



# International Journal of Pharmacology

ISSN 1811-7775

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## Anti-inflammatory, Analgesic and Antipyretic Activities of *Dicliptera verticillata*

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**Abstract:** The methanol extract of *Dicliptera verticillata* stems with leaves was investigated for its anti-inflammatory, analgesic and antipyretic activities in animal models. The extract was found to reduce significantly ( $p < 0.001$ ) the formation of oedema induced by carrageenan after 3 h. In the acetic acid induced writhing model, the extract has shown a good analgesic effect characterised by a significant reduction ( $p < 0.001$ ) in the number of writhes with all the doses used when compared to the control untreated group. In the antipyretic assay, the extract was efficient on yeast-induced hyperpyrexia in mice, mainly 2 h after drugs administration. The results give a scientific basis to the traditional uses of *Dicliptera verticillata*, mainly those involving pain, fever and inflammation.

**Key words:** *Dicliptera verticillata*, anti-inflammatory, analgesic, antipyretic

### INTRODUCTION

*Dicliptera verticillata* (Forssk) C. Christens (Acanthaceae) is a yearly herbaceous plant widely distributed through-out tropical Africa precisely in sudanian and sahelian zones (Berhaut, 1971; Nacoulma, 1996).

In central Burkina Faso, it is used in traditional medicine for the treatment of fever, diarrhoea, inflammation, malaria, debility, epilepsy and whooping cough (Berhaut, 1971; Nacoulma, 1996). In the western province of Cameroon, the aqueous extract of the mixture of *Aloe buettneri*, *justicia insularis*, *hibiscus macranthus* and *Dicliptera verticillata* are used for the treatment of dysmenorrhoea, sterility and regulation of the menstrual cycle (Telefo *et al.*, 1998).

The presence of coumarins, glycosides and flavonoids has been reported in this plant (Telefo *et al.*, 2004). In addition, our previous study has shown that *Dicliptera verticillata* possesses one of the highest flavonoids content (2.33%) in plants belonging to the Acanthaceae family in Burkina Faso (Sawadogo *et al.*, 2006).

However, except, its capacity to induce estradiol production both *in vivo* and *in vitro*, when mixed with *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus* (Telefo *et al.*, 1998, 2004), little is known on its biological properties.

The present study was undertaken to investigate the anti-inflammatory, analgesic and antipyretic potentials of

*Dicliptera verticillata*, as, most of its traditional uses are linked to diseases producing inflammation, fever and pain.

### MATERIALS AND METHODS

The present study was carried out during the year 2005 at Institut de Recherche en Sciences de la Santé of Ouagadougou, Burkina Faso.

**Plant material:** Stems with leaves of *Dicliptera verticillata* were collected in the botanic garden of the Research institute in Health Sciences of Ouagadougou, August 2004.

The plant material was taxonomically identified by Prof. J. Millogo, a botanist from University of Ouagadougou. A voucher specimen (N° A01) was deposited in herbarium of University of Ouagadougou. The fresh stems with leaves (100 g) were ground in a mortar and extracted by maceration with 800 mL of methanol. Extract was filtered and concentrated in a rotary evaporator (Büchi RE111, Switzerland) at approximately 50°C until thick solution was collected. The thick solution was lyophilised using freeze drying system (Christ Alpha 1-2, Bioblock Scientific, France.) for phytochemical and biological investigations.

**Animals:** Male Wistar albino rats weighing 150-200 g and male Swiss albino mice weighing 30-40 g were used in this study. They were obtained from the animal breeding facilities of Centre International de Recherche

et Développement sur l'élevage en zone Sub-humide, CIRDES, Bobo-Dioulasso, Burkina Faso. The animals were grouped and housed in polyacrylic cages (38×23×10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25±2°C) and a 12/12 h dark-light cycle. They were allowed free access to standard dry pellet diet and given water ad libitum. The animals were acclimatized to laboratory conditions for 10 days before beginning the experiments. All procedures described were reviewed and approved by the Research Institute in Health Sciences of Ouagadougou (Burkina Faso).

**Chemicals and drugs:** Carrageenan (Sigma, St Louis, USA), acetic acid (Sigma, St Louis, USA), methanol (BDH) and Brewer's yeast (Sigma, St Louis, USA) were used in the study. The standard drugs used are aspirin (Panpharma S.A. France) and paracetamol (Perfalgan, Bristol-Myers Squibb, France).

**Phytochemical analysis:** Standard phytochemical test (Asongalem *et al.*, 2004) was used in screening the extract for different constituents. Briefly, Borntrager's reaction was used to characterize anthraquinones, FeCl<sub>3</sub> test for tannins, Dragendorff reaction for alkaloids, observation under UV light of alkalized extracts for coumarins, frothing test for saponins and the Liebermann-Buchard reaction for triterpenoids and steroids.

**Toxicity study:** The LD<sub>50</sub> was determined using the graphical method of Litchfield and Wilcoxon (1949), in mice. Briefly, different doses of the extract (100, 500, 1000, 1500, 2000, 2500 and 3000 mg kg<sup>-1</sup>) were administered i.p. to 7 groups of 6 mice each. Control group received normal saline (5 mL kg<sup>-1</sup> i.p.). Signs of toxicity and mortality within 24–72 h were recorded. Confirmatory test was carried out and the LD<sub>50</sub> was calculated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract, probit 5 being 50%.

**Anti-inflammatory activity :Carrageenan-induced rat paw oedema:** Seven groups of six rats each have received either plant extract (10, 25, 50, 100 or 200 mg kg<sup>-1</sup> b.w., i.p.), aspirin (100 mg kg<sup>-1</sup>, i.p.) or vehicle control (0.9 % NaCl, i.p.) 1 h before the induction of inflammation. Acute inflammation was produced by the sub-plantar administration of 0.1 mL of 1% carrageenan in normal saline in the right paw of the rats. The paw volume was measured at 0 h and 3 h after carrageenan injection using plethysmometer (Model Ugo Basil, N°7141, Italy) (Winter *et al.*, 1962). The average volume of the right hindpaw of each rat was calculated from three readings which did not deviate more than 4% (Asongalem *et al.*, 2004).

The anti-inflammatory effect of the extract was calculated by the following equation: Anti-inflammatory activity (%) = (1-D/C)×100, where D represents the percentage difference in paw volume after extract was administered to the rats and C represents the percentage difference of volume in the control groups (Gupta *et al.*, 2005).

**Analgesic activity: Acetic acid-induced writhing response in mice:** To evaluate the analgesic effects of the plant extract, the method described by Veerappan *et al.* (2005) was used. Different groups of six mice each have received normal saline solution (5 mL kg<sup>-1</sup> i.p.) (control), paracetamol (100 mg kg<sup>-1</sup>, i.p.), or plant extract (25, 50, 100, 200 and 300 mg kg<sup>-1</sup>, i.p.). Sixty minutes later, a 0.6% acetic acid (10 mL kg<sup>-1</sup>) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptive response.

**Antipyretic activity: Yeast-induced hyperpyrexia in mice:** To induce hyperpyrexia, 10 mg kg<sup>-1</sup> b.w. of an aqueous suspension of brewer's yeast (20% in distilled water) was injected subcutaneously in the back below the nape of the mice (Sakandé *et al.*, 2004). The animals were fasted during the experiment, but they were given water ad libitum. Control temperatures were recorded with a thermometer (Model BAT-12, Physitemps, Sorsortek inc. USA) before the yeast injection and 16 h later to evaluate the pyretic response. The temperature measurements in febrile animals prior to drug administration were used as pre-drug control. The extract (100, 200 and 300 mg kg<sup>-1</sup> b.w.) and paracetamol (150 mg kg<sup>-1</sup> b.w.) which served as the reference drug, were given intraperitoneally 16 h after the yeast injection. The temperatures were recorded at 1, 2, 3 and 4 h after the drugs administration.

**Statistical analysis:** Values were expressed as mean±SEM (n = 6). Statistical significance was determined using the Sigma Stat 2.0 Jandel Scientific software (one way ANOVA followed by turkey test). Values of p<0.05 were considered significant.

## RESULTS

**Phytochemical analysis:** Phytochemical screening of the extracts revealed the presence of flavonoids, triterpenoids, steroids, coumarins, tannins and saponins.

**Toxicity study:** The LD<sub>50</sub> value of the extract was estimated to be 2555.44±14.94 mg kg<sup>-1</sup> body weight i.p. in mice.

Table 3: Antipyretic activities of methanol extract of *D. verticillata* stem with leaves and paracetamol (150 mg kg<sup>-1</sup>) on brewer's yeast-induced pyrexia in mice

Group	Dose	Rectal temperature (°C) before drug administration			Decrease of rectal T°C after drug administration			
		-16 h	0 h	1 h	2 h	3 h	4 h	
Control (saline)	5 mL kg <sup>-1</sup>	37.70±0.23	39.78±0.34	0.15±0.07	0.16±0.05	0.28±0.01	0.38±0.03	
Paracetamol	150 mg kg <sup>-1</sup>	37.88±0.31	39.03±0.34	0.13±0.08	0.38±0.09	0.81±0.13	1.61±0.20	
<i>D. verticillata</i>	100 mg kg <sup>-1</sup>	37.7±0.07	39.47±0.14	0.20±0.11	0.52±0.14	0.75±0.09	0.95±0.18	
<i>D. verticillata</i>	200 mg kg <sup>-1</sup>	37.92±0.37	39.1±0.21	0.26±0.08	0.86±0.12	1.08±0.23	1.12±0.15	
<i>D. verticillata</i>	300 mg kg <sup>-1</sup>	37.78±0.2	39.67±0.2	0.47±0.12	1.5±0.15	1.77±0.07	1.82±0.16	

Table 1: Anti-inflammatory activities of methanol extract of *Dicliptera verticillata* stems with leaves and Aspirin (100 mg kg<sup>-1</sup>) on carrageenan-induced oedema in the right hind-limb paw of rats

Group	Dose	Volume of paw oedema (mL)	Inhibition (%)
Control	5 mL kg <sup>-1</sup> (Normal saline)	0.69±0.08	-
Aspirin	100 mg kg <sup>-1</sup>	0.28±0.08	59.42
<i>D. verticillata</i>	10 mg kg <sup>-1</sup>	0.37±0.08	46.37
<i>D. verticillata</i>	25 mg kg <sup>-1</sup>	0.31±0.07	55.07
<i>D. verticillata</i>	50 mg kg <sup>-1</sup>	0.24±0.06	65.21
<i>D. verticillata</i>	100 mg kg <sup>-1</sup>	0.11±0.06	84.05
<i>D. verticillata</i>	200 mg kg <sup>-1</sup>	0.05±0.01	92.75

Table 2: Effect of the methanol extract of *D. verticillata* stem with leaves on writhing induced by acetic acid in mice

Group	Dose	Number of writhing	Inhibition (%)
Normal saline	5 mL kg <sup>-1</sup>	63.17±2.64	-
Paracetamol	100 mg kg <sup>-1</sup>	28.67±1.21	54.61
<i>D. verticillata</i>	25 mg kg <sup>-1</sup>	37.83±1.72	40.11
<i>D. verticillata</i>	50 mg kg <sup>-1</sup>	20.17±2.79	68.07
<i>D. verticillata</i>	100 mg kg <sup>-1</sup>	17.17±1.72	72.81
<i>D. verticillata</i>	200 mg kg <sup>-1</sup>	5.00±1.41	92.08
<i>D. verticillata</i>	300 mg kg <sup>-1</sup>	3.67±1.21	94.19

**Anti-inflammatory activity:** The anti-inflammatory activity of the extract was measured at the dose of 10, 25, 50, 100 and 200 mg kg<sup>-1</sup> b.w. against acute paw oedema induced by carrageenan. A strong inhibition of the paw oedema was observed with the different doses of the extract and with aspirin (100 mg kg<sup>-1</sup> a b.w.). All the doses tested have produced a significant (p<0.001) anti-inflammatory activity with the doses of 100 mg kg<sup>-1</sup> (84.05% inhibition) and 200 mg kg<sup>-1</sup> (92.75% inhibition) being more active than aspirin (59.42% at 100 mg kg<sup>-1</sup> b.w.) which were used as reference anti-inflammatory drug (Table 1).

**Analgesic activity: Acetic acid-induced writhing in mice:** *D. verticillata* at the doses of 25, 50, 100, 200 and 300 mg kg<sup>-1</sup> b.w. and paracetamol (100 mg kg<sup>-1</sup> b.w.) have induced a significant (p<0.001) decrease in the number of writhes when compared to the control untreated group (Table 2). In the acetic acid-induced writhing test in mice, the extract of *D. verticillata* at doses of 50, 100, 200 and 300 mg kg<sup>-1</sup> b.w. exhibited a higher antinociceptive power (68.07, 72.81, 92.08, 94.19% inhibition, respectively) than paracetamol (100 mg kg<sup>-1</sup>) used as control reference drug (54.61%).

**Antipyretic activity: Yeast-induced hyperpyrexia in mice:** Subcutaneous injection of yeast suspension

markedly elevated the rectal temperature 16 h after administration.

In the antipyretic test, the methanolic extract of *Dicliptera verticillata* has significantly decreased the yeast-induced elevation rectal temperature only at the dose of 300 mg kg<sup>-1</sup> after 1 hour while paracetamol at 100 mg kg<sup>-1</sup> does not have such a significant effect after the same period. However, at 2, 3 and 4 h after the hyperpyrexia, the extract at all the doses as well as paracetamol used as reference have significantly reduced the rectal temperature (Table 3).

## DISCUSSION

This study has shown that the methanol extract of *Dicliptera verticillata* possesses a significant anti-oedematogenic effect on paw oedema induced by carrageenan at 3 h. The extract was found to be more active than aspirin when used at the same or highest doses.

As the carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa *et al.*, 1995), these results are an indication that *Dicliptera verticillata* can be effective in acute inflammatory disorders.

In acetic acid-induced abdominal writhing which is the visceral pain model (Vycklicky, 1979), the results has shown that all the doses of the extract produce significant analgesic effect. This study has also shown that the extract of *Dicliptera verticillata* is more effective than paracetamol, when similar or highest doses were used. This analgesic effect of the extract can be attributed, at least in part, to its anti-inflammatory effect as, in the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism (Franzotti *et al.*, 2002). Therefore, we assume that the inhibition of the acute inflammation by these extracts, leads to their inhibitory effect on pain development process.

The extract was also found to possess a significant antipyretic effect in yeast-induced elevation of rectal temperature in rats mainly 2 h after hyperpyrexia.

The phytochemical analysis of the extract revealed that it contains terpenoids, steroids, alkaloids, flavonoids, saponins and coumarins. Of these, flavonoids and

saponins are well known for their ability to inhibit pain perception. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Oweyele *et al.*, 2005). This hypothesis is strongly supported by our previous study, which has shown that *Dicliptera verticillata*, possesses one of the highest flavonoids content ( $2.33 \pm 0.33\%$ ) in plants belonging to the Acanthaceae family in Burkina Faso (Sawadogo *et al.*, 2006).

Finally, this study confirms that *D. verticillata* has analgesic, antipyretic and anti-inflammatory properties and thus, gives scientific basis to its traditional uses. The combination of these three properties and the lack of toxicity, could help to support the usefulness of the plant in the treatment of malaria and whooping cough. The present study is a preliminary investigation on biological properties of *Dicliptera verticillata* and that is the main reason for administering the extracts intra-peritoneally.

Attempt is under way to further examine the various constituents identified in the methanol extract by oral route and determine their mechanism of action.

#### ACKNOWLEDGEMENTS

We are grateful to the Département Médecine, Pharmacopée Traditionnelle, et Pharmacie/ IRSS Burkina Faso for providing financial support.

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