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# Oleaginous extract from the fruits *Pterodon pubescens* Benth induces antinociception in animal models of acute and chronic pain

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ABSTRACT

*Ethnopharmacological relevance:* *Pterodon pubescens* Benth is a medicinal plant commonly used for therapeutic purposes in folk medicine for rheumatic diseases' treatment. In the present work we analyzed the chemical composition of the oleaginous extract of *P. pubescens* Benth (OEPp) and extended the antinociceptive effect of OEPp evaluating its role on animal models of acute and chronic pain.
*Materials and methods:* The antinociceptive and antiedematogenic effects of OEPp (3–100 mg/kg, i.g.) were evaluated in the formalin test; mechanical allodynia in the postoperative pain and complex regional pain syndrome type-I (CRPS-I) animal models; and thermal hyperalgesia was induced by plantar incision. Finally, we performed a phytochemical analysis of OEPp.
*Results:* The chemical composition of OEPp was analyzed by mass spectrometry (GC/MS) and eight sesquiterpene compounds were identified, i.e. three major sesquiterpene (E-cariofilene, $\gamma$-muurolene, biciclogermacrene), and nine vouacapane diterpenes, four of which showed in major concentration (6$\alpha$-acetoxyvouacapane, 6$\alpha$,7$\beta$-dimetoxivouacapan-17-ene, 6$\alpha$-acetoxy,7$\beta$-hidroxyvouacapane, 6$\alpha$,7$\beta$-diacetoxycouacapane). Furthermore, the results of the present study demonstrate, for the first time, that the OEPp reduced mechanical allodynia in the postoperative pain and CRPS-I animal models. OEPp also increased the paw withdrawal latency in hot- and cold-plate tests in the postoperative pain model. In addition, the present work confirms and extends previous data from literature showing that systemic administration of OEPp caused significant inhibition against both phases of pain response to formalin intraplantar injection and edema formation.
*Conclusions:* Together, present and previous findings show that OEPp given intra-gastrically caused significant inhibition against both phases of formalin intraplantar injection and effectively inhibited mechanical and thermal hyperalgesia in the postoperative pain and CRPS-I animal models.


## 1. Introduction

It is estimated that the economic burden of treating chronic pain that develops from acute pain in a 30 year-old individual, over a life time, could be as much as $1 million (Cousins et al., 2000). The prevention and effective relief of acute pain may improve clinical outcomes, save health care resources and improve quality of life. Surgical incision in postoperative patients leads to mechanical hyperalgesia, therefore, mechanical evoked pain after surgery is important for postoperative pain, and the underlying mechanisms must be studied to improve postoperative analgesia and the outcome of patients after surgery (Pogatzki and Raja, 2003).

Chronic post-ischemic pain (CPIP) is an animal model of complex regional pain syndrome type I (CRPS-I). Clinically, CRPS can begin after an injury, usually in the extremities. This trauma may be a distal fracture, nerve lesion, post-surgical injury and others. The syndrome is divided into two types: with major nerve injury (type II) or without (type I) (Feliu and Edwards, 2010). Studies in rats show that hind paw ischemia and reperfusion (IR) causes microvascular injury (Coderre and Bennett, 2010) and symptoms that are similar to CRPS-I in humans. These findings reinforce the evidence that CRPS-I may depend, in part, on tissue ischemia (Coderre et al., 2004) in patients with microvascular pathologies that lead to chronic tissue inflammation (Laferrière et al., 2008).

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Medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, especially to treat pain and inflammatory processes (Calixto et al., 2004). The medicinal plant *Pterodon pubescens* Benth is popularly known as "sucupira-branca" or "faveira" because its fruits form small "honeycombs" where its essential oil is stored. It is a native tree specie widely distributed throughout central Brazil (Lorenzi, 1998). This vegetal specie is used for therapeutic purposes, in folk medicine, for rheumatic diseases' treatment (Carvalho, 2004). It has been demonstrated that the hydroalcoholic extract of *P. pubescens* seeds (HEPp), administered orally, effectively inhibits edema and pain caused by collagen-induced arthritis (Sabino et al. 1999). Furthermore, Pinto Coelho et al. (2001) have demonstrated that the oral administration of HEPp reduced the arthritic index when compared to control group and concluded that HEPp presents antiarthritic activity, with no toxic effect in mice. Here, we examined: (i) the chemical composition of OEPp; (ii) the possible antihyperalgesic effect of OEPp in standard rodent models of acute and chronic pain, i.e., the intraplantar injection of formalin, postoperative pain-induced by plantar incision surgery (PIS), and chronic post-ischemic pain-induced by prolonged hind paw ischemia and reperfusion.

## 2. Material and Methods

### 2.1. Animals

All animal care and experimental procedures were carried out in accordance with the National Institutes of Health Animal Care Guidelines (NIH publications no. 80–23), and were approved by the Ethics Committee of the Universidade do Sul de Santa Catarina (protocol number 11.008.4.06.IV). Experiments were conducted using Swiss male mice (25–35 g), obtained from the Biotério Central da Universidade do Sul de Santa Catarina. Animals were housed at $22 \pm 2$ °C under a 12 hours light/12 hours dark cycle (lights on at 06:00) and with free access to food and water. Animals were acclimatized to the laboratory for at least 1 hour before testing and were used only once throughout the experiments. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments.

### 2.2. Extraction of vegetal material

The fruits of *P. pubescens* Benth were acquired in the medicinal garden of the Florianopolis' Health Pastoral, Santa Catarina, Brazil and identified by Dr. Rafael Trevisan (Department of Botany, Federal University of Santa Catarina, Florianópolis, Brazil). The dried fruits (with seed) of *P. pubescens* (100 g) were powdered and submitted to maceration with alcohol cereals (1 L) at room temperature. After 30 day, the extract was filtered and the solvent was evaporated under reduced pressure in a rotative evaporator to yield a oleaginous extract of *P. pubescens* (OEPp) (20,716 g) as previously described (Coelho et al., 2005).

### 2.3. Phytochemical analysis of the extract from P. pubescens

The oil was analyzed by chromatography-mass spectrometry (GC–MS). The analysis was performed in a GC–MS QP5050 Shimazdu (70 eV) apparatus, using DB-5 column (30 m × 0.25 mm × 0.25 μm) and helio carrier gas. The column temperature was programmed 60–280 °C (3 °C min) with isotherm 290 °C to 5/ min and flux of 1 mL/min. The data spectral were compared to the literature. The separation of major compounds was by flash chromatography column eluted with 20% hexane:acetate. And isolated compounds were subjected to spectrometry of $^{1}H$, $^{13}C$ NMR and GC/MS analyses.

### 2.4. Formalin test

Formalin-induced nociception was measured as previously described (Hunskaar et al., 1985; Martins et al., 2011). Animals were injected with 20 μL of a 2.5% formalin solution (0.92% formaldehyde in saline) intraplantarly (i.pl.) in the ventral surface of the right hindpaw. Animals were observed for 30 min; the time between 0 and 5 min represented the neurogenic phase and the time between 15 and 30 min represented the inflammatory phase. Time spent licking the injected paw was recorded and was indicative of nociception. To determine the effects of OEPp administration on nociception, animals were treated with OEPp (1–100 mg/kg, i.g.) 1 hour before formalin injection in the right hindpaw. Control animals were treated with vehicle (5% tween 80 in saline, 10 mL/kg, i.g.) 1 hour before formalin injection. After the formalin injection, animals were immediately placed in individual glass cylinders (20 cm diameter). Antinociception was expressed as a reduction of time that treated group spent licking the paw in relationship to the control group.

The antiedematogenic effect of EOPp was assessed by measuring the external diameter of the hind paw using a vernier caliper (Starrett: 125MEB-6/150) after the assessment of licking caused by formalin. Measurements were obtained from each group of mice, and the edema difference for each animal was generated by subtracting the diameter of the left hind paw (saline injection) from the right hind paw (formalin solution).

### 2.5. Plantar incision surgery

The plantar incision surgery (PIS) was performed as previously described (Pogatzki and Raja, 2003). Briefly, mice were anesthetized with 1%–2% isoflurane delivered via a nose cone. After sterile preparation of the right hind paw, a 5 mm longitudinal incision was made through skin and fascia of the plantar surface using a number 11 scalpel blade. The incision started 2 mm from the proximal edge of the heel and extended toward the toes. The underlying muscle was elevated with curved forceps, leaving the muscle origin and insertion intact. After wound homeostasis, the skin was apposed with a 6.0 mm nylon mattress suture, and the wound was covered with 10% povidone–iodine solution. Control animals were anesthetized but no incision was made. Animals were allowed to recover in their cages, and sutures were removed on the second postoperative day.

### 2.6. Chronic post-ischemic pain

Chronic post-ischemic pain (CPIP) is an animal model of CRPS-I developed using a 3 hours ischemia-reperfusion injury of the rodent right hind paw. CPIP induction was performed as described previously (Millecamps et al., 2010). CPIP mice were generated following exposure to prolonged hind paw ischemia and reperfusion (IR). Mice were anesthetized over a 3 hours period with a bolus (7%, 0.6 mL/kg, i.p.) of chloral hydrate and 20% of the initial volume at the end of the first and second hour. After induction of anesthesia, an elastic O-ring for braces (Elástico Ligadura 000–1237, Uniden) with 1.2 mm internal diameter was placed around the mouse's right hind limb just proximal to the ankle joint. The O-rings were selected to provide a tight-fit that produced ischemia. They were left on the limb for 3 hours as initially described with larger O-rings. The O-ring was always positioned at a point on the limb just proximal to the medial malleolus and its application was standardized by sliding it off the outside of a 100 μL pipette tip (that had 4 mm of the