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Assessing the potency of *Pedilanthus tithymaloides* latex against *Plasmodium berghei berghei* infected mice

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ABSTRACT

The latex of *Pedilanthus tithymaloides* is used in traditional medicine for treating malaria attack. This usage was investigated by testing for *in vivo* antiplasmodial activity of the latex against *Plasmodium berghei berghei* in mice. Curative effect against established infection and suppressive activity against earlier infection of the parasite was evaluated. Results show that the latex exhibited potent activity against the parasite by exhibiting both curative and suppressive activity (36 – 79%) against the parasite. The results demonstrated the scientific basis for the traditional usage, and the potential of the plant in malaria chemotherapy.

Key words: Ethnomedicine, Plant tincture, Malaria.

INTRODUCTION

Pedilanthus tithymaloides (L.) Poit. (Euphorbiaceae) (Syn: *P. terthymeloides*) is a plant known to have wide range of medicinal properties that include anti-inflammatory, antibiotic, antiseptic, antihaemorhagic, antiviral and antitumor effect (Heinrich et al., 1992; Zamora-Martinez and Pola, 1992; Norhanom and Yadav, 1995; Abreu et al., 2006; Vidotti et al., 2006). Some useful compounds and active agents that include cycloartenone, dammaronol A, dotriacontan-1-ol, friedelanol, hentriacontan-1-ol and sitosterol have been isolated from the plant (Dhar et al., 1973; Mukherjee et al., 1992; Seshagirirao, 1995). The plant can resist drought and other environmental changes (Bricage, 1988; Reddy et al., 2003), and is domesticated by traditional medical healers in Midlu, Adamawa State, Nigeria, as a remedy against malaria attack (Mulmasha – Personal communication). To the best of our knowledge, the scientific basis for such usage has not been elucidated. We therefore investigated the traditional use of the plant as an antimalarial by testing the activity of the plant's dilute latex (tincture) against *Plasmodium berghei berghei in vivo* in mice, by evaluating its curative effect against infected mice and suppressive activity against earlier infection. The safety of the latex was also tested.

MATERIALS AND METHODS

Plant material

Pedilanthus tithymaloides was collected in August 2006 at Midlu-Jevi, Adamawa State, Nigeria. It was authenticated

at the Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD # 5932) was deposited at the herbarium of the Institute. The latex (a milky liquid) of the plant was carefully collected from the stem into a glass sample bottle and stored in a refrigerator. The latex was diluted to 2.5 – 10% v/v with distilled water (as used in traditional practice) prior to each study as the working concentration.

Animals

Swiss albino mice sourced from the Animal Facility Centre (AFC), NIPRD, Abuja were used for the study. The mice were housed in standard polypropylene cages and have access to food and water *ad libitum*. The animals were used in accordance with *NIH Guide for the Care and Use of Laboratory Animals [NIH Publication No. 83-23 revised (1985)]. The National Academic Press, Washington, DC, (1985)*, as outlined in NIPRD's Standard Operational Procedures (SOPs) for animal usage.

Acute toxicity test

The safety of the latex was assessed using Lorke's (1983) method. Ten ml/kg of 1, 10, 100% v/v of the aqueous latex was administered to four groups of mice (n = 3), i.p. Another group received normal saline and serve as the control. All the mice were kept under same conditions and observed for toxic signs and mortality within 24h. Toxic level of the latex was assessed as the square of the lowest lethal dose and the highest non-lethal dose from the second stage of dosing (Vongtau et al., 2004).

Inoculation of *P. berghei berghei*

The rodent parasite (*Plasmodium berghei berghei*) was obtained from National Institute for Medical Research, Lagos, Nigeria. The parasites were kept alive by continuous re-infection (Calvalho et al., 1991). An infected mouse was anaesthetised with chloroform. One ml of blood was collected with needle and syringe through cardiac puncture and made up to 20ml with normal saline. The study mice received 0.2ml of the inoculums, i.p. (Adzu et al., 2007).

Curative test on infected mice

Twenty five mice were inoculated as describe above, and kept untreated for three days. On the fourth day, they mice were grouped into five groups (n = 5). Groups 1 – 3 were treated with 0.2 ml, p.o./day of 2.5, 5 and 10% v/v of the diluted latex respectively; Group 4 was treated with chloroquine (5 mg/kg, p.o.), while Group 5 was left untreated to serve as the negative control. Same treatment continued daily until the seventh day when blood was collected from the tail vein of each mouse and smear unto a microscopic slide to make a film. The films were fixed with methanol, stained with 4% Giemsa at pH 7.2 for 45 min (Adzu et al., 2007) and examined using a microscope (Kirby et al., 1993). Inhibition of parasitaemia by the latex was calculated as:

$$\frac{PC - PT}{PC} \times 100$$ (Hilou et al., 2006);

where PC = Parasitaemia in control and PT= Parasitaemia in treated group.

Suppressive activity against earlier infection

The test was performed using a modified 4–day early infection method (Okokon et al., 2006). Mice were grouped into five (n = 5) as indicated in the curative test above, but treatment started immediately and continued daily until the fourth day when blood smears were collected, and examined for parasitaemia.

Statistical Analysis

Results were expressed as mean ± SEM. Analysis was performed using ANOVA followed by Dunnett's test using GraphPad Prism Version 4.00 for Windows, GraphPad software, San Diego California USA, (www.graphpad.com).

RESULTS AND DISCUSSION

Results showed that the latex of *P. tithymaloides* exhibited potency against *P. berghei* infected mice by showing a curative effect against the infected mice (Figure 1). It also showed efficacy on suppressive test as shown by the significant reduction in the level of parasitaemia (Table 1). *P. berghei* is used in mice as an *in vivo* model of studying the activity of potential antimalarials (Thomas et

al., 1998). The *in vivo* model has an advantage of taking into account any prodrug effect and the likelihood of immune system in controlling infection (Waako et al., 2005). Plant substances with effect against *P. berghei* infected mice were known for antimalarial activity (Calvalho et al., 1991; Elufioye and Agbedahunsi, 2004). The doses used are within safe limit. This is because the LD_{50} was established to be 10 ml/kg of 25% v/v of the tincture. Conclusively, the latex of *P. tithymaloides* has demonstrated potent antiplasmodial activity, justifying its folkloric usage and potential lead for possible development into phytodrug for chemotherapeutic treatment of malaria.

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Table 1: Suppressive effect of dilute latex of *Pedilanthus tithymaloides* against *Plasmodium berghei berghei* infected mice.

Treatment (0.2 ml, p.o./day)		Parasitaemia count (D_4)	% Inhibition
Control	-	24.8 ± 3.51	-
Latex	2.5%	15.8 ± 1.56	36.29*
	5%	7.6 ± 1.03	69.35*
	10%	5.2 ± 0.97	79.03*
CQ	5 mg/kg	1.8 ± 0.73	92.74*

* Indicates significant difference ($p < 0.05$), compared to control;
D_4 = Day 4 after inoculation; CQ = chloroquine.

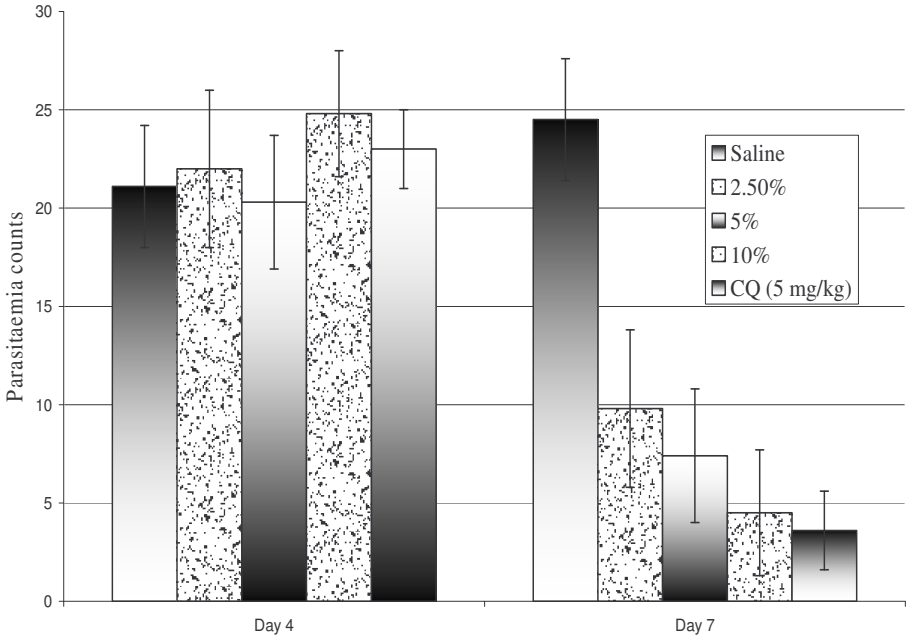

Figure 1: Curative effect of *Pedilanthus tithymaloides* dilute latex (0.2 ml, p.o./kg) against *Plasmodium berghei berghei* infected mice.

REFERENCES

Abreu P, Matthew S, Gonzalez T, Costa D, Segundu MA, Fernandes E. 2006. Anti-inflammatory and antioxidant activity of a medicinal tincture from *Pedilanthus tithymaloides*. *Life Sciences,***78**: 1578-1585.

Adzu B, Haruna AK, Salawu OA, Katsayal UA, Anoka N. 2007. *In vivo* antiplasmodial activity of ZS-2A: a fraction from chloroform extract of *Zizyphus spina-christi* root bark against *Plasmodium berghei berghei* in mice. *Int. J. Biol. Chem. Sci.,* **1**(3): 281–286.

Bricage P. 1988. The isoperoxidase pattern changes and the pigment changes of *Pedilanthus tithymaloides* L. Variegatus Calli as a result of sucrose concentration and phytoregulator content of culture medium and daily temperature differences. *Plant Science,* **55**: 169 – 173.

Calvalho LH, Brandao MGL, Santos-Filho D, Lopes JLC, Krettli AU. 1991. Anti-malarial activity of crude extracts from Brazilian plants studied in vivo in *Plasmodium berghei*-infected mice and in vitro against *Plasmodium falciparum* in culture. *Braz. J. Med. Biol. Res.,* **24**: 1113–1123.

Dhar ML, Dhar MN, Dwawan BN, Mehrotra BN, Srimal RC, Tandon JS. 1973. Screening of Indian plants for biological activity. Part IV. *Indian J. Exp. Biol*., **11**: 43 – 54.

Elufioye TO, Agbedahunsi JM. 2004. Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crosspteryx ferbrifuga* (Rubiaceae) on mice in vivo. *J. Ethnopharmacol*., **93**: 167 – 171.

Heinrich M, Rimpler H, Barrera, NA. 1992. Indigenous phytotherapy of gastro-intestinal disorders in lowland Mixe community (Oaxaca, Mexico): ethnopharmacologic evaluation. *J. Ethnopharmacol*., **36**: 63 – 80.

Hilou A, Nacoulma OG, Guiguemde TR. 2006. In vivo antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. *J. Ethnopharmacol*., **103**: 236-240.

Kirby GC, Khumalo-Ngwenya NB, Grawehr BA, Fison TW, Warhust DC, Phillipson JD. 1993. Antimalarial activity from 'Mhera' (*Uapaca nitida* Mull – Arg.) a Tanzanian tree. *J. Ethnopharmacol*., **40**: 47–51.

Lorke D. 1983. A new approach for acute toxicity testing. *Arch. Toxicol*., **54**: 275-287.

Murherjee KS, Laha S, Manna TK, Chakraborty CK. 1992. Chemical investigation on *Limnophila rogusa* and *Pedilanthus tithymaloides*. *J. Indian Chem. Soc*., **69**: 411– 412.

Norhanom AW, Yadav M. 1995. Tumor promoter activity in Malaysian Euphorbiaceae. *Brit. J. Cancer,* **71**: 776–779.

Okokon JE, Ita BN, Udokpoh AE. 2006. Antiplasmodial activity of *Cylicodiscus gabunensis*. *J. Ethnopharmacol*., **107**: 175–178.

Reddy AR, Sundar D, Gnanam A. 2003. Photosynthetic flexibility in *Pedilanthus tithymaloides* Poit, a CAM plant. *J. Plant Phy.,* **160**: 75 – 80.

Seshagirirao K. 1995. Purification and partial characterization of a lectin from *Pedilanthus tithymaloides* latex. *Biochem. Arch.,* **11**: 197 – 201.

Thomas AM, Van Der Waal AM, Thomas AW, Janse CJ, Waters AP. 1998. Transfection systems for animal models of malaria. *Parasitol. Today,* **14**: 248–249.

Vidotti GJ, Zimmermann A, Sarragiotto MH, Nakamura CV, Dias Filho BP. 2006. Antimicrobial and photochemical studies on *Pedilanthus tithymaloides*. *Fitoterapia* **77**: 43 – 46.

Vongtau HO, Abbah J, Mosugu O, Chindo BA, Ngazal IE, Salawu AO, Kwanashie HO, Gamaniel KS. 2004. Antinociceptive profile of the methanolic extract of *Neorautanania mitis* root in rats and mice. *J. Ethnopharmacol*., **92**: 317 - 324.

Waako PJ, Gumede B, Smith P, Folb PI. 2005. The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foelida* Schumch. Et Thonn. *J. Ethnopharmacol*., **99**: 137-143.

Zamora-Martinez MC, Pola CNP. 1992. Medicinal plants used in some rural populations of Oaxaca, Puebla and Veracruz, Mexico. *J. Ethnopharmacol*., **35**: 229–257.