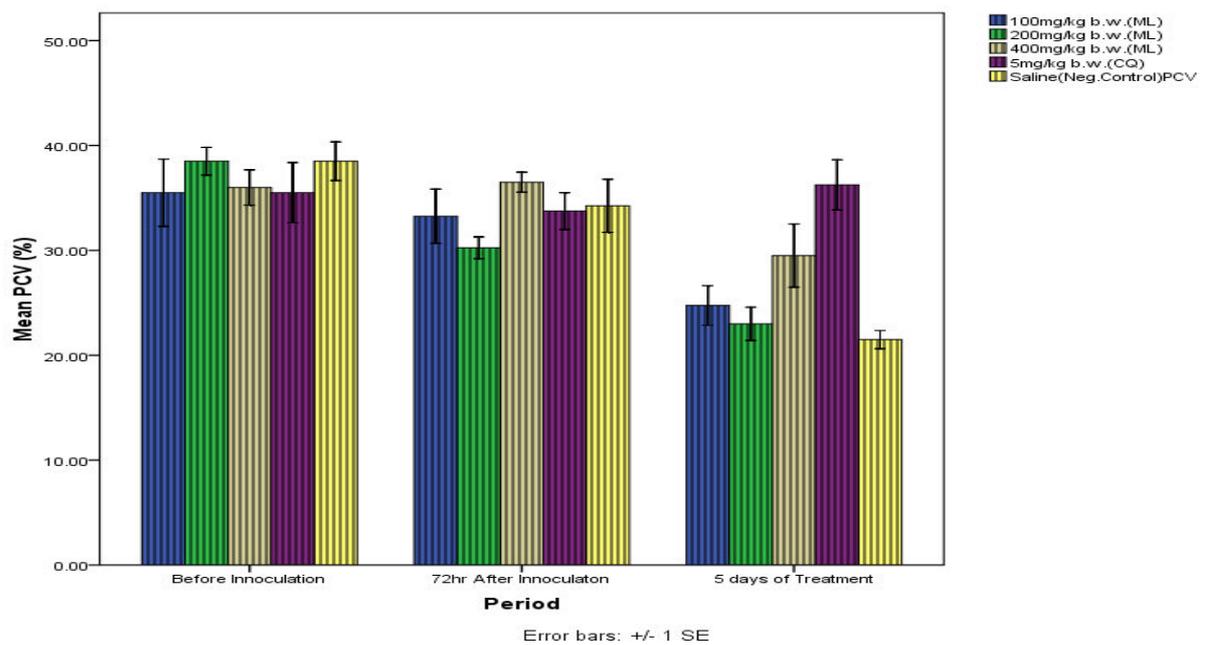


Fig. 5: Antiplasmodial effect of methanolic root extract of *Morinda lucida*



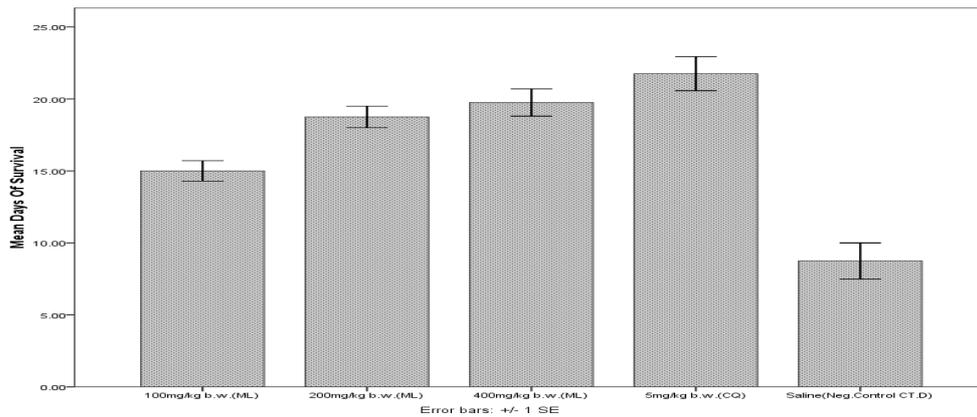
**Fig. 6: Average weight of mice, before infection, 72hours after infection, and after 5 days of treatment for curative test**

M.L: Crude methanolic root extract of *Morinda lucida*, CQ: Chloroquine



**Fig. 7: Average Pack Cell Volume (PCV) of Mice, before infection, 72hours after infection, and after 5 days of Treatment**

M.L: Crude methanolic root extract of *Morinda lucida*, CQ: Chloroquine



**Fig. 8: Mean days of survival of *Plasmodium berghei* infected mice treated with methanolic root extract of *Morinda lucida***

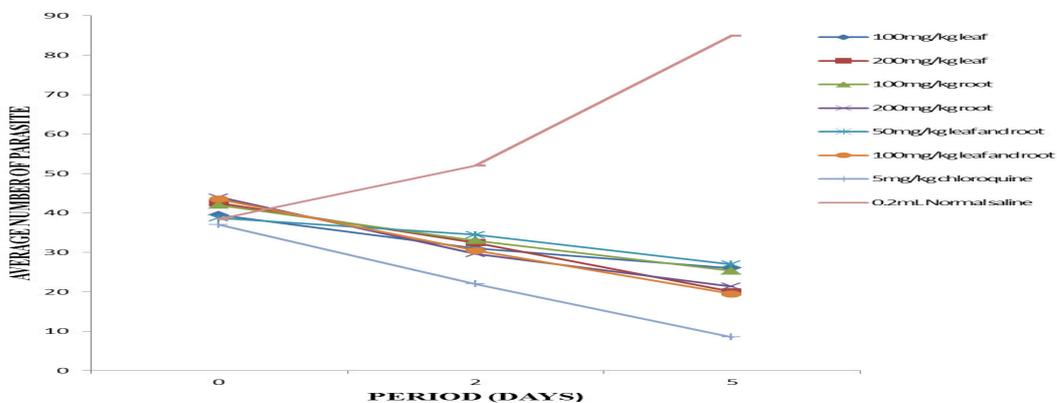
M.L: Crude methanolic root extract of *Morinda lucida*, CQ: Chloroquine

### 3.2 *In vivo* Antiplasmodial activity of combination of methanolic root and leaf extracts of *Morinda lucida*

The chloroquine treated group showed remarkable parasite inhibition at the end of the experiment ( $8.50 \pm 1.26$ ) compared to all other test groups. There was no significant difference in parasite inhibition between the groups treated with 200mg/kg b.w leaf alone ( $20.00 \pm 1.29$ ), 200mg/kg b.w root alone ( $21.35 \pm 1.85$ ) and 100mg/kg b.w combination of methanolic root and leaf extracts of *Morinda lucida* ( $22.50 \pm 1.29$ ) (Fig. 9).

There was no significant difference in weight of mice in all the test groups between the periods before and after the experiment (Fig. 10).

In all the experimental groups, there was significant decrease in PCV levels after infection with *Plasmodium berghei* and after treatment, except for chloroquine treated group, where there was a decrease in PCV after infection, but subsequently increased after 5 days of treatment (Fig. 11).



**Fig. 9: Average number of parasite of mice treated with a combination of the methanolic root and leaf extracts of *Morinda lucida***

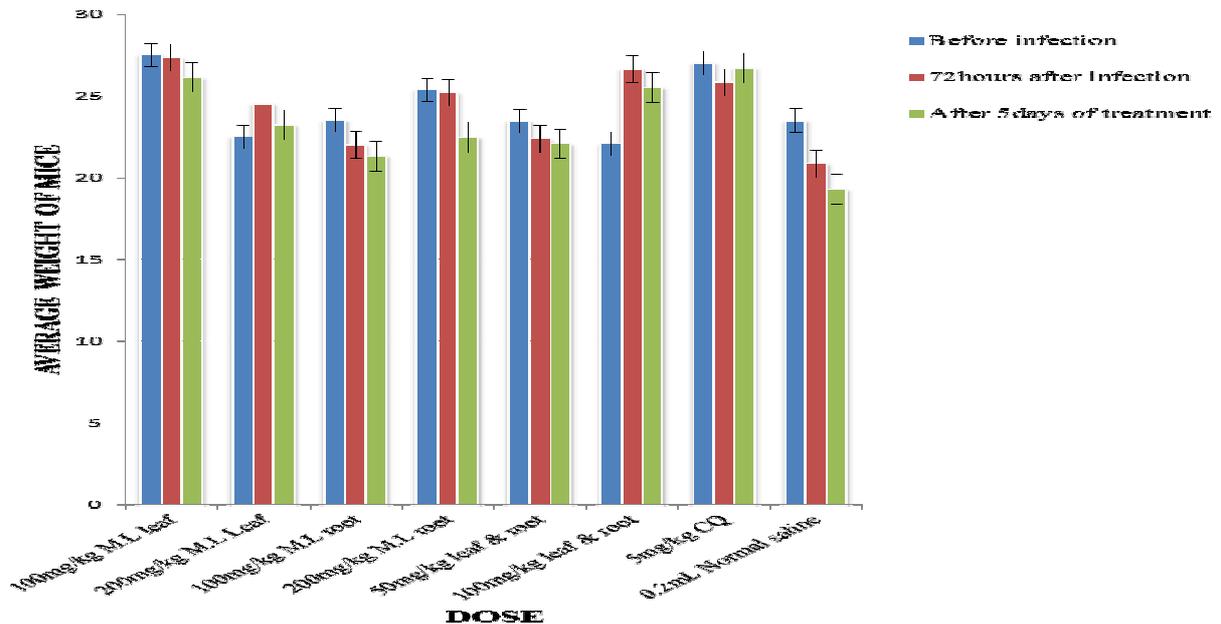


Fig. 10: Average weight of mice treated with a combination of the root and leaf methanolic extracts of *Morinda lucida*

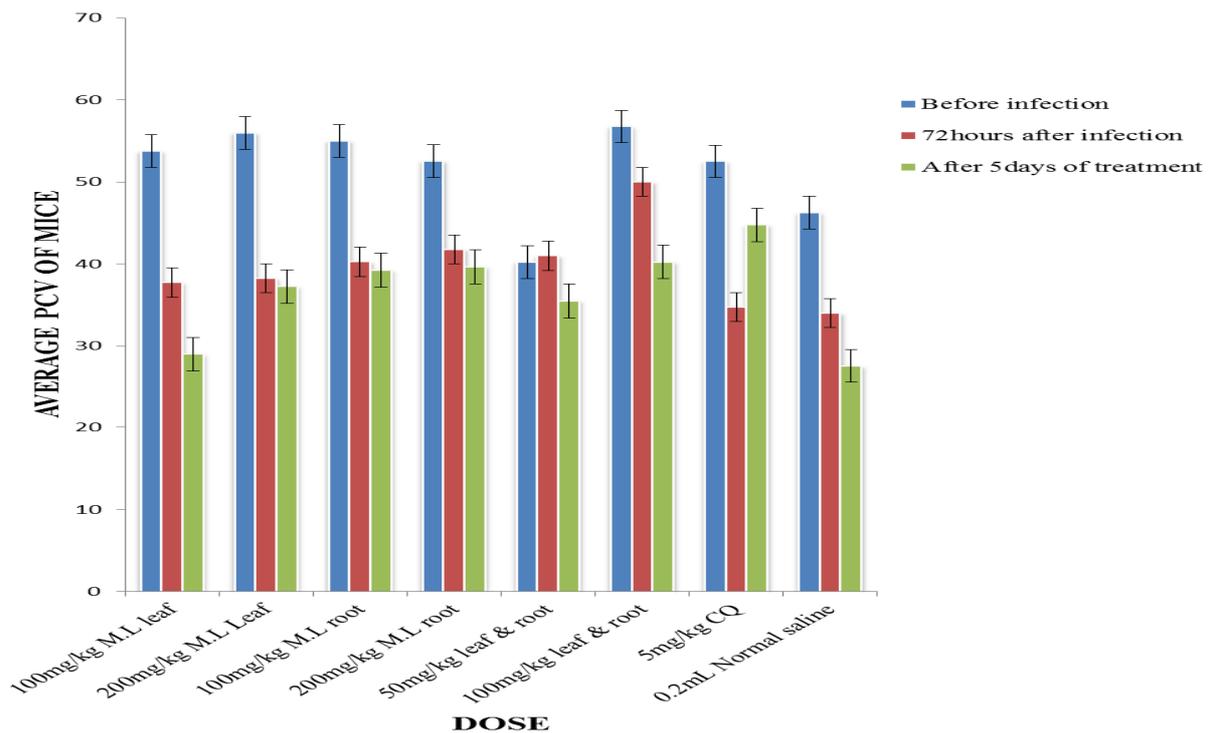


Fig. 11: Average PCV treated with a combination of the root and leaf methanolic extract of *Morinda lucida*

#### 4.0 Discussion

*Morinda lucida*, commonly known as “Ugigo” by the Ebara people in Kogi State North-Central Nigeria, is used in the treatment of malaria, fever, amongst other ailments.

The in vivo Antiplasmodial studies carried out on the methanolic root and leaf extracts, have confirmed the works of others on the Antimalarial activity of extracts of the plant parts (leaf, stem bark and root) (Asuzu and Chineme, 1990;

Makinde and Obih, 1985; Koumaglo *et al.*, 1992). It has been reported that the stem bark infusion is used as an antimalarial (Burkill, 1997).

It has been reported that the petroleum ether extract and fractions of the leaf samples were evaluated for antimalarial effects against *Plasmodium falciparum* using the Rabbit in vivo technique (Awe and Makinde 1998). It was observed that the extract and some fractions inhibited the maturation of a drug sensitive strain of *Plasmodium falciparum*. Active anthraquinones were isolated, the most active being damnacanthal.

Despite the level of work that has been carried out on *Morinda lucida* plant, more areas of research on the plant still needs to be explored. This research work aimed at studying the antimalarial activity of crude hot methanol extract of the root parts and a combination of the hot methanol root and leaf of *Morinda lucida*.

The four-day suppressive test carried out on hot methanol root extract, showed appreciable suppressive effect (fig.1), as the mice in two groups out of the three groups treated with the crude methanolic root extract survived beyond twenty days (fig.4). There was no significant difference in weight for all the groups, before and after treatment ( $p>0.05$ ).

The curative test showed appreciable curative effect (fig.5), though none of the groups treated with the extract survived up to 28 days, when compared with the experimental animals in the chloroquine treated group, where one of the animals survived beyond 28days. There was no significant difference ( $p>0.05$ ) in the body weight of all the test animals, before infection, after 72 hours of infection, and after 5 days of treatment, when compared with the negative control group, where there was significant ( $p<0.05$ ) drop in body weight, during the test period. There was significant ( $p<0.05$ ) difference in the pack cell volume (PCV) in all the groups, throughout the test period. There was increase in the PCV of the chloroquine treated group after 5days of treatment, compared to all the extract treated groups and the negative control group, where there was drop in PCV, though the negative control group being drastic (fig. 7). The drop in the PCV that is responsible for malarial anaemia occurs both through an increase in the rate at which old Red blood cells are broken and a decrease in the rate at which new ones are produced. Plasmodium not only causes the rupture of parasitized red blood cells, but stimulates the activity of macrophages in the spleen, which then destroys both parasitized and unparasitized red blood cells.

Curative test carried out on the combination of the methanolic extract of the root and leaf showed that parasite reduction in mice was more when treated with the combination of the two extracts, than when treated single extract (fig. 9).

The presence of flavonoids and other bioactive constituents, is believed to have contributed to the observed antiplasmodial activity of *Morinda lucida* root and leaf extracts.

#### **Acknowledgement**

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