



In vivo* antimalarial activity of methanol leaf extract of *Bombax buonopozense* in mice infected with *Plasmodium berghei

Godwin Christain AKUODOR^{1*}, Chukwuemeka Christian MBAH²,
Uchenna Anthony MEGWAS³, Nwakaego Chituru IKORO⁴, Joseph Linus AKPAN⁵,
Benjamin Obinna OKWUOSA¹ and Uche Alex OSUNKWO¹

¹Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, P.M.B 21 Garki, Abuja, Nigeria.

²Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, P.M.B 21 Garki, Abuja, Nigeria.

³Department of Optometry, Imo State University, Owerri, Nigeria.

⁴Department of Optometry and Optical Technology, Federal University of Technology, Owerri, Nigeria.

⁵Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria.

* Corresponding author, E-mail: goddyakuodor@yahoo.com, Tel: +234 803 6725237

ABSTRACT

Bombax buonopozense is used in ethnomedical practice for the treatment of fever. The methanol leaf extract of this plant was evaluated for *in vivo* antiplasmodial activity against chloroquine sensitive *plasmodium berghei* in mice. The antiplasmodial effect during early and established infections was investigated. The extract (200-600 mg/kg, *p.o*) exhibited significant ($P < 0.05$) antimalarial activity both in four-day early and in an established infection tests. The LD₅₀ of the extract was established to be greater than 5000 mg/kg, *p.o* in mice. The result suggests that *B. buonopozense* leaf extract possesses significant ($P < 0.05$) antiplasmodial activity thus confirming its traditional use in malarial therapy.

© 2011 International Formulae Group. All rights reserved.

Keywords: *Bombax buonopozense*; Herbal medicine; *Plasmodium berghei berghei*; Mice.

INTRODUCTION

Malaria is one of the most important parasitic diseases in the world. It remains a major public health problem in Africa responsible for annual death of over one million people (David et al., 2004; Martin et al., 2004). *Plasmodium falciparum*, the most important human parasitic infection (Greenwood et al., 2005), is becoming

increasingly resistant to standard antimalaria drugs which necessitate a continuous effort to search for new drugs, particularly with novel modes of action (Muregi et al., 2003). One of the areas for the search of new antimalarial agent is the traditionally claimed antimalarial plant from the African flora (Whitefield, 1995). For a long period of time medicinal plants have been the focus of many anti-

© 2011 International Formulae Group. All rights reserved.

DOI : <http://dx.doi.org/10.4314/ijbcs.v5i5.2>

infective drugs and alternative sources of antimalarial agents in different parts of the world. Studies have been conducted on the *in vivo* antimalarial properties of several plant extracts in mice (Agbaje and Onabanjo, 1991; Perez et al., 1994; Andre-Neto et al., 2003). Interestingly, some of the plants used have shown real antiparasitic activity (Hilou et al., 2006) and most of them are relatively safe (Ajaiyeoba et al., 2006; Kaou et al., 2008).

Bombax buonopozense P.beauv. (*Bombacaceae*) is a large tropical tree that grows to 40 metres in height with large buttress roots that can spread 6 metres. The bark is covered in large conical spines, especially when young but shedding them with age to some degree. The branches are arranged in whorls; the leaves are compound and have 5-9 leaflets and 5-25 secondary veins. The individual leaflets have entire margins and are also large. The undersides of the leaflets may be glabrous or puberulous (Beentje and Sara, 2001). It is widely distributed in Africa from Ghana to Sierra Leone, Uganda and Gabon. Many parts of the plant are used for medicinal purposes, as food, as a source of clothing fibre, as a building material, as cotton wool and as dye. The fruits are eaten by animals such as water chevrotain (Dubost, 1984). A decoction of the leaf is used for feverish conditions, diarrhoeal and pains. Root decoction is also used for antimicrobial and stomach aches. Therefore, this study aims at investigating the *in vivo* antiplasmodial activity of the leaf extract of *B. buonopozense* against *P. berghei* infection in mice.

MATERIALS AND METHODS

Plant material

The leaves of *B. buonopozense* were collected at Chaza Village, Suleja, Niger State, Nigeria in April 2009. The plant was identified and authenticated by Mrs Grace Ugababe, a taxonomist in the Department of

Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (No.6402) was deposited at the herbarium of the Institute.

Preparation of plant extract

The plant material was air-dried at room temperature and pulverized into a dry powder, and macerated with 70% methanol in water for 72h with constant shaking. The resultant mixture was filtered using whatman (No. 1) filter paper and the filtrate concentrated using rotary evaporator and dried on a water bath to give a yield of 7.7% (w/w). The extract was reconstituted in normal saline at appropriate concentration for the various experiments conducted.

Animals

Adult male and female Swiss albino mice (18-25 kg) maintained at the Animal facility Centre of NIPRD was used. The animals were maintained with standard diet and had water *ad libitum*. The studies were carried out following the ethical norms of 'NIH Guide for the Care and Use of Laboratory Animals; NIH publication (No 83-23), The National Academic Press, Washington, DC (1985).

Acute toxicity study

The median lethal dose (LD₅₀) of the extract was estimated *p.o.* following Lorke's (1983) method. Dose levels used ranged from 10-5000mg/kg. The acute toxicity LD₅₀ was calculated as geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Phytochemical analysis

Phytochemical screenings were performed using standard procedures (Trease and Evans, 1989; Sofowora, 1993).

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins

To 0.5 g of the extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for terpenoids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a lower layer. The formation of a reddish-violet colour in the chloroform layer indicates the presence of terpenoids.

Test for flavonoids

Three methods were used to test for flavonoids. Firstly, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated H₂SO₄ (1 ml) was added. A yellow colouration that disappears on standing indicates the presence of flavonoids. Secondly, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Thirdly, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for alkaloids

0.5 g of the extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute

ammonia. 5 ml of chloroform was added and shaken gently to extract alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for steroids

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colour at the interface indicates the presence of steroidal ring.

Test for carbohydrates

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of concentrated sulphuric acid was added and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates.

Parasite inoculation

The chloroquine-sensitive *Plasmodium berghei berghei* (NK65 Strain) was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained in NIPRD by continuous reinfestation (*i.p.*) (Calvalho et al., 1991) every 4 days. Blood was collected from a donor infected mouse through cardiac puncture and diluted with normal saline. The mice received 0.2 ml of diluted inoculum *i.p.* consisting of 10⁷ parasitized red blood cells.

Evaluation of Schizonticidal activity on early infection

Tests were performed in a 4 day suppressive standard test using the methods of Peters et al. (1993) and David et al. (2004) with slight modifications. Thirty Swiss albino mice of both sexes weighing (18-25 g) were

inoculated intraperitoneally with infected red blood cells (0.2 ml) containing 1×10^7 *P. berghei*. The animals were divided into five groups (n=6). Group 1 which served as negative control received normal saline (10 ml/kg) *p.o.* Group 2- 4 received (200, 400 and 600 mg/kg) of *B. buonopozense* extract orally, while group 5 received 10 ml/kg of chloroquine as the positive control *i.p.*, all on the first day (D_0). Treatment continued daily for four days (D_1 - D_4). On the fifth day, blood was collected from the tail of each mouse and thick films made. The films were fixed with methanol, stained with Giemsa for 30 min and parasitaemia examined microscopically for blood parasite suppression.

Curative test

Evaluation of curative potential of *B. buonopozense* leaf extract was done using a method described by (Ryley and Peters, 1970) with slight modifications. Thirty mice were selected and intraperitoneally injected with standard inoculums of 1×10^7 *Plasmodium berghei* infected red cells on the first day. Seventy two hours after (D_4), the animals were divided into five groups (n=6). Group 1 received normal saline 10 ml/kg as the negative control. Groups 2-4 received (200, 400 and 600 mg/kg) of the extract, all administered orally. Group 5 received 10 mg/kg chloroquine as the positive control *i.p.* Treatment continued daily until the seventh day (D_4 - D_7) when blood was collected from the tail of each mouse and thick films made. The films were fixed with methanol, stained with Giemsa for 30 min and parasitaemia density determined (Akuodor et al., 2010b). The survival time of the mice in each group over 30 days was noted (Saidu et al., 2000).

Statistical analysis

Results obtained were expressed as mean \pm Standard Error of Mean (S.E.M). The data was analyzed using student's t-test. $P < 0.05$ was considered significant.

RESULTS

Phytochemical screening

A phytochemical analysis of the crude extract gave positive reaction for each of the following secondary metabolites; Tannins, saponins, terpenes, steroids, flavonoids, alkaloids and carbohydrates.

Acute toxicity tests

There was no lethality observed in mice upon oral administration, even at dose as high as 5000 mg/kg signifying that the LD_{50} was greater than 5000 mg/kg. Apart from sedation and weakness, *B. buonopozense* did produce any sign of toxicity in mice during 72 h observation period. This indicates that the experimental doses used are relatively safe.

4-days test

The methanol extract of *B. buonopozense* produced a dose-dependent chemosuppressive activity at doses employed, (200, 400 and 600 mg/kg/day) with chemosuppression of 65%, 78% and 86% respectively. However, the standard drug chloroquine 10mg/kg/day caused 90% suppression which was significantly ($P < 0.05$) more than that of the extract treated groups (Table 1).

Curative test

On established infection, the extract caused a significant ($P < 0.05$) and dose-dependent reduction in the mean parasitemia in mice, similar to chloroquine 10 mg/kg. In the extract groups that received 400 and 600 mg/kg orally, only three deaths each were recorded and none in chloroquine group throughout the 30 days observation period of the study while the remaining mice recovered fully. However, all the mice in the saline group were lost within 10 days of the experiment (Table 2).

Table 1: Suppressive effect of *B. buonopozense* against *P. berghei* in mice.

Treatment	Dose (mg/kg)	Mean parasitaemia density		% inhibition
		D ₅		
Control	10 ml/kg	38.23 ± 1.87		—
Extract	200	13.3 ± 0.33		65*
	400	8.43 ± 0.52		78*
	600	5.72 ± 0.55		86*
CQ	10	3.78 ± 1.07		90*

D₅=Day five, CQ=chloroquine. *significantly different from control at P<0.05

Table 2: Curative effect of *B. buonopozense* against *P. berghei* in mice

Treatment	Dose (mg/kg)	Mean parasitemia density		Survival time (Days)
		Pre- (D ₃)	Post-(D ₇)-treatment	
Control	10 ml/kg	34.26 ± 3.11	40.32 ± 1.21	10.0 ± 2.0
Extract	200	27.53 ± 2.30	12.63 ± 0.47*	25.4 ± 1.0
	400	30.43 ± 3.15	8.4 ± 0.29*	28.0 ± 0.8
	600	32.46 ± 4.13	4.67 ± 0.59*	29.2 ± 0.5
CQ	10	29.30 ± 1.21	2.9 ± 0.61*	30.0 ± 0.0

3=Day three, D₇=Day seven, CQ=chloroquine. *significantly different from control at P<0.05

DISCUSSION

In this study, phytochemical screening and evaluation of antiplasmodial activity of the leaf extract of *B. buonopozense* were carried out. Alkaloids, flavonoids and terpenes which have been implicated in antiplasmodial activities of plant substances (Phillipson and Wright, 1990; Christensen and Kharazmi, 2001) are found in the extract studied.

The results of this study suggest that *B. buonopozense* contains active substances with potential values in the treatment of malaria as shown from chemosuppression obtained during the 4-day early infection test. However, on established infection, the extract exerted a significant (P<0.05) curative activity. Agents with suppressive activity against *P. berghei* were known for antimalarial activity (Calvalho et al., 1991). The antiplasmodial activity of the extract at all doses during early and established infections was not comparable to chloroquine, the referenced drug. There was a dose-dependent chemosuppression of

parasitemia observed with the extract. The fact that some of the mice in the group administered 400 and 600 mg/kg of the plant extract survived throughout the 30 days observation period similar to the chloroquine-treated group shows a high level of efficacy, indicative of its potential as a chemotherapeutic antimalarial agent. Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby et al., 1989) depending on their phytochemical constituent. *B. buonopozense* could have exerted its action through either of the two mechanisms mentioned or by other unknown mechanism. In addition, our earlier studies have shown that methanolic leaf extract of *B. buonopozense* has analgesic effect (Akuodor et al., 2011). Agents with such activity were

reported to provide relief to malaria patients (Addae-Kyereme et al., 2001).

The results of the present study have shown that the methanolic extract of *B. buonopozense* possesses antiplasmodial activity as seen in its ability to suppress *Plasmodium berghei* infection in the two evaluated animal models. The study strongly supports the traditional use of this plant against malaria.

ACKNOWLEDGEMENT

The authors are grateful to Ngozi Anyalewechi A. for her secretarial assistance.

REFERENCES

- Addae- Kyereme J, Croft S, Kendrick H, Wright CW. 2001. Antiplasmodial activities of some Ghanaian plants traditionally used for fever/ malaria treatment and of some alkaloids isolated from *Pleiocarpa mutica* in vivo antimalarial activity of *Pleiocarpine*. *J. Ethnopharmacol.*, **76**: 99-103.
- Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbale NO, Ashidi JS, Happi CT, Akinboye DO. 2006. *In vitro* cytotoxicity studies of 20 plants used in Nigerian antimalaria ethnomedicine. *Phytomedicine*, **13**: 295-298.
- Akuodor GC, Idris-Usman Maryam, Ugwu Theresa C, Akpan JL, Ghasi SI, Osunkwo UA. 2010b. *In vivo* schizonticidal activity of ethanolic leaf extract of *Gongronema latifolium* on *Plasmodium berghei berghei* in mice. *Ibnosina J. Med. Biomed. Sci.*, **2**(3): 118-124.
- Akuodor GC, Idris- Usman M, Ibrahim JA, Chilaka KC, Akpan JL, Dzarma S, Muazzam I and Osunkwo UA. 2011c. Anti-nociceptive anti-inflammatory and antipyretic effects of the methanolic extract of *Bombax buonopozense* leaves in rats and mice. *Afri. J. Biotechnol.*, **10**(16): 3191-3196.
- Andre-Neto VF, Brandao MGL, Stehmann JR, Oliveira LA, Krettli AU. 2003. Antimalarial activity of Cinchona like plants used to treat fever and malaria in Brazil. *J. Ethnopharmacol.*, **87**: 253-256.
- Agbeje EO, Onabanjo AO. 1991. The effects of extracts of *Enantia chlorantha* in malaria. *Ann. Trop. Med. Parasitol.*, **85**: 588-590.
- Beentje H, Sara S. 2001. Plant systematic and Phytogeography for the understanding of African Biodiversity. *Systematic and Geography of Plants*, **71**(2): 284-286.
- Calvaho LH, Brandao MGL, Santos-Filho D, Lopes JLC, Krettli UA. 1991. Antimalaria activity crude extract from Brazilian plants studied in vivo in Plasmodial berghei-infected mice and in vitro against *Plasmodium falciparum* in culture. *Braz. J. Med. Biol. Res.*, **24**: 1113-1123.
- Christensen SB, Kharazmi A. 2001. Antimalaria natural product isolation, characterization and biological properties. In *Bioactive Compound from Natural Sources: Isolation, Characterization and Biological Properties*, Tringali C (ed). Taylor and Francis; 379-432.
- David F, Philip JR, Simon IC, Reto B, Solomon N. 2004. Antimalaria drug discovery: Efficacy model for compound screening. *Nature Reviews*, **3**: 509-520.
- Dubost G. 1984. Comparison Of the diets of frugivorous forest mammals of Gabon. *Journal of Mammalogy*, **65**(2): 298-316.
- Etkin NL. 1997. Antimalarial plants used by Hausa in northern Nigeria. *Tropical Docto.*, **27**: 12-16.
- Greenwood BM, Bojang K, Wihitty CJM, Targett GAT. 2005. Malaria. *The Lancet*, **365**: 1487-1898.
- Hilou A, Nacoulma OG, Guiguemde TR. 2006. In vivo antimalaria activities of extracts from *Amaranthus spinosus* L and *Boerhaavia erecta* L. in mice. *J. Ethnopharmacol.*, **103**: 236-240.
- Kaou Am, Mahiou-Leddé V, Hutter S, Ainouddine S, Hassani S, Yahaya I, Azas N, Ollivier E. 2008. Antimalarial activity of crude extracts from nine

- African medicinal plants. *J. Ethnopharmacol.*, **116**: 74-83.
- Kirby JC, O'Neil NJ, Philipson JD, Warhurst DC. 1989. *In vitro* studies on the mode of action quassonoids with activity against chloroquine-resistant *Plasmodium falciparum*. *Biochem. Pharmacol.*, **38**: 4367-4374.
- Lorke D. 1983. A new approach to acute toxicity testing. *Archives of Toxicology*, **54**: 275-287.
- Martin SA, Bygbjerg IC, Joil GB. 2004. Are multilateral malaria researches and control programs the most successful ? Lessons from the past 100 years in Africa. *Am. J. Trop Med Hyg.*, **2**: 268-278.
- Muregi FW, Chhabra SC, Njagi, ENM, Lang'at-Thoruwa CC, Njue WM, Orago ASS, Omar SA, Ndiege IO. 2003. *In vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *J. Ethnopharmacol.*, **84**: 235-239.
- NIH. 1985. *Guide for the Care and Use of Laboratory Animals*. NIH publication no. 85-23. The National Academic Press: Washington, DC.
- Philips JD, Wright, CW. 1990. Antiprotozoal compounds from plants sources. *Planta Medica.*, **57**: 553-559.
- Perez HA, Dela Rosa M, Apitz R. 1994. *In vivo* activity of ajoene against rodent malaria. *Antimicrob. Agents Chemother.*, **38**: 337-339.
- Peters W, Robinson BL, Tovey G, Rossier JC, Jefford CW. 1993. The chemotherapy of rodent malaria. 1. The activities of some synthetic 1,2,4-trioxanes against chloroquine-sensitive and chloroquine resistant parasites. Part 3. Observations on 'Fenozan-50 F' a di-flurated 3, 3'-spirocyclopentane 1,2,4-trioxane. *Annals Trop. Med. Parasitol.*, **87**: 111-123.
- Ryley JF, Peters W. 1970. The antimalarial activity of some quinoline esters. *Annals Trop. Parasitol.*, **64**: 209-222.
- Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K. 2000. Antiplasmodial, analgesic and anti-inflammatory activities of the aqueous extract of the stem bark of *Erythrina senegalensis*. *J. Ethnopharmacol.*, **71**: 275-280.
- Sofowora A. 1993. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books: Ibadan; 150.
- Trease GE, Evans WC. 1989. *Textbook of Pharmacognosy* (13th edn). Bailliere Tindall: London; 176-180.
- Whiffeld PJ. 1995. Plant allelochemicals and the control of parasites. *Bull. Scand. Soc. Parasitol.*, **5**: 5-18.