Analgesic activity of *Ugni molinae* (murtilla) in mice models of acute pain

C. Delporte [a,*], N. Backhouse [a], V. Inostroza [a], M.C. Aguirre [a], N. Peredo [a], X. Silva [b], R. Negrete [a], H.F. Miranda [c]

[a] *Laboratory of Natural Products, Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Casilla 233, Santiago 1, Chile*
[b] *Chilean Public Health Institute, Marathon 1000, Santiago, Chile*
[c] *Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Clasificador 70.000, Santiago 7, Chile*

Abstract

Leaf extracts of *Ugni molinae* Turcz. (Myrtaceae) are used in Chilean folk medicine as analgesic and anti-inflammatory. The antinociceptive effect of dichloromethane (DCM), ethyl acetate (EA) and methanol (ME) leaf extracts was assessed by intraperitoneal, oral and topical administration in writhing, tail flick, and tail formalin tests in mice.

The extracts showed a dose-dependent antinociceptive activity in all the assays under different administration routes. The ED_{50} values for the different tests for the DCM, EA, ME extract and reference drug (ibuprofen) were as follows. Writhing test in acetic acid (i.p. administration): 0.21, 0.37, 1.37 and 0.85 mg/kg, respectively; tail flick test (oral administration): 199, 189, 120 and 45.9 mg/kg. The EC_{50} values for tail flick test were (topical administration): 2.0, 0.35, 1.4 and 8.2% (w/v), respectively; and the topical analgesic effects were (formalin assay) 75.5, 77.5, 31.6 and 76.5%, respectively.

Ugni molinae extracts produce antinociception in chemical and thermal pain models through a mechanism partially linked to either lipooxygenase and/or cyclooxygenase via the arachidonic acid cascade and/or opioid receptors. Flavonoid glycosides and triterpenoids have been isolated from the plant and can be associated with the observed effect. Our results corroborate the analgesic effects of *Ugni molinae*, and justify its traditional use for treating pain.

Keywords: Analgesic activity triterpenoids; Tail flick assay; Tail formalin test; *Ugni molinae*; Writhing test; Myrtaceae

1. Introduction

Ugni molinae Turcz (*Myrtus ugni* Mol.) is a wild native plant occurring in the downhill of the southern mountains of Chile. This is an aromatic species of the Myrtaceae family widely used in folk medicine as analgesic for different types of pain and as anti-inflammatory (Montenegro, 2000). Previous chemical studies have demonstrated the presence of pentacyclic triterpenoids in the hexane, dichloromethane and ethyl acetate extracts of this species together with polyphenolic compounds (Rubilar et al., 2006).

In a previous work, we identified triterpenoids (asiatic, corosolic, alphitolic, betulinic, oleanolic and ursolic acid) and showed their anti-inflammatory activity (Aguirre et al., 2006). Other works reported the presence of flavonoid glycosides of quercetin, myricetin and kaempferol in the leaves of *Ugni molinae*. The isolated compounds are widely known for their anti-inflammatory and analgesic activity (Recio et al., 1995; Middleton et al., 2000; Toker et al., 2004; Rubilar et al., 2006).

The aim of this study was to assess the antinociceptive activity of different extracts obtained from *Ugni molinae* leaves, using chemical and thermal models of acute pain in mice.

2. Material and methods

2.1. Plant material and extraction

Ugni molinae leaves (200 g) were collected in Chanco, south-central Chile (35°45′S, 72°33′W) in April 2004. A voucher sample (SQF-22230) is kept at the Herbarium of the Faculty

* Corresponding author. Tel.: +56 29781697; fax: +56 22227900.
E-mail address: cdelpor@uchile.cl (C. Delporte).

of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. The dried leaves of *Ugni molinae* were ground and successively extracted with dichloromethane (DCM), ethyl acetate (EA) and methanol (ME). After concentration under reduced pressure, the w/w extraction yields of the extracts were 8.0, 5.9 and 20.4% for the DCM, EA and ME, respectively.

For topical administration, the different extracts were dissolved in dimethylsulfoxide (DMSO) at different concentrations. For i.p. and per os administration the different extracts were dissolved in saline solution and 2% Tween 80 in water, respectively, just before use.

2.2. *In vivo assays animals*

CF-1 male mice weighing 25–30 g were housed under a 12 h light–dark cycle at $22 \pm 2\,^{\circ}$C with *ad libitum* access to food and water. Experiments were performed in accordance with current Guidelines for the Care of Laboratory Animals and Ethical Guidelines for investigation of experimental and approved by the Animal Care and Use Committee of the Faculty of Medicine, Faculty of Chemical and Pharmaceutical Sciences, University of Chile and Chilean Public Health Institute. Animals were acclimatized to the laboratory environment for at least 2 h before testing, and were used only once during the experimental protocol and killed by cervical dislocation immediately after the algesiometric test. The number of animals considered was at the minimum compatible with consistent effects for the treatments.

2.3. *Analgesic activity*

2.3.1. *Writhing test*

Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test)—a chemical visceral pain model (Miranda et al., 2006). Mice were injected intraperitoneal (i.p.) with 10 mL/kg of 0.6% acetic acid solution after 30 min of the i.p. administration of the different extracts (at the doses of 100, 30, 10, 3, 1, 0.3 and 0.1 mg/kg) time at which preliminary experiments showed occurrence of the maximum effect. Starting 5 min after acetic acid administration, the number of writhes was counted in a 5 min period. Antinociceptive activity was expressed as inhibition percent of the usual number of writhes observed in control animals. Dose–response curves were obtained for DCM, EA and ME extracts using groups of 8 animals for a single dose and groups of 16 control animals were treated similarly, but they did not receive the samples. Another group of eight animals was pretreated with ibuprofen, drug used as reference. The ibuprofen doses were 3, 1, 0.3 and 0.1 mg/kg. Least-squares linear regression analysis of the log-dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) for each extract and reference drug.

2.3.2. *Tail flick test*

A radiant heat automatic tail flick algesiometer (U. Basile, Comerio, Italy) was used to measure response latencies according to a previous report (Miranda et al., 2003). The intensity of the light beam was focused on animal tails 2–2.5 cm from the tip and adjusted for baseline readings between 2 and 3 s. An 8 s cut-off was imposed to avoid tail damage by heat. Control reaction was recorded twice with 20 min intervals between readings. After oral or topical administration of the extracts, the reaction time was tested at 30 min (time of peak effect) and the difference reaction time was recorded (Δlatency). For topical administration, the mouse tail was immersed for 3 min in DMSO containing the extracts at different concentrations (5, 3, 1 and 0.1%, w/v). For oral administration, a gastric catheter was used for the different doses (200, 100, 50 and 25 mg/kg). Tail flick latencies were converted to maximum possible effect (MPE), according to the following formula: MPE (%) = 100 × (postextract latency − preextract latency)/(cut-off time − preextract latency). Dose–response and concentration–response curves for p.o. and topical administration, respectively were obtained for DCM, EA and ME, using groups of 8 animals for a single dose and groups of 16 control animals were treated similarly, but they did not receive the extracts. Another group of eight animals were pretreated with ibuprofen-reference drug. For topical administration, 30, 15, 7.5 and 3.75% (w/v) concentrations were used. For oral administration, the reference drug doses were 30, 10, 3 and 1 mg/kg. Least-squares linear regression analysis of the log-dose (p.o.)– or log-concentration (topical)–response curves allowed the calculation of the dose or concentration that produced 50% of antinociception (ED_{50} and EC_{50}, respectively) for each extract and reference drug.

2.3.3. *Tail formalin test*

A modified formalin test was used, as described by Koleniskov et al. (2004). The different extracts at 5% (w/v) concentration were applied by topical administration as described in the tail flick test, and mice were immediately intra-dermically injected 20 μL of a formaldehyde solution to 10% into dorsal surface of their tail, using a tuberculin syringe. Then the mouse was located into a chamber, with mirrors walls to enable clear observation of animal tails for 5 min. The nociceptive behavior is directly proportional to the licking time of the tail, which is a monophasic process (Koleniskov et al., 2004). The time-course observation was restricted to 5 min, the length of time during which pain occurs. Antinociceptive activity (A) was expressed according to the following formula: A (%) = 100 − [(T1 × 100)/T2], where T1 stands for the mean licking time postextract and T2 is the mean licking time control.

For each concentration of the different extracts, the topic analgesic activity was evaluated using groups of 8 animals for a single dose and groups of 16 control animals were similarly treated, but they did not receive the extracts. Another one group of eight animals were pretreated with ibuprofen, reference drug at 5, 2.5, 1.2 and 0.6% (w/v) concentrations.

2.3.4. *Statistical analysis*

Results are presented as $ED_{50} \pm$ S.E.M. or $EC_{50} \pm$ S.E.M. except for the tail formalin test. The statistical difference between theoretical and experimental values was assessed by Wilcoxon test for independent data and p values less than 0.05 ($p \leq 0.05$) were considered significant.

Table 1
Analgesic activity of different extracts of *Ugni molinae* and reference drug

Sample	ED_{50wip} (mg/kg) ± S.E.M.	ED_{50tpo} (mg/kg) ± S.E.M.	EC_{50tt} (%, w/v) ± S.E.M.	$\%E_{ft}$ ± S.E.M.
DCM	0.21* ± 0.02	199* ± 16.0	2.0* ± 1.1	
EA	0.37* ± 0.03	189* ± 10.2	0.35* ± 0.1	
ME	1.37* ± 0.82	120* ± 6.8	1.4* ± 0.2	
Ibuprofen	0.85* ± 0.26	45.9* ± 12.0	8.23* ± 0.1	
DCM (5%, w/v)				75.5* ± 5.3
EA (5%, w/v)				77.5* ± 3.9
ME (5%, w/v)				31.6* ± 12.2
Ibuprofen (5%, w/v)				↑76.5* ± 6.3

*$p \leq 0.05$; $p > 0.05$. ED_{50wip} = dose that produces 50% antinociception in acetic acid writhing test (i.p. administration). ED_{50tpo} = dose that produces 50% antinociception in tail flick test (p.o. administration). EC_{50tt} = concentration that produces 50% antinociception in tail flick test for topical administration. $\%E_{ft}$ = percent of analgesic effect in tail formalin assay for topical administration. DCM, dichloromethane extract; EA, ethyl acetate extract; ME, methanol extract; ↑, maximum effect. Each group represents the mean ± S.E.M. of eight animals pretreated with extracts or reference drug.

3. Results

DMSO solution provides an effective way of solubilizing the different extracts and facilitating their transport through skin. DMSO alone has no effect on topical tail flick and formalin assays.

3.1. Writhing test

The i.p. administration of DCM, EA and ME induced a dose-dependent antinociceptive activity and the ED_{50wip} values ± S.E.M. for the extracts are shown in Table 1, where DCM and EA are the most active extracts with ED_{50wip} (0.21 and 0.37 mg/kg, respectively) smaller than the reference drug (0.85 mg/kg).

3.2. Tail flick test

The antinociceptive activity induced by oral or by topical administration of DCM, EA and ME was dose-dependent; Table 1 shows the ED_{50tpo} (p.o.) and EC_{50tt} (topical administration) values using the tail flick test. ME (120 mg/kg) and EA (0.35%, w/v) showed stronger analgesic activity for p.o. and topical administration, respectively. For topical administration, all extracts exhibited greater analgesic effects than ibuprofen.

3.3. Tail formalin test

In the tail formalin test, the percent analgesic effect ($\%E_{ft}$) to DCM, EA and ME by topical administration induced an antinociceptive activity which was dependent of concentration (data not shown); the values of the selected concentration (5%) are shown in Table 1. No differences were observed for DCM and EA, and their effects were similar to the maximum effect of the reference drug.

4. Discussion

The current study demonstrated that systemic, either i.p. or p.o., as topical administration of the DCM, EA and ME of *Ugni molinae* leaves produced dose-dependent antinociceptive activity according to two chemical and one thermal models for nociception. The writhing test allows us to identify central and peripheral analgesic compounds (Le Bars et al., 2001). The tail formalin test is a recent algesiometric assay in which the only behavior suggestive of pain is the licking of the tail. The lack of the two distinct phases after the administration of formalin in the tail may be due to a different pattern of the release of the chemical pain mediators at both the spinal and peripheral levels and this method mainly identifies peripheral analgesic (Koleniskov et al., 2004). The thermal model of the tail flick test is considered to be a spinal reflex, but could also involve higher neural structures and this method identifies mainly central analgesic (Jensen and Yaksh, 1986; Le Bars et al., 2001). The fact that DCM, EA and ME at the doses tested produced analgesia in all nociceptive models is indicative that they possess both central and peripheral antinociception and the mechanism of action of the active principles of these extracts could be partially related to lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade and/or opioid receptors (Deraedt et al., 1980; Le Bars et al., 2001). The analgesic effect of DCM, EA and ME of *Ugni molinae* on i.p. writhing and topical tail flick tests is high, which is explained by the ED_{50} and EC_{50} that are respectively closer or lower to ibuprofen, a widely known analgesic, anti-inflammatory and antipyretic drug (Goodman, 1996). On the other hand, comparisons of the above tests with per os tail flick permits to observe a higher ED_{50}, than ibuprofen, which implies a lower analgesic effect. In relation to tail formalin assay, DCM and EA were the most active analgesic extracts, yielding a similar effect to the maximal effect obtained with ibuprofen, all at the same concentration.

Moreover, the antinociceptive activity of *Ugni molinae* extracts for EA and ME may be due to the presence of myricetin, quercetin and kaempferol glycosides since these compounds are known for their antinociceptive properties and these phenolic compounds are present in a high percentage in EA and ME (Bittar et al., 2000; Middleton et al., 2000; Toker et al., 2004; Rubilar et al., 2006). Besides, our previous results showed that EA exhibits anti-inflammatory activity mainly due to the presence of several pentacyclic triterpene acids, including the 2-α-hydroxy derivatives alphitolic, asiatic, and corosolic acids (Aguirre et al., 2006). These compounds could present anal-

gesic activity since the mechanism of the anti-inflammatory and analgesic activities may be related with both prostanoids (PGE_2 and $PGF_{2\alpha}$) and lipooxygenase products (Deraedt et al., 1980; Sawynok, 2003).

For the triterpenoids presented in DCM as betulinic, oleanolic and ursolic acids (Aguirre et al., 2006) several recent reports on the analgesic activities of plant extracts have ascribed them to these compounds (Liu, 1995; Krogh et al., 1999; Tapondjou et al., 2003; Maia et al., 2006). The ursolic acid is a selective inhibitor of cyclooxygenase-2 (Ringbom et al., 1998). In relation to the analgesic effect of the oleanolic acid, this effect involved an opioid mechanism, and possibly, a modulatory influence on vanilliod receptors (Maia et al., 2006).

5. Conclusion

The data of the present study demonstrated that DCM, EA, and ME of *Ugni molinae* leaves produce a dose-dependent antinociceptive activity, as observed through different algesiometric tests. This antinociceptive effect may be partially related to the lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade. Our results account for the analgesic effects of *Ugni molinae*, and could be the reason for its wide use in folk medicine to treat different types of pain.

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