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IN VIVO ANTIPLASMODIAL EFFICACY OF FRACTIONS OF CRUDE METHANOLIC ROOT EXTRACT OF *MORINDA LUCIDA*

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ABSTRACT

Significant progress has been made in the control of malaria, but effective treatment of the disease remains a big challenge. Development of antiparasitic drugs from medicinal plants continues to be a very appealing option. Therapeutic effects of the crude methanol extract of *Morinda lucida* root and its fractions were evaluated in *Plasmodium berghei* - infected mice by assessing the curative effect and mean survival period after administration of the extracts. Preliminary toxicological study of the crude extract showed the LD_{50} to be 3800 mg/kg, while phytochemical screening showed the presence of saponins, flavonoids, alkaloids and tannins. The crude extract and two fractions obtained from it (fractions 3 and 4) exhibited significant curative antiplasmodial effects that manifested in a longer survival period for the treated mice compared to the control (29.25±1.43, 11.25±0.75, 11.75±1.60, 24.25±1.11, 28.50±1.32, and 5.75±2.14, for crude extract, fraction 1, fraction 2, fraction 3, fraction 4, and control groups respectively). However, the group of infected mice treated with Chloroquine drug demonstrated a longer survival period (over 28 days) and a higher percentage (%) suppression of parasites. It is concluded that the methanol extract of *Morinda lucida* root and two of its fractions are potentially useful for the development of antimalarial drugs.

Keywords: *Morinda lucida*, *Plasmodium berghei*, LD_{50}, % suppression, Survival period, Antimalarial.

INTRODUCTION

Malaria is a mosquito-borne infectious disease caused by a eukaryoticprotist 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 researcherson 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 fractionation of the crude root extract.

MATERIALS AND METHODS

Plant Materials

The root 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.

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.

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 cardiac puncture 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 $1x10^7$ parasitized erythrocytes.

Preparation of Crude Extract

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.

Column Chromatography

The crude extract was partially purified by Column Chromatography using the method described by Blessing *et al*., 2011. This was done by gradually increasing the polarity of solvent used at each step. Three solvents were used; hexane, ethyl acetate and methanol, starting with 100% hexane and gradually increasing the polarity, by varying the percentages of the three solvents. At the end, nineteen fractions were obtained, which were then subjected to Thin Layer Chromatography, in order to pull together those fractions with similar Rf (Refractive index). In the end, four fractions were obtained, which were tested for Antimalarial activity.

Acute Oral Toxicity Test (Determination of Median Lethal Dose, LD_{50})

The acute toxicity of the extract was determined by establishing its median lethal dose (LD_{50}) using the Lorkes method (Lorke, 1983). The test was carried out in two phases. Phase 1: Nine mice were divided into three groups (A, B and C) of three mice each. The three groups were administered orally with graded concentrations (10, 100 and 1000 mg kg^{-1} weight, respectively) of the crude hot methanolic extract of *Morinda lucida*. The animals were monitored for mortality, negative physical signs such as depression, loss of appetite, change in respiration, for a 24hour period. In the phase 2 experiment, nine mice were divided into three groups (D, E and F), each group consisting of three mice. These received graded concentration of 1600, 2900 and 5000 mg kg^{-1} body weight of the extract, respectively. All the Animals were monitored for pains, distress, behavioural alterations and most importantly, death for a period of 24hours.

Evaluation of Antimalarial Activity of Extract (Curative Test)

Evaluation of the curative potential of the extract was done using the method described by Ryley and Peters, 1970; Chandel and Bagai, 2010. On the first day (D_0), standard inoculums of about $1x10^7$ *P. Berghei berghei* infected red blood cells were injected intraperitoneally into the mice. Seventy-two hours later, seven groups (consisting of four mice each) were set up. 400mg/kg/day of the crude extract and 400mg/kg of the four fractions were administered orally to five groups. Chloroquine (5mg/kg/day) was given to the positive control group and 0.2 mL of normal saline to the negative control group. The drug/extracts were given once daily for 5 days. Thin blood smears were prepared from tail of each mouse every other day, 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_0-D_{27}).

Statistical Analysis

Data obtained from the study were analysed statistically using Analysis of variance (ANOVA) and values of $p<0.05$ were considered significant.

RESULTS

Partial Purification

The partial purification of the crude hot methanol extract of the root of morinda lucida, by column chromatography and thin layer chromatography gave a total of four fractions (as shown on plates I-1V).

Determination of Median Lethal Dose (LD_{50})

The crude extract (1000-5000mg/kg) produced physical signs such as gasping for air, palpitation, depression, decreased respiratory rate, loss of appetite, feeling sleepy, and death depending on the dose. One death was recorded in the group treated with 5000mg/kg body weight of the crude extract. The LD_{50} of the crude extract in mice was calculated to be 3800mg/kg.

Curative Test

It was observed that, there was a daily reduction in the levels of parasitaemia in all the test groups, as well as that of the positive control (chloroquine) group. However, the reverse was the case for the negative control group, as there was a daily increase in parasitaemia level. After 5 days of treatment, the average parasitaemia were 11.50, 20.20, 16.45, 14.40, 12.05, 6.65 and 100.87 per µl of blood for 400mg/kg/day of crude extract,

fraction 1, fraction 2, fraction3, fraction 4, 5mg/kg/day of chloroquine, and control groups respectively (as shown in Fig. 1). The mean survival time (MST) of the treated groups were significantly longer than that of control and was comparable to that of the standard drug, chloroquine ($p<0.05$). The values are given in Table 2. The mice treated with crude extract and fraction 4 survived throughout the duration of the study, but died 28-30 days post treatment. Chloroquine - treated mice on the other hand, survived beyond 30 days.

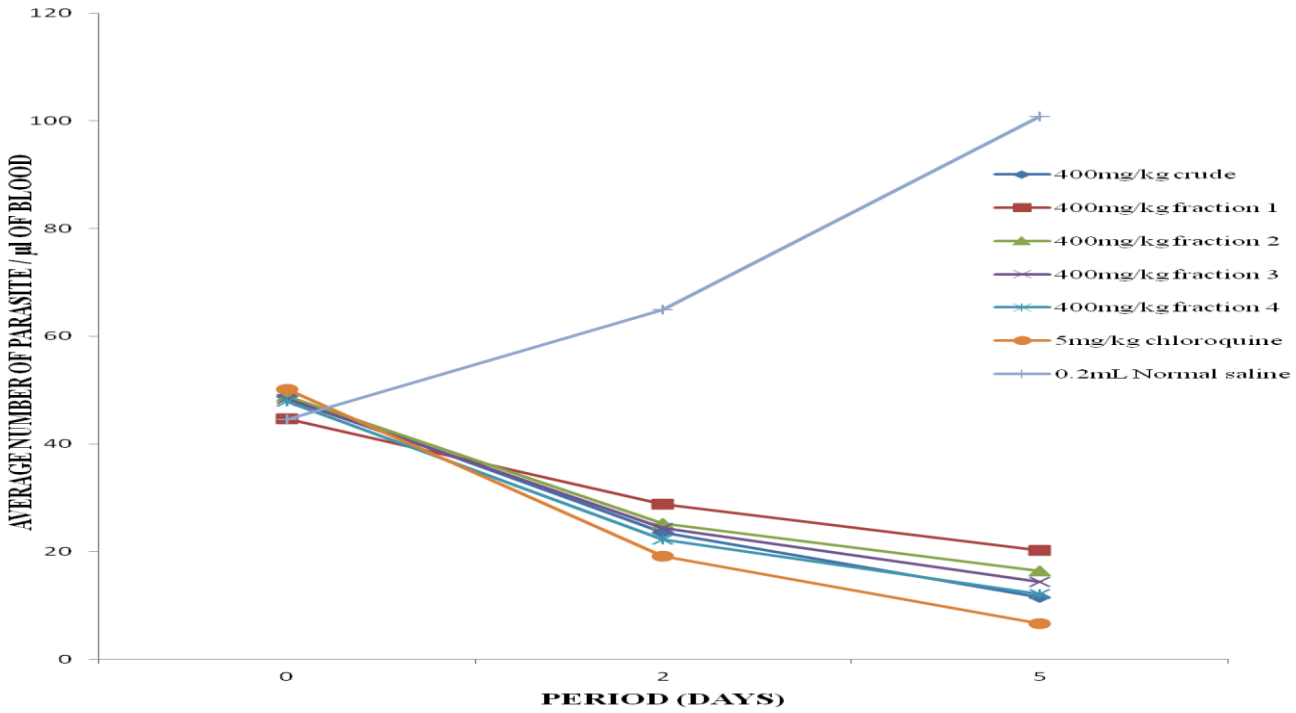

Figure1: Effect of the crude and various fractions of methanolic root extract of *Morinda lucida*on *Plasmodium berghei* infected mice

Table 1: Mean survival time of mice treated with crude and fractions of methanolic root extract of *Morinda lucida*

Extract(400mg/kg)	Mean survival time (days)
Crude methanolic root extract	29.25±1.43
Fraction 1	11.25±0.75
Fraction 2	11.75±1.60
Fraction 3	24.25±1.11
Fraction 4	28.50±1.32
Chloroquine	28.00±0.00
Normal saline	5.75.00±2.14

Values are ± standard error of mean for four replicate (n)

DISCUSSIONS

The results show that, crude methanol extract of *Morinda lucida* rootis slightly toxic, as seen in its LD50 value which was calculated to be 3800mg/kg. However this value is far above the curative dose of 400mg/Kg bodyweight which demonstrated a significant antiplasmodial activity as evident from its curative effect in infected mice compared to that of the control ($p<0.05$). It was also observed that two out of the four fractions (fractions 3 and 4) exhibited appreciable antimalarial activity as the crude extract, comparable to that of the standard drug,

chloroquine as shown in the mean survival time of treated mice (29.25±1.43, 24.25±1.11, 28.50±1.32, and 28.00±0.00) for crude extract, fraction 3, fraction 4, and chloroquine-treated groups respectively). For the chloroquine group which was used as the reference, two of the mice, out of four used in the experiment, survived beyond 30 days. The antimalarial activity of the fractions 3 and 4 indicates that they may be the active components responsible for the activity of the crude extract and so could be candidates for further research.

It is well documented that *Morindalucida* plant parts have antimalarial properties (Burkill, 1985; 1997, Tona et al., 1999, Aomoet al., 1992; Asuzu and Chineme, 1990; Makinde and Obih, 1985; Koumaglo., 1992). 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.

The result of this work and previous studies further justifies the use of *Morinda lucida* by traditional medical practitioners in the treatment of people suffering from malarial infection. It is therefore concluded that the crude methanol extract of *Morinda lucida* root as well as its fractions have appreciable antimalarial activity that can be exploited for the production of modern pharmaceuticals.

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