FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 6, December, 2024, pp 72 - 76
DOI: https://doi.org/10.33003/fjs-2024-0806-2574

IN VIVO EVALUATION OF THE ANTIPLASMODIAL EFFICACY OF *MANGIFERA INDICA* LEAF EXTRACT IN *PLASMODIUM BERGHEI*-INFECTED MICE

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ABSTRACT

The menace of malaria has been and is still one of the most devastating in the world. Chemotherapy, the main control strategy, is under threat as the parasite develops resistance to currently available antimalarial drugs. Therefore, the search for new compounds with novel mechanisms of action and targets to treat malaria is inevitable as nature is a constantly evolving source of compounds with medical importance. The present study aims to investigate the toxicological and *in vivo* antiplasmodial effects of *Mangifera indica* extract on albino mice infected with *Plasmodium berghei* (ANKA). Swiss albino mice (15-25g), chloroquine sensitive *P. berghei* and Fresh *M.indica* leaves were collected, air dried, ethanol extract obtained and used for the study. The modified Lorke method was adopted to determine acute toxicity, with 3 groups of mice intraperitoneally administered varying doses of the extracts in 2 phases. The malaria parasite *plasmodium berghei* was inoculated into the apparently healthy Swiss albino mice. The curative evaluation of grouped mice used at various concentrations (150mg/kg, 300mg/kg, and 1200mg/kg) alongside negative (distilled water) and positive (10mg/kg chloroquine) controls were performed. The parameters of body weight, packed cell volume and parasitemia were determined using Ranes' and Peters test. No toxicity was observed at a maximum dose of 4000mg/Kg. A four-day curative test was performed with mice inoculated intraperitoneally with $1x10^7$ parasitized erythrocytes per mL. Chloroquine (10mg/kg) and distilled water (untreated) were administered as positive and negative controls, respectively, while the plant extract concentrations of 150mg/kg, 300mg/kg and 1200mg/kg were administered orally to the treatment groups. The average parasitemia for these concentrations decreased from 14.5±0.29 to 3.6±0.49, from 15.1±0.15 to 2.1±0.10 and from 13.2±0.53 to 0.8±0.03, respectively, while the packed cell volume increased from 54.3±1.10 to 56.4±0.60, from 50.3±0.50 to 59.0±0.20 and from 57.7±0.20 to 67.7±0.60, respectively. The crude extract prevented weight loss from 16.3±0.40 to 20.1±0.10, from 20.3±0.70 to 24.3±0.60 and from 19.4±0.20 to 25.7±1.20, on the last day of the treatment, respectively. The crude extract of *M. indica* showed significant antiplasmodial activity against *P. berghei*, depending on the dosage. This study suggests that the leaf extract of *M.indica* possess antiplasmodial activity against *plasmodium berghei* in a dose dependent manner, and prevented loss of weight and increase packed cell volume which establish a scientific justification on the traditional use of *M.indica* in management of malaria.

Keywords: Antiplasmodial activity, *Mangifera indica*, *Plasmodium berghei*, Leaf extract

INTRODUCTION

Malaria, a mosquito-borne infectious disease of humans and other animals, is still a deadly disease and a global problem despite tremendous interventions (White, 2011). Chemotherapy, which is currently the most important measure to combat malaria (Kpanon et al., 2021; Nyandwaro et al., 2020), is threatened by the development of resistance to existing antimalarial drugs (Abdulrazaq et al., 2020). The growing threat of parasite resistance to currently administered antimalarial drugs necessitates the discovery of new antimalarial drugs with novel mechanism of action and/or new targets to avert the problem of drug resistance (Tajbakhsh et al., 2021). Plasmodium Berghei, one of the four plasmodium species (Plasmodium berghei, Plasmodium chabaudi, Plasmodium vinckel, and Plasmodium yoelii) that have been described in African murine rodents (Janse et al., 2012), causes malaria (Nadjm and Behrens, 2012).

Plants have proven to be a rich source of medicines over the years and have been traditionally used in many cultures and societies. They remain an ever-evolving source of compounds with medicinal significance, providing a safe and effective source of medicines (Abdulrazaq et al., 2020). Mangifera indica (mango) is one of the most important fruits in the tropical and subtropical regions (Joyce et al., 2014). Its readily available leaves have earned its use in traditional medical practice for the treatment of malaria (Tsabang et al., 2012). Although there is little scientific information on the antiplasmodial effect of M. indica (Olayode et al., 2015), its ethnomedicinal use suggests that the plant may have an antimalarial effect. This study aims to investigate the in-vivo antiplasmodial activity of the crude extract of M. indica in mice infected with P. berghei.

MATERIALS AND METHODS

Mice and Plant material source

Swiss albino mice (15-25g) and chloroquine sensitive P. berghei used for this study were obtained from the animal house of the Department of Pharmacy, and Pharmacology and Therapeutic respectively in Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Kaduna state, Nigeria. The mice were acclimatized for 7 days prior to their randomization into the various experimental groups. The animals were maintained on standard commercial diets and water given ad libtium.

Plant Sample Collection, Identification and Processing of Leaves

Fresh leaves of M. indica were sourced within the institution of Ahmadu Bello University Zaria, and taken to the Department of Biological Science, Ahmadu Bello University, Zaria for proper identification (ABU0950212). M. indica leaves were air-dried for 2 weeks at room temperature. The

dried leaves were then pulverized to coarse powder using a dry mechanical mill. The powder was packed in a tight container and stored a cool dry place.

Extraction

The extraction of M. indica leaves was carried out by the use of soxhlet apparatus; 70g of the powder leaves was weighed. 600ml of ethanol was transferred into a round bottom flask another 200ml was transferred into the condenser. The soxhlet apparatus was set and allowed to run for 16-24 hours after which the ethanol extract was collected and put in a water bath to evaporate at 64°C to dry. The dried extract (18.69g) was weighed and transferred into a sample container and kept in a refrigerator for subsequent use. The percentage yield calculated (26.7%) (Ihekwereme et al., 2016).

Acute Toxicity of the Extract

The modified Lorke method was adopted to determine the LD50 of the crude extract of M. indica. This was performed using male mice. They were subjected to fasting overnight and divided into three groups of 3 mice pergroup. In the first phase, the mice received 10, 100, and 1000mg/kg of the extract intraperitoneally, and closely observed for the first 6-24hours for signs of toxicity and mortality. For the second phase, the 3 groups of mice per cage received 1600, 3200, and 4000mg/kg of the extract intraperitoneally, and observed for signs of toxicity and mortality at regular intervals for 24hours, 48hours, and 72hours (Ihekwereme et al., 2016).

Experimental design

The mice were grouped into five (5) groups of three (3) as follows: Group 1: Positive control group treated with 10mg/kg chloroquine (PC), Group 2: Negative control group treated with distilled water (NC), Group 3: Infected group treated with 150mg/kg body weight of M. indicia crude extract (IT150MI), Group 4: Infected group treated with 300mg/kg body weight of M. indicia crude extract (IT300MI), Group 5: Infected group treated with 1200mg/kg body weight of M. indicia crude extract (IT1200MI).

Inoculation of Experimental Animals

Blood was intraocularly collected via punch from two donor mice with rising parasitaemia of 25% and 27% in heparinized syringes. Standard inoculums of 1×107 P. berghei (ANKA) infected erythrocyte in 0.2 ml were prepared by diluting infected blood with 0.9% normal saline. Each mouse was intra-peritoneally injection with the blood suspension (0.2ml) containing 1×107 parasitized erythrocytes.(Okokon et al., 2017).

Curative Test

This evaluation was performed according to the method described by (Ryley and Peters, 1970). Twenty (20) mice were used for curative experiment. Infected animals were divided into 5 groups (n = 5) of 4 mice each. The infected mice were kept for 72hours for their body to pick up the parasite. Two control groups were used namely, positive (infected and treated with 10mg/kg of chloroquine), and negative (infected and treated with distilled water). Three different concentrations of 150mg/kg, 300 mg/kg, and 1200mg/kg body weight of M. indica extract was administered to groups 3-5 respectively. All treatments were administered orally using feeding cannula and the treatment lasted for 4 consecutive days. Blood samples were collected from the tip of the tails of the animals on each day to check for the parasitaemia level.

Estimation of Parasitaemia

A small drop of blood was collected from the tail of each of the animals in each group, using clean, non-greasy slides to make thin blood smears and allowed to air-dry. The thin films were then fixed using few drops of methanol. The films were left for about 15-20 minutes to air-dry. Thereafter they were washed off and stained with Giemsa stain for 45 minutes. The stain was washed off and slides were left to air-dry, before they were viewed under light microscope using oil immersion objective. Each of the blood films prepared was mounted and viewed under the microscope using the ×100 objective. The number of parasitized red blood cells that were seen per film was counted and recorded. The average percentage parasitaemia was calculated using the formula (Bantie et al., 2014)

$$\%\ \text{parasitaemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes counted}} \times 100$$

Determination of Packed Cell Volume (PCV)

The tip of the tail of each mouse was cut using a sterilized scissors. Blood was collected by gently milking the tail using heparined microhaematocrit capillary tubes and sealed at their dry end with flame. The tubes were then placed in a microhaematocrit centrifuge with the sealed end outwards. The blood was centrifuged at 12000rpm for 5min. the packed cell volume was determined using the following relation (Bantie et al., 2014)

$$\text{PCV} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100$$

Monitoring of Body Weight Changes

For the curative test, body weight of each mouse was measured before inoculation, 3 days after inoculation, and all the treatment days using a sensitive digital weighing scale. In order to rule out the effect of the extract on body weight, and PCV; the crude extract was administered to healthy mice at the doses used for four days treatment.

Data Analysis

Data obtained were expressed as mean ± standard deviation (SD). Data were analyzed using one way analysis of variance (ANOVA) with the help of statistical package for social science (SPSS) version 23 for window. Duncan post hoc test was done to compare the differences among the mean of the various treatment groups. P. value less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

LD50 Toxicity Test

The results of the acute toxicity showed that the extract caused no mortality within the dose range of 10-4000 mg/kg (Table 1). This shows a clear indication that the doses used were safe. This proposes that the LD50 of the extract is greater than 4000 mg/kg.

Table 1: LD50 toxicity of plant extract

Phase 1	Dose(mg/kg)	Mortality	Phase2	Dose(mg/kg)	Mortality
Group1	10	0/3	Group1	1600	0/3
Group2	100	0/3	Group2	3200	0/3
Group3	1000	0/3	Group3	4000	0/3

Effect of M. indica Crude Extract on % Parasitaemia of P. berghei Infected Mice

The mean parasitaemia of the P. berghei infection in the mice was calculated from the % parasitaemia of the total number of mice. From the figure 1, there was a significant increase in the parasitaemia level in all the groups after inoculation. The negative control (NC) had the highest parasitaemia level of 28.00±1.00 at the end of the treatment days. There was a significant decrease in parasitaemia level in all the groups that received M. indica crude extract. The group that received the highest dose of the extract 1200mg/kg exhibited the lowest mean parasitaemia amongst all the groups that received the extract as treatment which is comparable to the positive control group which received 10mg/kg of chloroquine, the standard drug.

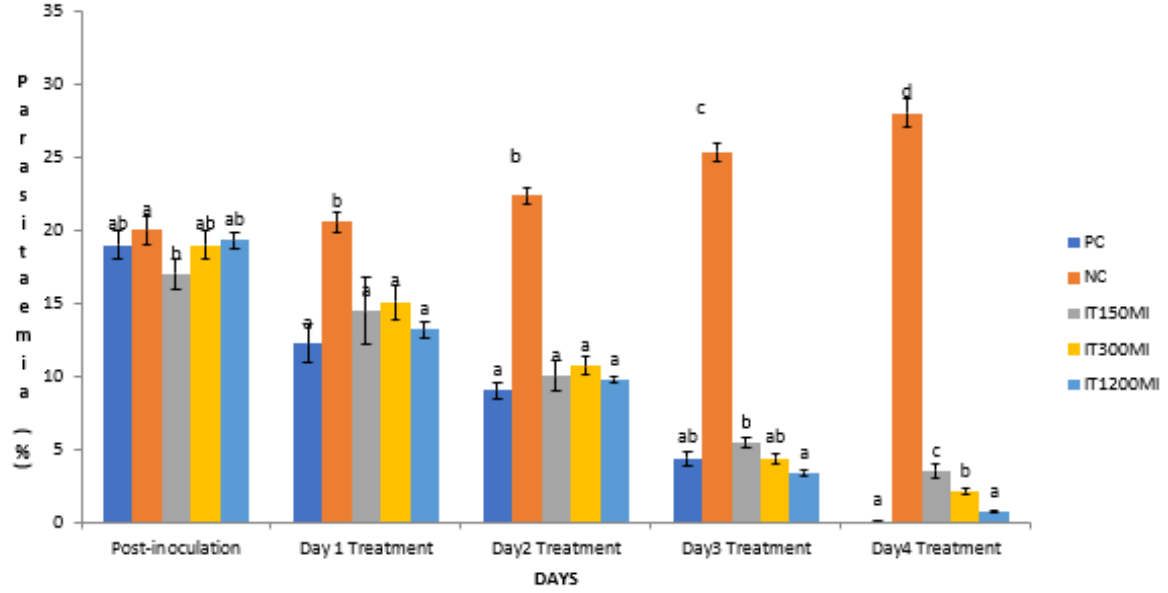

Figure 1: Effect of M. indica extract on parasitaemia level in P. berghei infected mice.
Values are presented as mean of triplicates ± standard deviation. Values with different superscripts per day differ significantly ($p \leq 0.05$).

Effect of Manifera indica Crude Extract on Weight of Plasmodium berghei Infected Mice

The mean weight of the P. berghei infected mice was calculated from the weight of the total number of mice in each group. From the figure 2, there was a significant decrease in body weight after inoculation. The negative control had the lowest weight of at the end of the treatment days. The groups that received the doses of M. indica crude extract and positive control group which received 10mg/kg of chloroquine, the standard drug, all had significant increase in body weight. The crude extract significantly prevented weight loss in the P. berghei infected mice which is comparable to the standard drug (chloroquine).

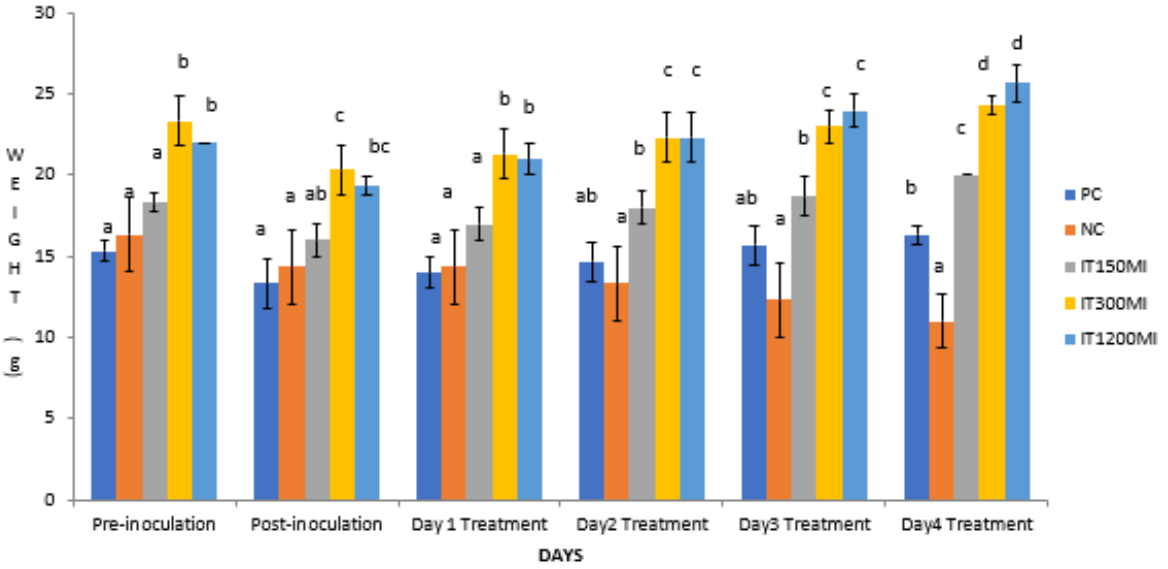

Figure 2: Effect of M. indica crude extract on body weight of P. berghei infected mice.
Values are presented as mean of triplicates± standard deviation. Values with different superscripts per bar differ significantly ($p \leq 0.05$).

Effect of M. indica Crude Extract on Packed Cell Volume (PCV) of P. berghei Infected Mice

The mean PCV of the P. berghei infected mice was calculated from the PCV of the total number of mice. From the figure 3 , NC had the lowest PCV level of at the end of the treatment days. There was a significant decrease in the PCV level in all the groups after inoculation. There was a significant increase in the PCV level of all the groups that received the M. indica extract as compared to the group that received the standard drug, Chloroquine. M. indica crude extract significantly prevented reduction in PCV level in the P. berghei infected mice which is comparable to the standard drug (chloroquine).

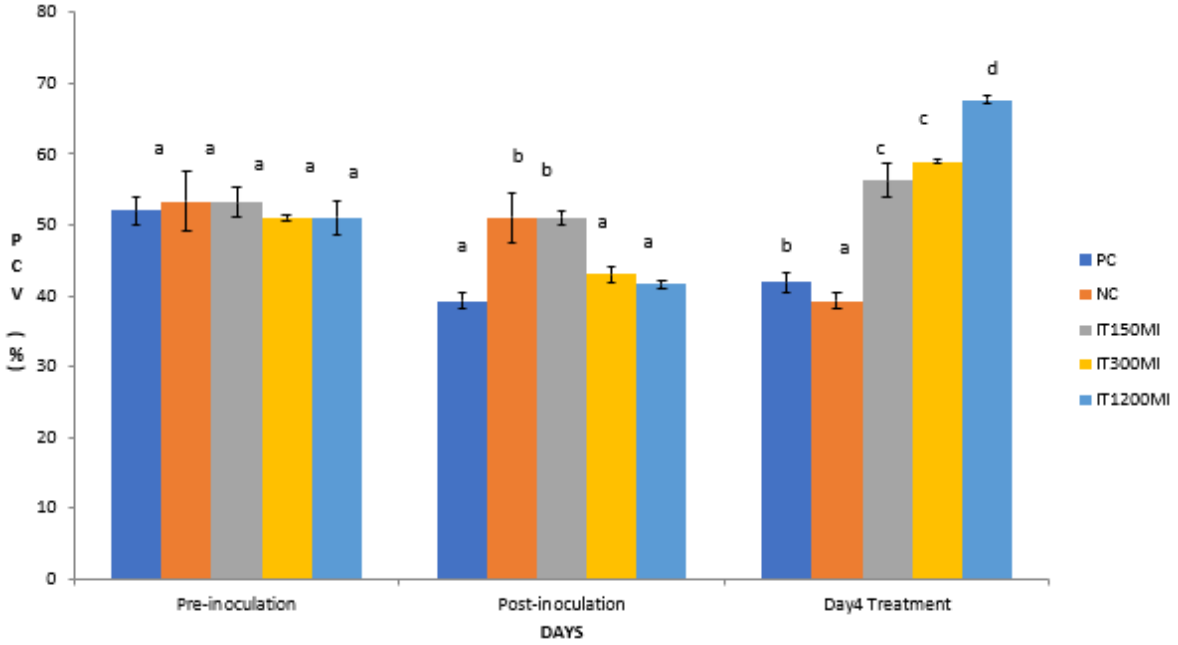

Figure 3: Effect of of M. indica leafextract on PCV of P. berghei infected mice.
Values are presented as mean of triplicates ± standard deviation. Values with different superscripts per day differ significantly ($p \leq 0.05$).

Discussion

The results for the acute toxicity assay showed that the extract had no significant toxicity at the maximum concentration used (4000 mg/kg/day).

A gradual reduction was observed in the body weight of P. berghei-infected mice untreated which is in conformity with the study of Yeshanew and Mekonnen, (2013). There was a gradual increase in the body weights of the mice in the extract treated and chloroquine groups. One of the features of rodent malaria infections is body weight loss (Perlmann et al., 2002). (Nwinuka et al, 2008) reported a gradual increase in the body weight, following the administration of extract of M. indica. These are in accordance with the findings in this study. This also conforms to the findings of (Sellamuthu et al, 2009).

The results also showed a dose dependent manner and when compared with the positive control group, the treated group with the highest dose had a similar reduction ability with the positive control. This significant reduction of percentage levels of parasitemia by M. indica was dose dependent. The presence of triterpenoid in M. indica might have inhibited the stages of infection, thereby reducing the percentage parasitemia. Triterpenoid possesses dual antimalarial activity as inhibitor of erythrocytic and liver stages of Plasmodium infections (Ramalhete et al, 2014). Chloroquine reduced the number of parasitized red blood cells in circulation and this was in accordance with a previous study by (Georgewill and Ebong, 2012) as above.

Anaemia has been reported as one of the symptoms of malarial infection in mice (Airaodion et al., 2020). Anaemic condition has been reported to be sequel to haemolysis (Airaodion et al., 2019). From the results of this present study, the packed cell volume (PCV) of P. berghei infected but untreated mice (negative control) showed significant decrease in PCV after 3 days of infection (Figure 3). This revealed that P. berghei infection significantly reduced red blood cells of animals. Treatment of infected mice with 150 mg/kg and 300 mg/kg of M. indica leaf extract was able to reverse the effect of P. berghei infection on the PCV of the mice. Also, treatment of infected mice with 1200mg/kg of M. indica leaf extract as well as 10 mg/kg of chloroquine (positive control) showed significant increase in PCV after 4 days of treatment. This is indicative that treatments with the doses of the extract (150mg/kg, 300mg/kg, and 1200mg/kg) all have the propensity to ameliorate the effect of P. berghei infection on the PCV of infected mice. The group treated with 1200mg /kg is comparable with the group treated with antimalarial standard drug (chloroquine) in this study.

CONCLUSION

The LD50, measured at over 4000 mg/kg, suggests that the substance is relatively safe and exhibits negligible toxicity. The results revealed that the crude extract significantly reduced % parasitaemia confirming the antiplasmodial activity of M. indica. The results indicated that the extract effectively prevented both significant weight loss and a significant drop in PCV in Plasmodium berghei-infected mice.

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