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Case Report

In-vivo antiplasmodial activity of crude n-hexane and ethanolic extracts of *Moringa oleifera* (LAM.) seeds on *Plasmodium berghei*

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Studies were carried out to determine the antiplasmodial activity of crude n-hexane and ethanolic seed extracts of *Moringa oleifera* using cold extraction method. Twenty-four albino mice (*Mus musculus*) induced intraperitoneally with chloroquine sensitive *Plasmodium berghei* strain were divided into 4 groups and treated at three concentrations viz: 50, 100 and 200 ml/kg. Positive control was set up with chloroquine diphosphate while negative control was set up with olive oil. The mice models were treated for 72 h. For the ethanolic extract, a parasite inhibition rate of 61% was observed at concentration 50 ml/kg, 65% at concentration 100 ml/kg and 100% at concentration 200 ml/kg in day 3 after treatment. In n-hexane extract of the seeds of *M. oleifera,* plasmodial inhibition rate of 61% was observed at concentration 50 ml/kg, 70% at concentration 100 ml/kg and 97% at concentration 200 ml/kg after treatment for 72 h. A 100% inhibition rate was observed for mice treated with 25 mg/kg of standard chloroquine diphosphate after day 3 of treatment while parasitaemia increased from 48 on day 0 to 86 after day 3 for mice treated with olive oil. Overall, crude ethanolic extract of *M. oleifera* seed showed higher parasite inhibition activity than the crude n-hexane extract.

Key words: Antiplasmodial, parasitaemia, *Moringa oleifera,* n-hexane, ethanolic.

INTRODUCTION

Malaria is a global health problem usually caused by the plasmodium parasite. It is one of the leading infectious diseases in many tropical regions, including Nigeria, a West African country where transmission occurs all year round. It is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world; variance is as a result of factors like the species of malaria parasite, their susceptibility to antimalarial drugs, distribution and efficiency of mosquito vectors, climate and other environmental conditions and level of immunity of the exposed human population (Bloland, 2001). This parasitic disease is transmitted by the bites of *Anopheles* mosquitoes infected with *Plasmodium* species, four of which infect humans: *P. falciparum* (most deadly one), *Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale* (Getie, 2010). *P. falciparum* being the most dominant and pathogenic is responsible for almost all malaria mortality in tropical and subtropical countries (Andare-Neto et al., 2004) where the temperature and rainfall are optimum for the development of vectors and parasites (Greenwood et al., 2008). The organism that causes the most severe form of malaria, *P. falciparum,* has developed resistance to nearly all synthetically manufactured drugs used for treatment. The next options in combating malaria are the development of new drugs and drug target in parasite. Disparities in development of new drugs for malaria vary due to public health importance of the disease and the amount of resources invested in developing new cures. *P. berghei* is a unicellular protozoan parasite of rodents transmitted by the mosquito *Anopheles dureni,* it is transmissible experimentally to other rodents. It occurs geographically in central Africa. These parasites are practical model organisms in the laboratory in the study of human malaria aimed at the development of new vaccines and treatment.

M*oringa* is a shrub plant, an angiosperm, dicot and perennial. It is also called drumstick tree, horseradish

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tree or Ben tree. It is a small to medium-sized (usually growing to 10 or 12 m in height), evergreen or deciduous tree. It is valued mainly for its edible fruits, leaves, flowers, roots, and seed oil. *Moringa* has been proven to be useful sources of food, medicinal products, fuelwood, renewable polymer products, animal and aquaculture feeds (Gidamis et al., 2003). Drumstick tree continues to have an important role in traditional Asian and West African medicine. Some of the earliest studies on the therapeutic effects of *M. oleifera* extracts are that of Kohler et al. (2002). In Nigeria, it is locally used as tonic and aphrodisiac, and in the treatment of intestinal worms and asthma. It is the most used because it is the most widely distributed, popularly known and most utilized of the *Moringa* species (Gidamis et al., 2003). In traditional medicine various parts of the tree are used therapeutically, including for treatment of rheumatism, venomous bites, and as cardiac and circulatory stimulants, cholera, scurvy, respiratory ailments, tumours and they are also applied externally to cure inflammatory swellings. Juice extracted from the leaves has antibacterial and antimalarial properties. Seeds of *Moringa* contain 19 to 47% of oil commercially known as Ben oil (Roloff et al., 2009).

Studies on the various therapeutic effects of *M. oleifera* has been reported among which includes: antibiotic (Fahey et al., 2002; Haristoy et al*.,* 2005), anticancer (Guevara et al*.,* 1999: Bharali et al., 2003), antiulcerogenic (Akhtar and Ahmad, 1995), analgesic (Rao and Ojha, 2003), antiurolithiatic (Bennett et al., 2003), antitrypanosomal (Atawodi and Shehu, 2010) and larvicidal activities (Sharma et al., 2006). However, compared to these other therapeutic activities, there has been no much report on its antimalarial tendencies. It is in light of this that the study was carried out using ethanolic and n-hexane extracts of *M. oleifera* seeds.

METHODOLOGY

Sample preparation and extraction

Seeds of the plant were harvested, de-shelled and dried under the shade or in open air in the laboratory. Dried materials were pounded in laboratory mortar into a fine powder. The fine powder was then sieved in the laboratory and stored in airtight containers in the dark until extraction. Three grams (3 g) of the dried powder plant material was measured using an electrical measuring scale (Adventurer ™ OHUAUS, China) and put into two 500 ml beakers and were extracted with 300 ml each of solvents (Durmaz et al., 2006) by using cold extraction technique for 24 h at room temperature (Omodamiro et al., 2012). After 24 h, filtration using a filter paper was done (the supernatant was decanted from the residue into another beaker; another 300 ml of solvent was added again and left for 24 h again). This was done for 3 consecutive days (72 h). During the extraction, the beakers were covered with aluminium foil paper to avoid evaporation of solvent. Using a rotary evaporator the liquid filtrate were evaporated and concentrated at 40°C water bath until condensation of the solvent stopped dropping for different solvents timing varied; for n-hexane, extraction time was 40mins and for ethanol, extraction time was for 1hr and 15 mins. The extract was then transferred into a sterile McCartney bottle. Extract from seed was gotten as oil and was stored at room temperature. Stocks of both extracts were prepared by dissolving 5 ml of the extract in 10 ml of olive oil (ratio 1:2). Both extracts were homogenized in olive oil at concentration 0.5 ml of extract per ml of olive oil to get a concentration of 5 ml/kg.

Test organisms

Twenty-four Albino mice, *Mus musculus* weighing between 10 to 18 g were purchased from the animal house, Lagos University Teaching Hospital, LUTH, Lagos. All animals were housed in standard animal cages and kept in the animal house at Covenant University, Ota, Ogun State, at room temperature. They were fed with standard Recommended Dietary Allowance (RDA) feed (pellet) and water was freely supplied. A chloroquine sensitive *P. berghei* (ANKA strain) was gotten from the animal house at National Institute of Medical Research (NIMR), Yaba, Lagos which was further maintained by further passage in mice. Ethical clearance was given by the Institutional Review Board of the Nigerian Institute for Medical Research (NIMR) and Covenant University Ethical Review Board (CUERB) in accordance with International Standard on the care and use of experimental animals.

Plasmodium strains were previously maintained prior to purchase and were not allowed to stay more than 72 h prior to passaging. Passage was considered necessary when parasitaemia was in the range of 16 to 32 parasites per field (usually 8 to 12 days post-infection in mice). In passaging, 1 × 104 parasites were introduced intraperitoneally or intramuscularly into rats in 0.1 - 0.2 ml blood / phosphate buffered salin (PBS) solution. About 0.05 ml of the blood collected as described earlier or blood (diluted with PBS to contain approximately 1 × 104 Parasite/ml) was injected into clean animals acclimatized under laboratory condition for at least two weeks.

Administration of extract

The mice were randomly and evenly divided into four groups of control, group 1, 2, and 3. There were six animals in each group; three received ethanolic extract while the other three received n-hexane extract. Extracts were administered orally once daily for 72 h using

Table 1. Average daily response of parasitaemia to seed extracts of *M. oleifera*.

Concentration (ml/kg)	Extract	Average daily parasitaemia			
		D_0	D_1	D_2	D_3
50	n-hexane	82	71	50	32
	ethanolic	64	49	38	25
100	n-hexane	54	40	22	16
	ethanolic	48	31	24	17
200	n-hexane	30	16	2	1
	ethanolic	54	19	2	0
Control	Chloroquine	32	13	4	0
	Olive oil	48	67	72	86

cannula on the 7th day of post- inoculation of mice and administered according to body weight of animal (Omodamiro et al., 2012) in a dose of 50, 100 and 200 ml/kg for group 1, 2 and 3 in that order. In the control group, mice were administered with chloroquine diphosphate at 25 mg/kg body weight and olive oil (positive and negative control respectively). According to Sattaur (1983) and Jahn (1988) dosages of between 50 and 500 mg/kg body weight have been reported to produce no toxic effects in rats.

Determination of parasitaemia

Parasitaemia was monitored in blood obtained from the tail, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at ×100. Blood was dropped on a slide, the slide allowed to air dry in a vertical position and examined using a light microscope. Parasitaemia was estimated by number of parasites on slide per 200 red blood cells.

RESULTS

Crude ethanolic extract of *M. oleifera* seeds significantly reduced parasitaemia at the three different concentrations. A parasite inhibition percentage of 61% was observed at concentration 50 ml/kg, 65% at concentration 100 ml/kg and 100% at concentration 200 ml/kg on day 3 after treatment. In n-hexane extract of the seeds of *M. oleifera,* anti-plasmodial activity was observed at the three different concentrations that is 50, 100, 200 ml/kg. An inhibition rate of 61% inhibition was observed at concentration 50 ml/kg, 70% at concentration 100 ml/kg and 97% at concentration 200 ml/kg on day 3 after treatment.

Table 1 show the average daily response of parasitaemia to the different concentrations and the two seed extracts of *M. oleifera* over a 72 h period. From the table, it can be deduced that parasitaemia level for both the n-hexane and ethanolic extracts of *M. oleifera* reduced considerably over the period of time. It also shows that the control, chloroquine diphosphate reduced the parasitaemia level over the period of time. However, the reverse was the case with olive oil as the parasitaemia level increase with time. Table 2 shows the percentage inhibition of the parasites to the different extracts. The table reveals a progressive positive correlation between the two extracts and concentration. Chloroquine and 200 ml/kg ethanolic extract treatment shows highest inhibition rate of 100% by the end of 72 h. IC_{50} values are 35 and 40 ml/kg for ethanolic and n-hexane extract respectively. Data obtained from results were considered significant at $P<0.05$.

DISCUSSION

The *in-vivo* anti-plasmodial activity of crude ethanolic and n-hexane seed extracts of *M. oleifera* was determined in this study. Positive activity against *P. berghei* was observed at three different concentrations that is, 50, 100, 200 ml/kg. The ethanolic extracts cleared the parasite completely at 200 ml/kg as observed in the case of positive control CQ diphosphate, a standard antimalarial drug, administered at a daily dose of 25 mg/kg body weight. Both crude ethanolic and n-hexane extracts of *M. oleifera* seeds showed promising inhibition of parasitaemia ranging from between 61 and 100% for ethanolic extract and between 61 and 97% for n-hexane extract.

As can be seen in Table 2, both extracts showed a progressive reduction in parasitaemia with time; the highest clearance observed on the third day. Considering that the extracts were administered once daily, it is likely that extracts of *M. Oleifera* has residual potency and so as more of the extracts were administered, there is a

Table 2. Average percent parasite inhibition.

Concentration (ml/kg)	Extract	Parasite inhibition (%)
50	n-hexane	61
	ethanolic	61
100	n-hexane	70
	ethanolic	65
200	n-hexane	97
	ethanolic	100
Control	Chloroquine	100

Mean % after 3 days.

cummulative antiplasmodial effect thus the reason for the progressive reduction in parasitaemia. This is a very promising feature in the potentiality of the use of seed extracts of *M. oleifera* as an antimalarial drug considering the difficulty in bringing down to very low levels, parasitaemia in malaria patients.

There is very little difference in the antiplasmodial effects of the two different solvents used for extraction despite the wide variation in their solvent polarity. Solvent polarity is the key characteristic of a liquid solvent that determines its overall capability to dissolve a certain gaseous, liquid or solid solute. Ethanol is a high polar solvent as opposed to n-hexane which is a low polar solvent. That there is a little difference in their antiplasmodial activity may mean that the chemical substances present in the high polar solvent are also present in the low polar solvent. A phytochemical screening may be necessary to ascertain what these active constituents are. However on the basis of the result obtained in this study, there may be no preference for the use of any of the two solvents in further studies involving seed extracts of *M. oleifera*.

A high percentage (%) parasite inhibition was observed in this study; 100 and 97% for ethanolic and n-hexane extracts respectively. This can be compared to previous reported studies of plant extracts like Getie (2010) who reported 86.21% inhibitory activity of the methanol extract of the *D. angustifolia* seeds on *P. berghei*. An increase in weight of about 1.5 to 2.0 g and 0.9 to 1.5 g for ethanolic seed extract and n-hexane seed extract respectively was also observed in this research which supports earlier claims (Fahey, 2005; Anwar et al., 2007; Kasolo et al., 2010; Ayushy et al., 2010) about the nutritive value of that *M. oleifera*.

The phytochemical diversity of a plant species is indicative of its high medicinal and therapeutic potentials. This is because these compounds form the basis of the pharmacologic effects of such plants (Haidet, 2003; Jigam et al., 2004). Alkaloids for example, which has been reported by Tende et al. (2011) in the leaves of *M. oleifera*, rank among the most efficient and therapeutically significant plant compounds. Pure alkaloids and their synthetic derivatives are used as basic medicinal agents for example, morphine is an analgesic, quinine is antiplasmodial, colchicines is used for gout, reserpine is a tranquilizer, vincristine and vinblastine have antitumor effects (Haidet, 2003).

The relatively high antiplasmodial activity of the seed extracts of *M. oleifera* probably explains its widespread use in herbal medicine. The extract can hence be standardized and packaged to be used as phytomedicine. Its long term consumption should however be weighed viz-a-viz the likelihood of adverse effects on organs as is the case with some reported plant species (Gamaniel, 2000).

The higher parasite suppression by crude extracts is significant in the sense that reports about the anti malarial effects of *M. oleifera* are not much especially in Nigeria. Crude plant extracts have generally been suggested to be more plasmodistatic than plasmodicidal probably because unpurified bioactive principles may require initial conversions which time lag allows for parasite proliferation (Noedl et al., 2003). The observed potency of crude *M. oleifera* extracts in mice is a confirmation of the rationale for its use in malaria treatment among indigenous Nigerians.

The weakness and deaths observed in the mice of different groups with continuous administration of the extracts; even after parasites were eliminated from the blood stream suggest that the extracts may have some cumulative toxic effects at the high dose used. Earlier studies have found *M. oleifera* seed to be nontoxic and recommended its use as a coagulant in developing countries (Olsen, 1987). Oral test, acute and chronic toxicity tests on rats with both *Moringa stenopetala* and *M. oleifera* seeds (dosages 50 and 500 mg/kg body weight) have been reported to produce no toxic effects, but, rather increased the weights of the rats (Sattaur, 1983; Jahn, 1988). The highest dose (200 ml/kg) used in this study corresponds to about 666 mg/kg of both extract and was toxic to the test animals as death was recorded within 24 h (for ethanol extract) and 48 h (for n-hexane

extract) of administering the drug to the animals. However, put together, these results suggest that *M. oleifera* possess significant anti-plasmodial effect to warrant further detailed studies utilizing bioassay-guided fractionations under varied pharmacological conditions in order to unequivocally establish its therapeutic efficacy, active ingredient (s) and toxicological properties.

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