Antinociceptive and Anti-inflammatory Effects of Triterpenes from Pluchea quitoc DC. Aerial Parts

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INTRODUCTION

Pluchea quitoc DC., a medicinal plant used in folk medicine, like some other plants belonging to the same genus,[1‑5] is an aromatic shrub of the Asteraceae family, popularly known in Brazil as “quitoco”, “madre-cravo”, “cuculacug”, or “tabacarana.” It is used in traditional medicine for the treatment of inflammation as well as of digestive and respiratory diseases in the North and Central West of Brazil.[6,7] The phytochemical analysis of the less polar fractions of the hexane extract of this species afforded stigmasterol, a mixture of β-amyrin, taraxasterol and pseudo-taraxasterol, and six eudesmane derivatives obtained from hexanic extracts of other plants belonging to the same genus, like some “quitoco, “ “caculucage, “ “tabacarana” and “madre-cravo,” is indicated for inflammatory conditions such as bronchitis, arthritis, and inflammation in the uterus and digestive system. Objective: This study evaluated the analgesic and anti-inflammatory activities of the triterpenes compounds obtained from P. quitoc aerial parts. Materials and Methods: The triterpenes compounds β-amyrin, taraxasterol and pseudo-taraxasterol in a mixture (T); β-amyrin, taraxasterol and pseudo-taraxasterol acetates in a mixture (Ta); β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with pseudo-taraxasterol myristates (Tafe) were analyzed in the models of nociception and inflammation. The evaluation of antinociceptive activity was carried out by the acetic acid-induced writhing and tail-flick tests while leukocyte migration to the peritoneal cavity was used for anti-inflammatory profile. Results: The oral administration of T or Tafe (40 mg/kg and 70 mg/kg) and Ta (70 mg/kg) to mice reduced acetic acid-induced writhing. The tail-flick response of mice was not affected by T or Tafe (40 mg/kg). T or Tafe (40 mg/kg) and Ta (70 mg/kg) also inhibited peritoneal leukocyte infiltration following the injection of carrageenan. Conclusion: The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol that were decreased when these were acetylated; while the acetylated triterpenes in mixture with myristoyl triterpenes improved this activity. These compounds seem, at least in part, to be related to the plant’s reported activity. Key words: Anti-inflammatory, antinociceptive, Pluchea quitoc, triterpene

SUMMARY

The mixtures of hydroxylated, acetylated, and myristate triterpenes isolated from hexanic extracts of Pluchea quitoc DC. were analyzed in the models of nociception and inflammation in mice. The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol. This study showed too that the activity of triterpenes may be decreased by their being acetylated, while the acetylated triterpenes in mixture with myristate triterpenes improved this activity.

ABSTRACT

Background: Pluchea quitoc DC. (Asteraceae), a medicinal plant known as “quitoco,” “cuculacug,” “tabacarana” and “madre-cravo,” is indicated for inflammatory conditions such as bronchitis, arthritis, and inflammation in the uterus and digestive system. Objective: This study evaluated the analgesic and anti-inflammatory activities of the triterpenes compounds obtained from P. quitoc aerial parts. Materials and Methods: The triterpenes compounds β-amyrin, taraxasterol and pseudo-taraxasterol in a mixture (T); β-amyrin, taraxasterol and pseudo-taraxasterol acetates in a mixture (Ta); β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with pseudo-taraxasterol myristates (Tafe) were analyzed in the models of nociception and inflammation. The evaluation of antinociceptive activity was carried out by the acetic acid induced writhing and tail-flick tests while leukocyte migration to the peritoneal cavity was used for anti-inflammatory profile. Results: The oral administration of T or Tafe (40 mg/kg and 70 mg/kg) and Ta (70 mg/kg) to mice reduced acetic acid induced writhing. The tail-flick response of mice was not affected by T or Tafe (40 mg/kg). T or Tafe (40 mg/kg) and Ta (70 mg/kg) also inhibited peritoneal leukocyte infiltration following the injection of carrageenan. Conclusion: The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol that were decreased when these were acetylated; while the acetylated triterpenes in mixture with myristoyl triterpenes improved this activity. These compounds seem, at least in part, to be related to the plant’s reported activity. Key words: Anti-inflammatory, antinociceptive, Pluchea quitoc, triterpene

SUMMARY

The mixtures of hydroxylated, acetylated, and myristate triterpenes isolated from hexanic extracts of Pluchea quitoc DC. were analyzed in the models of nociception and inflammation in mice. The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of the triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol. This study showed too that the activity of triterpenes may be decreased by their being acetylated, while the acetylated triterpenes in mixture with myristate triterpenes improved this activity.

Abbreviations Used: T: Triterpenes compounds β-amyrin, taraxasterol, and pseudo-taraxasterol in a mixture, Ta: Triterpenes compounds β-amyrin, taraxasterol and pseudo-taraxasterol acetates in a mixture, Tafe: Triterpenes compounds β-amyrin, taraxasterol, pseudo-taraxasterol acetates in a mixture with pseudo-taraxasterol myristates, Ctrl: Control, Indo: Indomethacin, Dexa: Dexamethasone, ETGAc: Ethyl acetate, MeOH: Methanol.

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DOI: 10.4103/pr.pr_51_17
production, as well as MMP-1, MMP-3, MMP-13, iNOS, and COX-2 expression in chondrocytes and exhibited anti-inflammatory effects in IL-1 β-stimulated chondrocytes through the inhibition of NF-kB activation.[13] The triterpenes a and β-amyrin produced antinociceptive effect on rat model of orofacial pain induced by formalin or capsaicin.[14]

We have previously shown that the ethanolic extract of P. quitoc had anti-inflammatory and antinociceptive effects in mice and rats in various analgesic and inflammatory models.[17]

In this work, we evaluated the anti-inflammatory and antinociceptive effects of mixtures of hydroxylated, acetylated and myristate triterpenes isolated from hexanic extracts of P. quitoc in mice, to determine whether these compounds might be responsible for the plant’s reported activity.

MATERIALS AND METHODS

Plant materials

P. quitoc was collected at Peixe-Boi, Pará State, Brazil and identified by the botanist Dr. João Ubiratan Santos, from Museu Paraense Emílio Goeldi (Belém, Pará, Brazil) where a voucher specimen has been deposited (No. 147609).

Isolation of the compounds

The aerial parts of P. quitoc (7 kg) were air dried and extracted with hexane at room temperature. Part of the crude hexane extract (20 g) was subjected to chromatography on a silica gel column and eluted with solvents of increasing polarity in the order hexane, hexane-ethyl acetate (EtOAc), EtOAc, and MeOH. The fraction containing the esterified triterpenes (2257 mg) was obtained from 1% to 3% EtOAc in hexane and was subjected to further chromatography separation on a silica gel column eluted with mixtures of EtOAc in hexane 0.5% and 1% yielding 977 mg of a mixture of β-amyrin acetate (16.26%), taraxasterol acetate (21.35%), pseudo-taraxasterol acetate (5.33%) in mixture with β-amyrin myristate (16.26%), taraxasterol myristate (27.18%), pseudo-taraxasterol myristate (13.59%) (Tafe) and 313 mg of a mixture of β-amyrin acetate (16.66%), taraxasterol acetate (66.66%), and pseudo-taraxasterol acetate (16.66%) (Ta, 1:4:1). Part of the fraction Tafe was submitted to hydrolysis yielding the hydroxylated triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol and myristic acid, which structures were confirmed by 1H nuclear magnetic resonance (NMR), 13C NMR, and gas chromatography-mass spectroscopy spectral analysis. The acetates of the triterpenes (Ta) were identified by comparison of their 13C NMR spectral data with those reported in the literature.[18,19] The hydroxylated triterpenoid fractions were obtained from 5% to 10% EtOAc in hexane, yielding 127 mg of a mixture of β-amyrin (40.0%), taraxasterol (40.0%), and pseudo-taraxasterol (20.0%) (T, 1:1:0.5).[9]

Animals

Swiss mice (25–30 g), obtained from the Animal House of Federal University of Maranhão, were used. Animals were maintained under environmental conditions and had free access to a standard diet and water ad libitum. All experimental protocols were developed in accordance with the principles of ethics and animal welfare designated by the Brazilian College of Animal Experiments (COBEA) and the ethical guidelines for the investigation of experimental pain in conscious animals.[20]

Antinociceptive activity

Acetic acid-induced writhing test

The writhing test described previously[21] was used with adaptations.[22,23] T or Tafe (40 mg/kg and 70 mg/kg) and Ta (70 mg/kg) in 4% tween 80 and water (vehicle), indomethacin (10 mg/kg) or vehicle, were administered p. o. in mice (n = 5–9), 60 min before acetic acid (0.8% v/v, 0.1 ml/10 g). The response to intraperitoneal injection of acid was cumulatively counted for 20 min.

Tail-flick test

Experiments were carried out according to previously described methodology,[24] with modifications. Male and female Swiss mice (n = 5) were placed on the tail-flick unit (Ugo Basile) so that the tail occluded a slit over a photocell. The source of heat was applied by a 70-W lamp mounted in a reflector and adjusted to 55°C ± 2°C. When the animal moved its tail away from the slit light fell on the photocell, and the timer was automatically stopped. The apparatus was previously calibrated to produce tail-flick latencies of approximately 3–5 s in control animals. The mice were treated with vehicle, T or Tafe (40 mg/kg, p. o.), or morphine (20 mg/kg, s. c.). All mice were observed in control conditions (60 and 30 min before) and 30, 60, 90, 120, and 150 min after drug administration.

Anti-inflammatory activity

Carrageenan-induced peritonitis test

The acute carrageenan-induced inflammatory reaction was induced by modification of the technique previously described.[25] Male and female Swiss mice (n = 5) were pretreated with T or Tafe (40 mg/kg), Ta (70 mg/kg), dexamethasone (0.5 mg/kg) or vehicle, p. o., 30 min before the injection of carrageenan (0.25 ml, 1% w/v in saline), into the peritoneal cavity. Four hours after the application of the irritant agent, the mice were sacrificed by cervical dislocation. Ca++ and Mg++ free heparinized (10 IU/ml) phosphate-buffered saline (2 ml) was injected into the peritoneal cavity and after a gentle massage, peritoneal fluids were removed; total leukocytes were determined in a Neubauer chamber.

Statistical analysis

The data are expressed as means ± standard error of the mean for 5–9 animals per group. The statistical analysis between treatment groups was done by one-way analysis of variance followed by Newman-Keuls test. Comparison between individual groups was analyzed with Student’s t-test. Results were considered statistically significant when P < 0.05.

RESULTS

The triterpenes caused dose-dependent inhibition of the acetic acid-induced writhing response in mice [Figure 1]. In control animals treated with vehicle, the total number of writhing movements determined over 20 min was 22.6 ± 2.4 (n = 8). Pretreatment with T or Tafe (40 mg/kg and 70 mg/kg) inhibited the number of writhing by 68.2% and 98.9% or 58.5% and 91.2%, respectively, when compared with the control (P < 0.05). In comparison, indomethacin (10 mg/kg) reduced writhing movements by 63.8%. However, Ta (70 mg/kg) returned an inhibition of only 30.8%. The tail-flick test demonstrated that the mice treated with T or Tafe (40 mg/kg, p. o.) did not present a longer latency than the control animals. In the same conditions, the tail-flick latency of mice treated with morphine (20 mg/kg, s. c.) was increased 92.3% after 30 min. In the experiment involving carrageenan-induced inflammation in the peritoneal cavity, the leukocyte migration in control mice treated with the vehicle was 4.11 ± 0.23 × 10⁷/mm² ± 0.23 [Figure 2]. Previous treatment p. o. with T or Tafe (40 mg/kg) or Ta (70 mg/kg) reduced the migration by 56.5%, 44.6%, and 43.1%, respectively (P < 0.05). The animals treated with dexamethasone, a steroidal agent used as a positive control, exhibited an inhibition by 63.3%.
DISCUSSION

This study evaluated the antinociceptive and anti-inflammatory effects of a mixture of triterpenes from *P. quitoc* using different stimuli, including chemical agents (acetic acid, carrageenan) and heat (tail flick). The triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol in mixture, administered p. o. showed a significant and dose-dependent activity against the acetic acid-induced writhing response in mice and exhibited higher activity than the acetylated triterpenes (β-amyrin, taraxasterol, and pseudo-taraxasterol acetates); at the same dose (70 mg/kg), the effect of the former was 63 times higher. Nevertheless, the mixture of acetylated triterpenes with β-amyrin, taraxasterol and pseudo-taraxasterol myristates presented an effect comparable with that of the triterpenes. The hydroxylated triterpenes and acetylated triterpene with myristate presented an effect comparable with that of the triterpenes. The results demonstrate the anti-inflammatory and peripheral antinociceptive activity of triterpenes β-amyrin, taraxasterol, and pseudo-taraxasterol acetates and β-amyrin, taraxasterol and pseudo-taraxasterol myristates in mixture. This study showed too that the activity of triterpenes may be decreased by their being acetylated, while acetoxyled triterpenes in mixture with myristate triterpenes improved this activity.

CONCLUSION

The results demonstrate the anti-inflammatory and peripheral antinociceptive activities of the mixture of the triterpenes β-amirina, taraxasterol and pseudo-taraxasterol, that these activities were diminished in the mixture of triterpenes acetate, whereas these in mixture with triterpenos miristato improved these activities. These compounds appear, at least in part, to relate to the reported activity of the plant.

Financial support and sponsorship

The authors thank the Brazilian funding agencies CAPES, FINEP, CNPq, and FAPEMA for financial support.

Conflicts of interest

There are no conflicts of interest.
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