INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–830/2016/18–5–906–910
DOI: 10.17957/IJAB/15.0161
http://www.fspublishers.org

Full Length Article

In vivo Antiplasmodial Activity of Crude Ethanolic and N-hexane Extracts of *Moringa oleifera* Leaves

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Abstract

This study was carried out to determine the antiplasmodial activity of leaves of *Moringa oleifera*. Cold extraction method was carried out on grinded leaves to prepare the crude ethanolic and n-hexane extracts. Mice models (*Mus musculus*) were passaged with chloroquine resistant *Plasmodium berghei,* which are similar in morphology, physiology and life cycle to *P. falciparum* that infect humans. Stock solutions of 5 mg/mL 5% DMSO were prepared and the extracts were administered at different treatment concentrations, 50 mg/kg, 100 mg/kg and 200 mg/kg body weight over 4 days. Positive and negative control groups, Chloroquine diphosphate (25 mg/kg) and 5% DMSO, respectively were set up. Crude ethanolic and n-hexane extracts of *M. oleifera* showed anti-plasmodial activity at the three different concentrations used. Both crude ethanolic and n-hexane extracts of *M. oleifera* leaves showed a significant inhibition of parasitaemia ($p < 0.05$) ranging from 74.7 to 95.6% for ethanolic extract and 59.3 to 87.9% for n-hexane extract. EC_{50} value of crude ethanolic and n-hexane extracts were 32 mg/kg and 42 mg/kg body weight, respectively. *M. oleifera* showed potential for possible future use as an alternative to some conventional drugs.

Keywords: Antiplasmodial; Parasitaemia; *Moringa oleifera*; N-hexane; Ethanolic

Introduction

The consistent rise in resistance to majority of the anti-malarial drugs available, and the ability of malaria vectors to resist insecticides is responsible for the re-emergence of malaria in many parts of the world (Ridley, 2002). In the time past, traditional herbs enjoyed a wide acceptability in the treatment of malaria. As early as in the 17th century, infusions from the bark of the plant Cinchona (Rubiaceae) species have proven successful for the treatment of human malaria (Baird *et al*., 1996). With time, Quinine was isolated and characterized (Saxena *et al*., 2003), making it the most essential drug against malaria. According to Mokuolu *et al*. (2007), since 2005 till date, there has been a formal adoption of Artemisinin-combined therapies (ACT) as a first-line of treatment of uncomplicated malaria in Nigeria. Nevertheless, ACT has not been widely used. One reason for this is the high cost of the drug. Another reason is that production of artemisinin derivatives that meets toxicity and Good Manufacturing Practices (GMP) standards is limited (Haynes, 2001 and Malomo *et al*., 2001; Adebayo and Malomo, 2002; Borstnik *et al*., 2002; Afonso *et al*., 2006; Boareto *et al*., 2008). There is awareness that conventional medicine is not widespread, as such there is a renewal of interest in native medicine all over the world. In countries where the standard of living is low, healthcare is often sustained by engaging culture-based alternatives. However, in many advanced countries, about 20% of patients use local herbs for the treatment of malaria (Willcox and Bodeker, 2004).

A combination of cultural, historical and economic reasons can be attributed to the sustained popularity and acceptability of herbal medicines. The use of plants as treatments for malaria has gradually increased all over the world lately, so also is the search for new plant chemicals that could potentially be developed and used as anti-malarial and other infectious diseases treatment (Willcox, 1999). The most widely cultivated species in the family Moringaceae is *Moringa oleifera* (Lam.) whose origin is in the sub-Himalayan tracts India and used by the old Egyptians, Romans and Greeks. Due to its wide cultivation, it has now become naturalized in many countries particularly in the tropics.

This fast-growing tree is known in English as the horseradish tree or drumstick tree. It is known as zogale in Hausa, ewe igbale and igi iyanu in Yoruba. It is a perennial pan-tropical tree; it is slender, short, drought-resistant and deciduous, which can grow to about up 10 m in height (Keay, 1989). In some parts of central and northern Nigeria, its leaves are widely consumed both as animal and human and food (Lockett *et al*., 2000), as well as in other parts of the world (Sena *et al*., 1998; Seshadri and Nambiar, 2003). It is used against diabetics in Indian folk medicine (Kar *et al*., 2003), while it is used locally as an aphrodisiac and as a

To cite this paper: Olasehinde, G.I., O.I. Ayanda, L. O. Egwari, A.A. Ajayi and T. Awofeso, 2016. *In vivo* antiplasmodial activity of crude ethanolic and n-hexane extracts of *Moringa oleifera* leaves. *Int. J. Agric. Biol*. 18: 906–910

tonic in Nigeria, and also in treating asthma and intestinal worms. Studies as recent as in the last decade have proven that the plant possess anti-bacterial activity (Kohler *et al*., 2002), chemomodulatory effect in hepatic metabolizing enzymes (Bharali *et al*., 2003), hepato- and radio-protective capacities (Rao *et al*., 2001) and thyroid hormone regulatory properties (Tahiliani and Kar, 2000). This study was therefore designed to assess whether or not the plant can show some promise in the treatment of malaria.

Materials and Methods

Collection and Processing of Leaves

The leaves of *M. oleifera* were collected at University of Ilorin Botanical Center, Kwara State, Nigeria and air dried for 2 weeks. The leaves were thereafter grinded using a mechanical grinder. Powdered leaves were then sieved to obtain fine powder.

30 g each of the powdered leaves were weighed using a measuring scale, and put into 500 mL beaker and was extracted with 300 mL of ethanol and n-hexane (Durmaz *et al*., 2006) using cold extraction method for 24 h at room temperature as described by Omodamiro *et al*. (2012). The mixtures were filtered with filter paper at every 24 h interval for 3 consecutive days. To obtain a quality yield, a rotary evaporator was used to concentrate and evaporate the liquid filtrate to dryness at 40˚C. The yield of each extract was calculated and recorded, and transferred into sterile McCartney bottles. The dry extracts/fractions were stored at room temperature prior to use.

Experimental Animals

Swiss albino mice (*Mus muluscus)* of both sexes, weighing between 12–18 g obtained from University of Lagos Teaching Hospital Animal House, Lagos, Nigeria, were used for these experiments. They were housed in standard cages in Covenant University Animal House, Ota, Ogun State, Nigeria and were maintained on a pellet feeds (RDA standard) and water *ad libitum*. A total of 24 mice were recruited into this study. The mice were divided into 4 groups of 50 mg/kg, 100 mg/kg and 200 mg/kg per body weight of the extract, respectively and the last group served as control group.

Ethical Consideration

For this experiment, scientific and ethical clearance was obtained from both the Covenant University Ethics Committee (CUEC) and the Nigerian Institute of Medical Research Institutional Review Board (NIMR-IRB). The guideline for the use of laboratory animals in research specified by the Animal Ethics Committee of both NIMR-IRB and CUEC was employed for the maintenance and care of the experimental animals.

Passaging

A chloroquine-sensitive strain of *Plasmodium berghei* (ANKA strain 65) was obtained from the National Institute of Medical Research (NIMR), Yaba, Lagos and was maintained by subpassage in mice. Each mouse used in the experiment was inoculated intraperitonially with 1 mL of infected blood, which contains about 0.5×10^5 *P. berghei* parasitized blood cells. This was prepared by finding both the percentage parasitaemia and the red blood cells count of the donor mouse and diluting the blood with PBS.

Preparation of Extract

The ethanolic and n-hexane extracts of the plant were solubilized in 5% DMSO at concentration of 5 mg of extract per ml 5% DMSO (5 mg/mL). Stocks of both extracts were prepared by dissolving 75 mg of the extract in 15 mL of 5% DMSO.

Administration of Extract

Peters four days suppressive test was used (Peters, 1965). Four groups of mice were administered four concentrations of the extract 50, 100, 200, 400 (mg/mL) of the extracts, respectively by oral dosage for four consecutive days *ad libitum* (D0-D3). The other two groups were used as positive and negative controls. Positive control was dosed with chloroquine (CQ) 25 mg/mL and negative control was given only 50% DMSO. Blood samples were taken from the tail of the mice and smeared on glass slides for the parasitaemia using Giemsa stains.

Microscopy

Tail blood smear was made from each mouse 24 h after each dosing for 4 days, stained with 10% Giemsa in phosphate buffer, pH 7.2 for 15 min and examined under a light microscope at 100X magnification. Parasitaemia was determined by counting the number of parasite against 200 erythrocytes. Inhibition of parasites was calculated for each extract by comparing the parasitaemia present in infected controls with those of test mice. The data obtained were considered significant at $P < 0.05$.

Results

The result showed that crude ethanolic and n-hexane extracts of the leaves of *M. Oleifera* had antiplasmodial activity. The two crude extracts showed positive activity against *P. berghei* at the different concentrations used. It also revealed that the higher the concentration, the higher the number of total parasitaemia cleared. In the overall, the n-hexane extract has more antiplasmodial activity in comparison to the other extract because it was able to completely clear more parasitaemia. The efficacy of the two leaf extracts for the three concentrations are presented in

Tables 1 to 3. In the least concentration, there was a 72% and 86.8% total clearance of parasitaemia for ethanolic and n-hexane extracts, respectively (Table 1). The ethanolic extract caused a progressive reduction in the number of parasitaemia in all mice. The n-hexane extract could completely clear the parasitaemia in two of the mice.

The antiplasmodial activity of the next higher concentration is presented in Table 2. Both extracts also showed a gradual reduction in the number of parasitaemia until the third day. The ethanolic and n-hexane extract for this concentration showed 89.7% and 95.8% clearance in total parasitaemia after three days.

A similar trend was observed in both extracts of the highest concentration (Table 3). The ethanolic and n-hexane extracts, respectively revealed 98.3% and 100% clearance of total parasitaemia. These various observations are graphically represented in Fig. 1.

Fig. 2 shows the relationship between parasite inhibition and the two leaf extracts of *M. oleifera*. From the graph, the IC_{50} value for the ethanolic extract was found to be 32 mg/kg b.w., while for the n-hexane extract, the IC_{50} value was found to be 42 mg/kg b.w.

Table 1: Mean parasitemia at 50 mg/kg body weight

Extract	Mouse Code	Weight (g)	Parasitemia			
			Before treatment	After treatment		
			Day 0	Day 1	Day 2	Day 3
Ethanol	1	14	87	59	37	19
	2	16	96	63	44	29
	3	12	71	47	31	22
Total count			254	169	118	70
Mean			85	56	39	23
n-hexane	1	16	95	67	49	37
	2	12	73	-	-	-
	3	18	113	-	-	-
Total count			281	67	49	37
Mean			94	67	49	37

Table 2: Mean parasitemia at 100 mg/kg body weight

Extract	Mouse Code	Weight (g)	Parasitemia			
			Before treatment	After treatment		
			Day 0	Day 1	Day 2	Day 3
Ethanol	1	14	84	46	23	9
	2	12	74	39	19	7
	3	12	76	41	21	8
Total count			234	126	63	24
Mean			78	42	21	8
n-hexane	1	12	79	46	24	11
	2	16	91	49	-	-
	3	16	93	51	33	-
Total count			263	146	57	11
Mean			88	49	29	11

Table 3: Mean parasitemia at 200 mg/kg body weight

Extract	Mouse Code	Weight (g)	Parasitemia			
			Before treatment	After treatment		
			Day 0	Day 1	Day 2	Day 3
Ethanol	1	12	73	-	-	-
	2	12	78	26	9	-
	3	14	89	37	13	4
Total count			240	63	28	4
Mean			80	32	14	4
n- hexane	1	12	76	-	-	-
	2	16	94	41	16	-
	3	16	98	-	-	-
Total count			268	41	16	0
Mean			89	41	16	0

Discussion

That both crude extracts of the leaves of *M. oleifera* were able to cause a progressive inhibition of parasite growth as concentration increased means there is a very promising potential if the leaves of the plant is to be considered as a treatment for malaria. This probably means the crude leaf extracts of the plant possess chemical substances that could resist the growth of the parasites. At concentration 200 mg/kg, all mice model died after the second dose at 48 h. This may be due to the high concentration of the extract and the solvent used for the extraction even though, parasite inhibition progressed as drug concentration increased. The leaf extracts of *M. oleifera* used in this study had higher activity against *P. berghei* when compared with the activity of some leaf extracts that have been previously used to inhibit the parasite *in vivo*. Ali *et al.* (2010) recorded that dichloromethane extract of *Boerhavia elegans* had the highest inhibitory activity on *P. berghei* (66.18%) at a concentration of 300 mg/kg (Ali *et al*., 2010), whereas, ethanolic extracts of *M. oleifera* showed highest inhibitory activity on *P. berghei* (95.6%) at concentration of 200 mg/kg.

Both ethanolic and n-hexane extracts of *M. oleifera* showed anti-plasmodial activity at a low concentration as compared to methanolic extract of both *Striga hermonthica* and *Tapinanthus sessilifolius* that had inhibitory activity against *P. berghei* at 400 mg/kg body weight (Okpako and Ajaiyeoba, 2004). Methanolic extract of *Cassia singueana* showed non-toxic anti-plasmodial activity on *P. berghei* at 200 mg/kg (Adzu *et al.*, 2003), while 200 mg/kg concentration of both ethanolic and n-hexane extracts of *M. oleifera* were toxic to the mice, which may be due to high concentration of the extracts.

It has been observed from previous studies that *M. oleifera* has potent antihelmintic activity against adult Indian earthworm *Pheritima posthuma* at 100 mg/mL, paralysis and death of worm occurred at 6 and 45 min, respectively (Trapti *et al*., 2009), also optimum anti-trypanosoma activity against *Trypanosoma brucei brucei* at concentration of 300 mg/kg (Atawodi and Shehu, 2010). *M. oleifera* also had broad spectrum activity against some bacteria associated with food-borne disease, with MIC value ranging between 2.0 and 4.0 mg/mL for all organisms tested (Bukar *et al*., 2010). It was also reported by Prabhu *et al*. (2011) that *M. oleifera* showed larvacidal activity at concentration on *Anopheles stephensi* which is a vector for malaria transmission. In addition to these reports, this study has also showed *M. oleifera* leaves

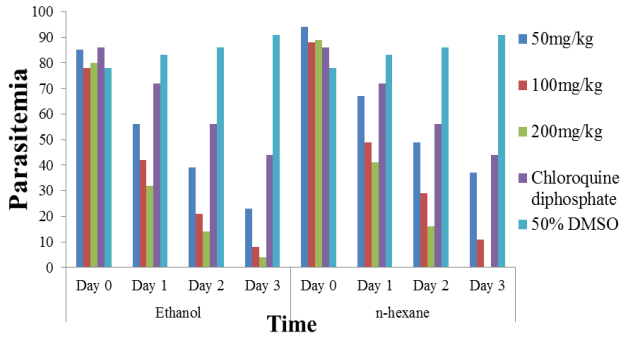

Fig 1: Relationship between parasitemia clearance and time for all concentrations of the two extracts

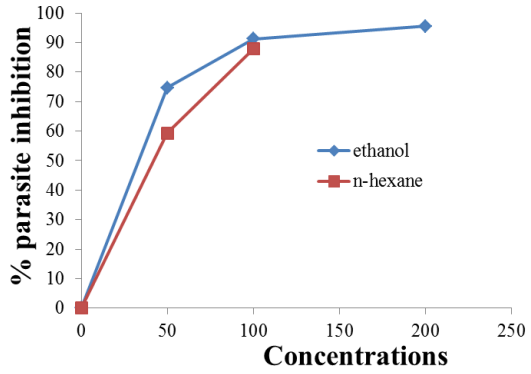

Fig. 2: Relationship between % parasite inhibition of ethanolic and n-hexane extract of *M. oleifera*

as having antiplasmodial effects. Olasehinde *et al*. (2012) had earlier reported these same effects using *M. oleifera* seeds.

Increase in the weight of the mice was also observed which supports the fact that *M. oleifera* leaves high nutritional value (Nambiar *et al*., 2003). For the mice treated with ethanolic extract, average increase in weight was observed to be 1.2 g and average weight increase of 0.8 g was observed for the n-hexane extract treated mice.

The IC_{50} is the concentration at which there was 50% parasite clearance. N-hexane extracts of *M. oleifera* has an IC_{50} value of 42 mg/kg body weight, while IC_{50} value of crude ethanolic extract is 32 mg/kg body weight (Fig. 2). That these values are below the least concentration tested makes this plant promising as an antiplasmodial substance; however, since there was no simultaneous effort to investigate the safety of these values via histopathology of major organs, it may be too early to make any major, definitive statements on these values.

Conclusion

This study has established that crude ethanolic and n-hexane extracts of *M. oleifera* leaves have high activity against *P. berghei*. Crude ethanolic has shown better activity against *P. berghei* and less toxicity on the animals than n-hexane extract. It may be necessary that the active compounds of *M. oleifera* should be identified, isolated and tested for their individual anti-plasmodial activities. Solvents other than ethanol and n-hexane should be used for leaves extraction of this valuable plant so as to know, which solvent would most suitable and has high activity against *P. berghei* as well as low toxicity on the mice model. For further studies, concentrations other than the ones used in this study may be tested while also factoring in histopathological analysis of the major organs of the animals used as it is possible that even the lowest concentrations may be toxic to some organs. Overall, it seems *M. oleifera* has the potential to be a good herbal treatment for malaria and research efforts in this direction should be encouraged.

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(Received 14 October 2015; Accepted 31 December 2015)