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Anti-inflammatory activity of ethanol extract and fractions from *Couroupita guianensis* Aublet leaves

Mariana M.G. Pinheiro [a], Sidnei B.O. Fernandes [b], Catarina E. Fingolo [b], Fábio Boylan [c,*], Patrícia D. Fernandes [a]

[a] Universidade Federal do Rio de Janeiro, Instituto de Ciências Biomédicas, Laboratório de Farmacologia da Inflamação e do Óxido Nítrico, Brazil
[b] Universidade Federal do Rio de Janeiro, Núcleo de Pesquisas de Produtos Naturais, Brazil
[c] School of Pharmacy and Pharmaceutical Sciences & Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland

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ABSTRACT

Ethnopharmacological relevance: *Couroupita guianensis* Aublet, 'macacarecuia', 'abricó-de-macaco', 'castanha-de-macaco' and 'amêndoa-dos-andes', is found in tropical regions and is widely used in the treatment of tumors, pain, and inflammatory processes.
Aim of the study: Ethanol extract and hexane and ethyl acetate fractions were evaluated in models of inflammatory pain (formalin-induced licking) and acute inflammation (carrageenan-induced peritonitis).
Materials and methods: Ethanol extract, hexane and ethyl acetate fractions (10, 30 or 100 mg/kg, p.o.) and the reference drugs dexamethasone (5 mg/kg), morphine (5 mg/kg, s.c.), and acetylsalicylic acid (100 mg/kg, p.o.) were tested in formalin-induced licking response and carrageenan-induced peritonitis.
Results: All three doses from *Couroupita guianensis* fractions significantly reduced the time that the animal spent licking the formalin-injected paw in first and second phases. However, only higher doses (30 and 100 mg/kg) were able to inhibit the leukocyte migration into the peritoneal cavity after carrageenan injection. In this model, the 100 mg/kg dose almost abolished the cell migration. It was also observed that protein concentration resulted from extravasation to the peritoneum and nitric oxide (NO) productions were significantly reduced. Cytokines production was differently affected by the treatment. TNF-α production was reduced after ethanol extract and ethyl acetate fraction pre-treatment whereas hexane fraction had effect only with 100 mg/kg dose. IL-1β production was inhibited only after hexane fraction pre-treatment.
The inhibitory effect observed was not due to a direct cytotoxic effect on cells nor to a NO-scavenger activity. The effect was due to a direct inhibition on NO production by the cells.
Conclusions: The results show that *Couroupita guianensis* fractions have anti-inflammatory effect, partly due to a reduction on cell migration and a inhibition on cytokines and inflammatory mediators production.

1. Introduction

There is a consensus that the inflammatory process in a beneficial response of the host to challenges or cellular injury that may result in the release of inflammatory mediators with the final objective of restoration of tissue structures and function. However, sometimes the perpetuation of the inflammation can be harmful and contributes to the pathogenesis of several diseases. Many cells involved in this process are potent secretory cells that release a diversity of mediators, including pro-inflammatory and cytotoxic cytokines, prostaglandins, reactive oxygen intermediates, and nitric oxide (NO), all of which have been implicated in the pathogenesis of tissue injury (Laskin and Pendino, 1995). The non-steroidal anti-inflammatory drugs (NSAID) are widely used to treat several inflammatory conditions, however the probability to cause many and severe adverse effects limit their use. In this regard, the traditional medicine continues to use medicinal plants as a substituent to allopathic medicines.

The Lecythidaceae family is composed of some 325 tropical trees divided among 15 genera (Pettit et al., 2004). *Couroupita guianensis* Aublet is one of the species of this botanic family largely found in tropical regions of South America (Lorenzi, 2000). It is popularly known in Brazil as 'macacarecuia', 'abricó-de-macaco', 'castanha-de-macaco' and 'amêndoa-dos-andes' (Lorenzi, 2000). Native people from Amazonian region and other states of North of Brazil use infusions or teas obtained from the leaves and flowers to treat hypertension, tumors, pain, and inflammatory processes (Revilla, 2002).

* Corresponding author. Tel.: +351 8964154.
E-mail address: fabio.boylan@tcd.ie (F. Boylan).

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Phytochemical studies revealed the presence of triterpenoid glucoside, saponins (Massiot et al., 1992), triterpenoid saponins (Das and Mahato, 1982), flavonol glycosides and indol constituents (Crublet et al., 2003). Some studies with this species had evidenced the presence of α-amirin, β-amirin, β-sitosterol, tannins (Row et al., 1966) and cetoesteroids (Anjaneyulu and Rao, 1998). In the leaves, triterpenoid esters of fatty acids such as palmitate β-amiryn were characterized. In the flowers, eugenol, linalool and (*E*,*E*)-farnesolwere identified (Eknat and Shivchandraji, 2002).

Recently, we demonstrated that ethanol extract of *Couroupita guianensis* Aubl. leaves as well as its fractions have antinociceptive action in the acetic acid-induced writhing, tail flick, and hot plate test mediated, in part, by opioid and cholinergic systems and nitric oxide pathway (Pinheiro et al., 2010).

The aim of this study was to investigate the anti-inflammatory activities of fractions obtained from *Couroupita guianensis* leaves using different models of inflammatory pain (i.e., formalin-induced licking) and acute inflammation (i.e., carrageenan-induced peritonitis).

2. Materials and methods

2.1. Plant material

Couroupita guianensis Aublet leaves (1200 g) were collected in the campus of the Federal University of Rio de Janeiro (Rio de Janeiro, Brazil), in December, 2004. The plant was identified by Dr. Rosana C. Lopes (Biology Institute, UFRJ) and a voucher specimen of this material was deposited at Herbarium of the Department of Botany, Federal University of Rio de Janeiro (number 13,150).

2.2. Preparation of fractions

The leaves of *Couroupita guianensis* Aublet were dried under airflow at 37 °C and reduced to a fine powder and extracted by static macerations with ethanol at room temperature. After filtration, the crude ethanol extract was concentrated in a rotary evaporator yielding a total of 59 g of dry extract. The dry ethanol extract was suspended in ethanol/water (1:4) and partitioned in hexane (H) and ethyl acetate (EA). All fractions were evaporated to dryness under reduced pressure yielding 7.7 and 12.0 g, respectively. This procedure is in accordance to Renno et al. (2008).

2.3. Animals

All experiments were performed with male Swiss mice (18–25 g) obtained from our own animal facilities. Animals were kept in a room with controlled temperature $22 \pm 2^{\circ}$ for 12 h light/dark cycle with free access to food and water. Twelve hours before each experiment animals received only water, in order to avoid food interference with substances absorption. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), approved by the Biomedical Science Institute/UFRJ, Ethical Committee for Animal Research, and received the number DFBCICB-015.

2.4. Drugs and Couroupita guianensis Aublet fractions administration

Dexamethasone was obtained from Aché (São Paulo, Brazil), acetylsalicylic acid, and carrageenan (type IV), were obtained from Sigma Chemical (St. Louis, MO, USA), morphine was obtained from Cristália (São Paulo, Brazil).

Couroupita guianensis fractions were dissolved in dimetylsulfoxide (DMSO) in order to prepare a stock solution at a concentration of 100 mg/ml. In all experiments, the final concentration of DMSO did not exceed 0.5% at which had no effect per se. The ethanol extract and hexane or ethyl acetate fractions were administered at concentrations of 10, 30 or 100 mg/kg in a final volume 0.1 ml. The control group was composed by vehicle (phosphate buffer saline [PBS] with the same amount of DMSO used in the highest dose). Positive control groups were composed by dexamethasone (5 mg/kg), acetylsalicylic acid (100 mg/kg), and morphine (5 mg/kg, s.c.). All drugs and fractions were dissolved in PBS just before use. The vehicle, ethanol extract, fractions, and acetylsalicylic acid were administered by oral gavage. Morphine was s.c. administered and dexamethasone was i.p. administered.

2.5. Formalin test

The procedure used was similar to the method described by Tsølsen et al. (1992) and with some adaptations done by Gomes et al. (2007). Briefly, mice received an intraplantar injection of 20 μl formalin (2.5%, v/v) into dorsal surface of the right hind paw. Immediately, the time (in seconds) that the animal spent licking the formalin-injected paw was recorded from 0 to 5 min (first phase or neurogenic phase) and 15–30 min (second phase or inflammatory phase) after formalin injection. Animals were pre-treated with vehicle (0.1 ml, p.o.), ethanol extract or fractions (10, 30 or 100 mg/kg, p.o.), morphine (5 mg/kg, s.c.) or acetylsalicylic (ASA, 100 mg/kg, p.o.) 60 min before intraplantar injection of formalin. The result was expressed as the time that the animal spent licking the formalin-injected paw.

2.6. Carrageenan-induced peritonitis

Carrageenan-induced peritonitis experiments were performed according to Da Silva Guerra et al. (2011) and Guimarães et al. (2012). The animals received oral treatment with ethanol extract or fractions of *Couroupita guianensis* (10, 30 or 100 mg/kg). After 60 min, inflammation was induced by intraperitoneal injection of 250 μl of carrageenan suspension (1% in sterile PBS). The positive control group was composed by mice i.p. pre-treated with dexamethasone (5 mg/kg) 60 min before carrageenan injection. The control group received p.o. the same volume of vehicle (PBS). After 24 h the animals were euthanized with hydrate chloral (1%, i.p.) and the peritoneal cavity washed with 1 ml of PBS. Exudates were collected, centrifuged at 11,000 rpm for 10 min at 4 °C and aliquots were stored at −20 °C until the dosages.

The total and differential cells counts were determined in the exudates using a pocH-100iV Diff (Sysmex) hematology analyzer. The protein levels were determined in the supernatant by the BCA method accordingly to the manufactures protocol (BCA™ Protein Assay Kit, Pierce).

2.7. Quantification of nitric oxide concentration

To determinate nitric oxide (NO) production, nitrate concentration in the exsudate was measured using the nitrate conversion protocol (Bartholomew, 1984) adapted by Raymundo et al. (2011) followed by the Griess reaction (Green et al., 1982). The absorbance was measured at 540 nm and the nitrite concentration was calculated using a standard curve of sodium nitrite. Results were expressed as μM of nitric oxide (NO).