Antinociceptive activity of *Quillaja saponaria* Mol. saponin extract, quillaic acid and derivatives in mice

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ABSTRACT

Ethnopharmacological relevance: Quillaja saponaria bark contains a high percentage of triterpene saponins and has been used for centuries as a cleansing and analgesic agent in Chilean folk medicine.
Aim of the study: The topical and systemic analgesic effects of a commercial partially purified saponin extract, 3β,16α-dihydroxy-23-oxoolean-12-en-28-oic acid (quillaic acid), methyl 3β,16α-dihydroxy-23-oxoolean-12-en-28-oate and methyl 4-nor-3,16-dioxoolean-12-en-28-oate.
Materials and methods: The samples were assessed in mice using the topical tail-flick and i.p. hot-plate tests, respectively.
Results: All the samples showed activity in both analgesic tests in a dose-dependent manner. The most active against tail flick test was commercial partially purified saponin extract (EC50 27.9 mg%, w/v) and more than the ibuprofen sodium. On hot-plate test, methyl 4-nor-3, 16-dioxoolean-12-en-28-oate was the most active (ED50 12.2 mg/kg) and more than the ibuprofen sodium.
Conclusions: The results of the present study demonstrated that *Quillaja saponaria* saponins, quillaic acid, its methyl ester, and one of the oxidized derivatives of the latter, elicit dose-dependent antinociceptive effects in two murine thermal models.

1. Introduction

It is well established that pain cannot be monitored directly in animals but can only be estimated by examining their responses (Le Bars et al., 2001).

Chronic and acute pain are conditions that require multimodal treatment, and clinicians routinely employ various combinations of pharmacological agents. Multimodal analgesia is needed for acute postoperative pain management due to the adverse effects of opioid analgesics, which can hinder recovery, a problem that is of increasing concern due to the rapid increase in the number of ambulatory surgeries. Still, a review of the literature on multimodal analgesia shows variable degrees of success, even between studies utilizing the same adjuvant medication (Buvanendran and Kroin, 2009).

Nonsteroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors consistently reduce postoperative opioid consumption. On the other hand, *N*-methyl-ᴅ-aspartate antagonists have produced inconsistent results, which may be due to the dose regimen and timing of drug administration. Alpha-2 adrenergic agonists have been found to be useful as adjuvants for regional analgesia but not when administered orally. Alpha-2-delta receptor modulators such as gabapentin have shown early promising results in multimodal analgesia. Local anesthetic injection at the surgical site, though not as a preemptive analgesic, has recently been demonstrated to be beneficial. However, there is a continuing need to explore new drug combinations to achieve all of the desired goals of multimodal anesthesia (Buvanendran and Kroin, 2009).

Many saponins tested have displayed significant antinociceptive, anti-inflammatory and antipyretic activities possibly due to their nonglycosidic moiety, the sapogenin, but also many diverse activities have also been reported such as antiallergic, antifungal, analgesic and others (Hostettmann and Marston, 1995; Milgate and Roberts, 1995; Lacaille-Dubois and Wagner, 1996; Francis et al., 2002). Moreover a variety of extracts have proved to be useful in animal models of analgesia, e.g., to cite recent examples, *Bauhinia racemosa* (Rinaldi et al., 2009), *Buddleja globosa* (Backhouse et al., 2008), *Tribulus terrestris* (Heidari et al., 2007), *Ugni*
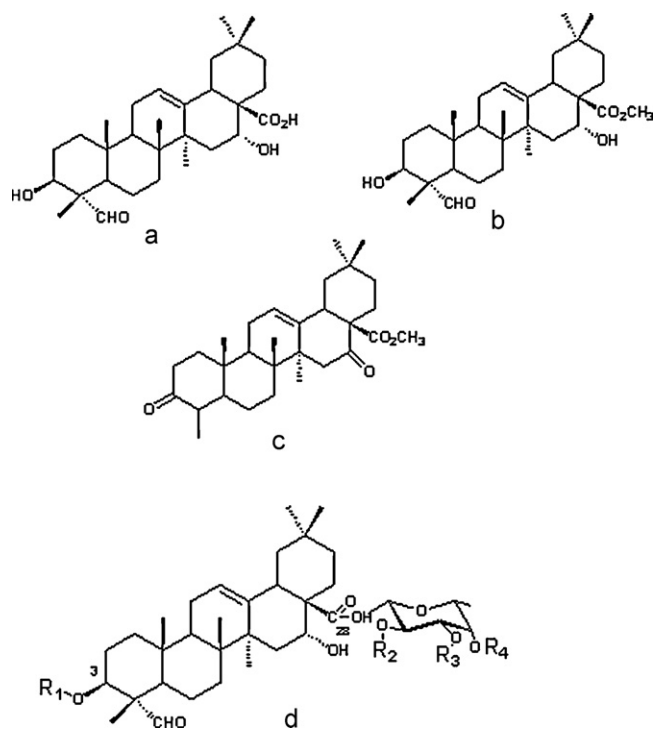
Fig. 1. Chemical structures of QA (a), ME (b), MO (c) and *Quillaja saponin* (d).

molinae (Delporte et al., 2007), *Cipura paludosa* (Greice et al., 2007), *Kalopanax pictus* (Choi et al., 2005). Choi et al. (2005) also reported that the antinociceptive and anti-inflammatory properties of the stem *of Akebia quinata* (Lardizabalaceae) could be attributed to the sapogenins oleanolic acid and hederagenin. The extracts of this plant material, their fractions, the isolated saponins and sapogenins obtained by hydrolysis of the saponins were subjected to antinociceptive activity tests using the hot-plate and tail-flick methods in mice (Choi et al., 2002).

The Chilean soapbark tree (*Quillaja saponaria* Mol.) grows in a wide range of habitats in the forests and scrubland of the Mediterranean climate zone of central Chile. In relation to the traditional use of quillay bark, mapuche indigenous people used it as analgesic for the relief toothache and as detergent agent (Zin and Weiss, 1980). This species is well known for its content of triterpene saponins (between 8.5 and 16.4%) that are widely used in industry, in personal care products and as vaccine adjuvants. Related triterpene glycosides and their aglycones have been shown to possess anti-inflammatory, antiallergic, antiviral, antifungal and cytotoxic properties (Roner et al., 2007).

Based on all of this background and with the conviction that there were more chances of finding analgesic-antiinflammatory properties mainly from the sapogenin and its derivatives than the saponin portion of the molecule, the aims of the present work assessed the antinociceptive activity of UD (65% of saponins), its major aglycone 3β,16α-dihydroxy-23-oxoolean-12-en-28-oic acid (quillaic acid, QA), and the chemical modification of QA molecule by metilation (ME): methyl 3β,16α-dihydroxy-23-oxoolean-12-en-28-oate and oxidation reactions (MO): methyl 4-nor-3β,16α-dioxoolean-12-en-28-oate, using models of acute pain in mice. Chemical structures are showed in Fig. 1.

Another purpose of this study was proving the significance of the acid group (C28) on the antinociceptive activity of QA. In regard to the demonstration of the importance of OH group on C3 and C16, ME was oxidized, but in addition to this chemical reaction a descarboxilated majority product (C4) was obtained, MO.

2. Materials and methods

2.1. In vivo assay animals

Male CF-1 mice (30 g) housed in groups of 8 males on a 12 h light–dark cycle at $22 \pm 2\,°C$ and with access to food and water *ad libitum* were used. Experiments were performed in accordance with current Guidelines for The Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile (Miranda et al., 2006). Animals were acclimatized to the laboratory environment for at least 2 h before testing, were used only once during the experimental protocol and were sacrificed immediately after each algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatment.

2.2. Analgesic activity

2.2.1. Tail flick test

The test protocol was similar to one described previously (Le Bars et al., 2001). A radiant heat, automatic tail flick algesiometer (Ugo Basile, Comerio, Italy) was used to measure response latencies. The light beam was focused on the animal's tail about 4 cm from the tip and the intensity was adjusted so that baseline readings were between 2 and 3 s. An 8 s cut-off time (T_{off}) was imposed to avoid damage to the tail. Control reaction time (latency of the response) was recorded twice, with an interval of 15 min between readings, the second reading being similar to the first. Only animals with baseline reaction times between 2 and 3 s were used in the experiments. Tail flick latencies were converted to % maximum possible effect (MPE) as follows:

$$\% \text{MPE} = 100 \times \left[\frac{L2 - L1}{T_{off} - L1} \right]$$

where L2 is the post drug latency and L1 is the pre drug latency or control reaction time. Drugs were topically administered immediately after control reaction time and their effects were measured 15 min after their topical administration, time at which preliminary experiments showed occurrence of the maximum effect. The reference drug was ibuprofen sodium and the concentrations tested were 2.8; 5.6; 11.3; 22.5; 75 mg% (w/v). The concentration that produced 50% of the MPE (EC_{50}) was calculated from the linear regression analysis of the curve obtained by plotting log dose versus %MPE.

2.2.2. Hot-plate test

A modification of the method of Menéndez et al. (1993) was used. In this case, the animals were free to move and the assay temperature was $45 \pm 1\,°C$. The behaviour of the animals considered as a sign of pain was licking the forelegs or jumping off the hot-plate. The baseline latency to this behaviour was recorded with a stopwatch. The cut-off time (T_{off}) was fixed at 30 s to avoid skin damage. Several measurements were performed with an interval of 3 min: two at baseline (without any drug) and two after i.p. administration of the test drug. The reference drug was ibuprofen sodium and the concentrations tested were 1.0; 1.5; 1.7; 2.0; 2.3 mg/kg.

Hot-plate latencies were converted to % maximum possible effect (MPE) with the same equation used in the tail-flick assays.

2.2.3. Drugs

Ibuprofen sodium (IBU, Sigma, U.S.A) was used as the reference drug, a commercial partially purified saponin extract (UD), Natural Response, Quillota, Chile); quillaic acid (QA); QA methyl ester (ME) and ME oxidation product (MO) were obtained in our laboratories starting from UD. All the samples were dissolved in water with the
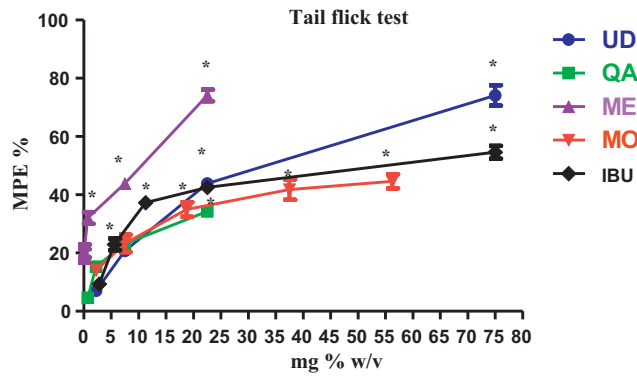
Fig. 2. MPE Maximum possible effect of topical analgesic on the tail-flick test; UD commercial partially purified saponins extract; ME methyl 3β, 16α-dihydroxy-23-oxoolean-12-en-28-oate; IBU sodium ibuprofen; QA quillaic acid; MO and methyl 4-nor-3, 16-dioxoolean-12-en-28-oate; $^*p < 0.05$.

Fig. 3. MPE Maximum possible effect of i.p. analgesic on the hot-plate test; UD commercial partially purified saponins extract; ME methyl 3β, 16α-dihydroxy-23-oxoolean-12-en-28-oate; IBU sodium ibuprofen; QA quillaic acid; MO and methyl 4-nor-3,16-dioxoolean-12-en-28-oate; $^*p < 0.05$.

aid of DMSO at a final concentration not exceeding 5% which had no effect *per se*. In the tail-flick test the animal tail is immersed in a warm solution (DMSO 5% in saline water 0.9%) at 22 °C containing the sample, during 5 min. Testing was performed on the portion of the tail immersed in the treatment solution.

2.3. Statistical analysis

All the results were expressed as mean ± SEM. The data were analyzed by ANOVA followed by the statistics software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA; $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Analgesic effects in the tail-flick test

The topical administration of UD, QA, ME and MO induced concentration-dependent responses in the tail-flick test with different potencies. UD showed the highest relative potency with a CE_{50} of 27.9 mg% (w/v). The concentration–response curves are displayed in Fig. 2.

The corresponding EC_{50} values are summarized in Table 1.

3.2. Analgesic effects in the hot-plate test

The intraperitoneal administration of UD, QA, ME and MO induced dose-dependent responses in the hot-plate test with different potencies. MO showed the most relative potency, with a DE_{50} of 12.2 mg/kg. The dose–response curves are displayed in Fig. 3.

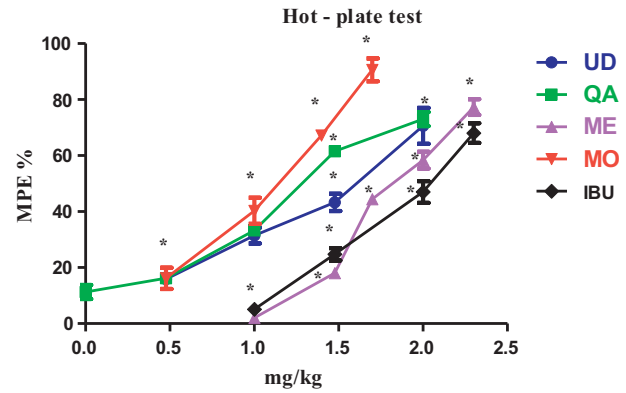The corresponding ED_{50} values are summarized in Table 2.

4. Discussion

The results obtained in the present study showed that UD, QA, ME and MO exert concentration or doses-dependent antinociceptive effects, against topical tail-flick and systemic hot-plate assays, respectively. The rank order of relative potencies in the tail-flick test was: UD > ME > IBU > QA > MO, and in the hot-plate test the order was: MO > QA > UD > ME > IBU. This means that UD and ME were more potent than the QA and MO in the tail-flick test, while MO and QA were the most active in the hot-plate test. Interestingly, ibuprofen sodium is less active than UD in both tests.

There is overwhelming evidence demonstrating that tail-flick and hot-plate tests are poorly affected by non-steroidal anti-inflammatory drugs (NSAIDs), but they are sensitive to the analgesic effects of opioid agents (Lavich et al., 2005).

Dogrul et al. (2007) point out that the mechanism of the local antinociceptive effects of NSAIDSs in tail-flick test remains to be clarified. These last authors emphasized that antinociceptive effects can only be observed after intradermal injection because the skin concentration of NSAIDs would be much higher by this route than when they are administered by other systemic route. It is important to highlight that radiant tail flick model involves nociceptive processing at spinal circuits with supraspinal modulation, so it seems reasonable to assume that the NSAIDs should not strongly modify nociceptive stimuli on this assay, since the primary site of action of NSAIDs is likely to be in the periphery.

However, our results showed that against topical tail flick test, all the samples were active as analgesic. Besides, the metilation of QA results on increases of the analgesic effect in the same assay and *Quillaja saponins* were the most active. In relation to MO and QA,

Table 1
EC_{50} values for the antinociceptive effects of topical administration of UD, QA, ME, MO and IBU using the tail-flick assay.

Sample	EC50 (mg%, w/v)	Relative potency	Ranking
UD	27.9 ± 0.7*	1.5	1
ME	32.8 ± 0.6*	1.3	2
IBU	42.5 ± 0.7*	1.0	3
QA	69.3 ± 0.8*	0.6	4
MO	77.3 ± 0.5*	0.5	5

UD commercial partially purified saponins extract; ME methyl 3β, 16α-dihydroxy-23-oxoolean-12-en-28-oate; IBU ibuprofen sodium; QA quillaic acid; MO and methyl 4-nor-3, 16-dioxoolean-12-en-28-oate; EC_{50} concentration (w/v) that produces 50% of the maximal antinociceptive effect; each group represents the mean ± SEM of eight animals pretreated with samples or reference drug.
  * $p < 0.05$.

Table 2
ED_{50} values for the antinociceptive effects of intraperitoneal administration of UD, QA, ME, MO and IBU using the hot-plate assay.

Sample	ED50 (mg/kg)	Relative potency	Ranking
MO	12.2 ± 0.7*	7.9	1
QA	20.7 ± 0.7*	4.7	2
UD	32.8 ± 0.6*	3.0	3
ME	72.7 ± 0.8*	1.3	4
IBU	97.1 ± 0.9*	1.0	5

UD commercial partially purified saponins extract; ME methyl 3β,16α-dihydroxy-23-oxoolean-12-en-28-oate; IBU ibuprofen sodium; QA quillaic acid; MO and methyl 4-nor-3,16-dioxoolean-12-en-28-oate; ED_{50} dose (mg/kg) that produces 50% of the maximal antinociceptive effect; each group represents the mean ± SEM of eight animals pretreated with samples or reference drug.
  * $p < 0.05$.

these compounds were the less active. It is remarkable that *Quillaja saponins* activities were stronger than IBU.

On the other hand, our results showed that MO and QA were the most active against i.p. hot-plate, surprisingly all the samples were most active than the reference drug. This issue might indicate that they would have a rather central pain inhibition.

In our study, it is possible that blockade of COX activity with topical administration of UD, QA, ME and MO significantly reduced the availability of prostaglandin in the tail skin, thus revealing a local role of prostaglandins—E2 receptors to modulate the response of vanilloid receptors (subtype VR1) through intracellular cascades in acute thermal nociception the same way that IBU does (Dogrul et al., 2007). The reason for the difference in the antinociceptive potencies may be related to the rank of potency of COX inhibition and the participation of peripherical opioid receptors. However, we cannot discard that these compounds have an activity at central level.

The highest potency of ME and MO against tail-flick and hot-plate respectively, could improve the pharmacokinetic parameters respect to QA.

On our laboratory it was proved that the anti-inflammatory activities of QA and ME (data not shown) and these results sustain our hypothesis that both compounds may be inhibitors of COX activity.

5. Conclusions

The results of the present study demonstrated that *Quillaja saponaria* saponins, quillaic acid, its methyl ester, and one of the oxidized derivatives of the latter, elicit dose-dependent antinociceptive effects in two murine thermal models. In the topical assay using the tail-flick test, the saponin mixture was more potent than the aglycone, its derivatives, and ibuprofen sodium. In the hot-plate test, after i.p. administration, an oxidized derivative of quillaic acid methyl ester was the most potent, but quillaic acid and the saponin mixture were still more potent than the reference drug.

These results support the traditional use of aqueous extracts saponins enriched of *Quillaja saponaria* bark as an analgesic, and are a stimulus for future investigation about the possible mechanisms of action of the compounds tested here and for future therapeutic formulations also.

Acknowledgments

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