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Short communication Pharmacology

Analgesic effects of the methylene chloride/methanol extract of the leaves of *Laportea ovalifolia (*Urticaceae*)*

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ABSTRACT
In the present work, the analgesic effects of methylene chloride/methanol (1:1) (CH_2Cl_2/CH_3OH) extract of *Laportea ovalifolia* (Urticaceae) were evaluated using acetic acid and formalin test. The anticonvulsant effects of the same extract were also investigated on seizures induced by pentylenetetrazol (PTZ) and picrotoxin. CH_2Cl_2/CH_3OH extract (100 -1000 mg/kg) exhibited protective effect reaching 59% of inhibition at a dose of 1000 mg/kg on the pain induced by acetic acid. The CH_2Cl_2/CH_3OH extract significantly reduced the first phase and second phase of pain induced by formalin reaching a maximum of 98.7% of inhibition (in the late phase). The extract at the doses of 100 -2000mg/kg did not exert anti-convulsant effect neither against PTZ or PIC induced seizures.

Key words: *Laportea avalofolia*, Urticaceae; analgesic activity; steroids; writhing test; formalin test.

INTRODUCTION

Laportea avalifolia is a ruderal paleotropical herbaceous plant belonging to the Urticaceae family [1]. It is widely distributed in African and asian pantropical regions, in Madagascar and Australia from Senegal to Indonesia [1]. In Cameroon, it is abundant from the forest to soudano-guinian zones. The leaves of this plant are used in traditional medicine to treat various ailment namely headaches, amoebic dysentery .This plant is also used for the treatment of abscess, gonorrhoea, haemorrhoid, pneumonia and urinary infectious diseases and epilepsy [1,2]
Although a number of secondary metabolites have been reported from various species belonging to the family of Urticaceae including alkaloids, terpenoids, peptides, lignans and flavonoids [3, 4] relatively little is known about the phytochemical and pharmacological activities of the Laportea genus.
The antidiabetic and hypolipidemic effets of *L. ovalifolia* has been demonstrated [5]. The present work was undertaken to evaluate the analgesic and anticonvulsant activities of the crude MeOH-CH_2Cl_2 (1:1) extract obtained from this plant by in vivo screening methods.

MATERIALS AND METHODS

Plant material

The stem of *Laportea ovalifolia* were collected in Ngoa-Ekelé, Yaoundé, Cameroon in September 2002 and identified by Dr L. Zapfack botanist at the Department of Plant Biology of the University of Yaoundé I Cameroon, where a voucher specimen had been deposited.

Extraction

The dried ground powder (2 kg) of *L. ovalifolia* was extracted by maceration at room temperature successively in CH_2Cl_2-MeOH (1:1) for 48h and in MeOH for 4h. From TLC analysis, the two filtrates were mixed and evaporated to dryness yielding 110g of dark viscous residue extract.
The pharmacological trials were carried out with the CH_2Cl_2-MeOH (1:1) extract dissolved in 25% Tween 80.

Animals

Swiss Albino mice (20–30 g) of either sex were used for this study. The animals were fed with rat pellet and water supplied ad libitum. All animals were acclimatized to the laboratory environment for at least 1 week before the experimental session.

Acetic acid-induced pain.

This test was performed as described by [6]. Acetic acid (0.7% v/v) was administered i.p. in a volume of 0.1 ml/10 g. Vehicle (saline), tramadol (20 mg/kg) and *L. ovalifolia* extract (LO; 100, 200 and 1000mg/kg), were administered p.o. 30 min before acetic acid injection. Each group was composed of 7 mice. The number of writhing and stretching produced in each group for the succeeding 15 min was counted and compared to the response in the control group. Immediately after the injection of the algic compound, each animal was isolated in an individual box (24 x 11 x 10 cm) to be observed during 15 min. The number of writhing and stretching was recorded and these data were used to express the percentage protection by using the following ratio:

(Control mean — treated mean) x 100/control mean.

Formalin induced paw licking in mice.

This test was carried out as described by [7]. Animals were injected subcutaneously with 20 µl of formalin into the dorsal hind paw. LO (100 and 200mg/kg), vehicle (saline 10 ml/kg) and tramadol (20 mg/kg) were administered p.o. 30 min before formalin injection. Each group was composed of 7 mice. The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by [8], two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 1-6 min after the injection of formalin and the second period (late phase) was recorded 20-30 min after the injection. The percentage inhibition of licking was calculated by the following formula:

% Inhibition = (C —T)/(C) x 100;

where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Pentylenetetrazol test

The method has been described previously [9]. In brief, clonic seizures were induced in male mice by the i.p. injection of 70 mg/kg pentylenetetrazol (PTZ). The effect of the LO (100, 200 and 2000mg/kg) was recorded. The time of onset of seizures in non-protected mice was also recorded. There were two control groups, one receiving placebo and a positive control group receiving 0.1 mg/kg clonazepam.

Picrotoxin test

The method has been described previously [10]. In brief, clonic seizures were induced in male mice by the i.p. injection of 7.5 mg/kg picrotoxin (PIC). The effect of the extract (100, 200 and 2000mg/kg) against PIC-induced clonic seizures was recorded. A 0.4 mg/kg dose of clonazepam was used as positive control. The observation period was 15 min.

Statistical analysis

The results were expressed as mean ± s.e.m. The statistical analysis involving two groups was performed by means of non parametric test of Mann-Whitney, whereas ANOVA, followed by Dunnett comparison test, was used in order to compare more than two groups. All data were processed with SPSS software (Sigma stat 2.03). A value of $P < 0.05$ significant was considered significant.

RESULTS

Effect of the L. ovalifolia extract on acetic acid-induced pain

As shown in Table 1, MeOH/CH_2Cl_2 (1:1) (Lo) significantly reduced the number of writhings and stretchings induced by a 0.7% acetic acid solution, from the dose of 100 mg/kg, the percentage of protection being 55.78 %. This protective effect decreased to 37.9 and 34.9% at the doses of 200 and 1000 mg/kg, respectively. Tramadol exerted a significant protective effect, inducing a protection of 93.73 % at a dose of 20 mg/kg.

Effect of the L. ovalifolia extract on formalin-induced pain

In the formalin test (Table 2) inhibition occurred in both early and late phase. LO significantly reduced formalin induced pain at the early phase with a percentage of inhibition reaching 51.07 % at 100 mg/kg, whereas 98.7 % of inhibition was observed at the late phase. Tramadol (20 mg/kg) was also more effective on the late phase (71.80% inhibition) than in the early phase (59.04%).

Effect of the L. ovalifolia extract on PTZ and PIC-induced seizures

The extract at the doses of 100 to 2000 mg/kg did not exert significantly any anti-convulsant effect neither against PTZ induced seizures (figure 1) nor against seizures induced by PIC (Table 2).

Table1: Antinociceptive effect of the MeOH/CH_2Cl_2 extract of *L. ovalifolia* on the acetic acid-induced nociception.

Group	Dose (mg/kg)	No. writings (Mean ± s.e.m)	% Inhibition
Control		50.50 ± 3.32	
Tramadol	20	03.30 ± 1.91*	93.00
L.ovalifolia	100	22.33 ± 5.89*	55.78
L. ovalifolia	200	32.83 ± 3.98*	34.98
L. ovalifolia	1000	31.33 ± 1.35*	37.95

Value are mean ± S.E.M; n = 7; * P< 0.05; as compared to the control.

Table 2: Antinoceptive effect of the MeOH/CH_2Cl_2 extract of *L. ovalifolia* on the formalin-induced nociception.

Group	Dose (mg/kg)	Licking times (s)		% inhibition	
		Early phase (1-6 min)	Late phase (20-30 min)	Early phase	Late phase
Control		91.15 ± 10.23	105.18 ± 19.44		
Tramadol	20	37.34 ±5.55 **	29.67± 9.49**	59.04	71.80
L. ovalifolia	100	44. 54 ± 9.65*	01.33 ± 1.33**	51.13	98.73
L. ovalifolia	200	47.31 ± 9.72 *	05.83 ± 5.8**	48.10	94.45

Value are mean ± S.E.M; n = 7; * P< 0.05; as compared to the control.

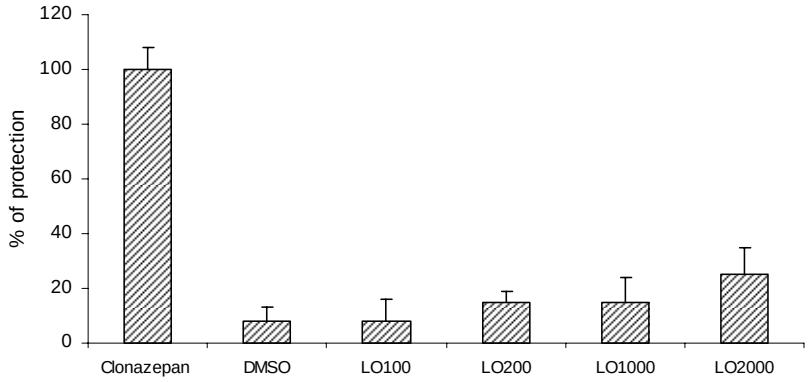

Figure 1: Effet of the *L. ovalifolia* (LO) extract on PTZ-induced clonic seizures in mice: the figure represents the percentage of animal protected by each treatment

DISCUSSION AND CONCLUSIONS

Analgesic activity was evaluated using two chemical stimuli (acetic acid and formalin). The writhing response of the mouse to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes algesia by liberating endogenous substances and many others that excite pain nerve endings [11]. A MeOH/CH_2Cl_2 (1:1) extract of *L. ovalifolia* showed significant inhibition effect on acid acetic-induced pain. The abdominal writhing induced by acetic

acid involves the production and release of arachidonic acid metabolites via cycloxygenase (COX) and prostaglandin biosynthesis [12]. It has been demonstrated that PGE_2 produced by COX-1 elicited acute writhing responses in noxious chemical stimuli [13] . Thus the effect of *L. ovalifolia* extract might be modulated by the inhibition of COX-1. The results led to the hypothesis that LO might play a role in the inhibition of prostaglandin synthesis. The mechanism of analgesic action of the LO extract could probably be due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similarly to NSAIDs.

The formalin test possesses two distinctive phases, possibly reflecting different types of pain. The earlier phase reflects direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects inflammation [7]. From this study, the *L. ovalifolia* extract showed significant analgesic activity on the early phase as well as on the late phase. This inhibitory effect in the early phase may be due to direct effects on nociceptor. The effect on the late phase of the extract indicates its inhibitory activity on pain arising from inflammation, which reflects the effect on the synthesis and/or release of PGs. Tramadol which is a centrally acting drug inhibited the two phases of formalin induced pain with a pronounced and significant response in the late phase, which suggests that it is a more morphine-like analgesic. Moreover recent studies reported that the antinociceptive effect of tramadol in the formalin test seems to be mediated by serotonergic transmission and that the opioid and noradrenergic systems play a minor role in this process [14].

The pentylenetetrazole test (PTZ) is one of the primary bioassays employed in the *in vivo* screening of new anticonvulsant compounds [15,16]. PTZ test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures [16,17]. As PTZ have been shown to interact with the GABA neurotransmitter and the GABA receptor complex [16,18], antagonism of PTZ-induced seizures suggests effects on GABA-ergic neurotransmission. The lack of the effect of the LO extract in these tests could therefore suggest lack of anticonvulsant effects against the above mentioned seizure types.

Absence of significant anticonvulsant activity in PIC test may suggest the absence of compounds in LO extract with non-competitive interaction at $GABA_A$ receptor, since PIC is a known convulsant and a non-competitive $GABA_A$ receptor antagonist [20]

The absence of an inhibitory effect on PTZ and PIC-induced convulsion does not necessarily indicate a lack of anti-convulsant activity. The active compound(s) present in the extract may act at other sites implicated in the seizure process.

The Phytosterols isolated in the LO extract might be the active principle responsible for the observed analgesic effect since there are numerous reports in literature on the analgesic and anti-inflammatory properties of this class of compounds [20-22]

It can be concluded that the extract of *L. ovalifolia* posseses analgesic activity, probably mediated by the inhibition of prostaglandin synthesis.

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