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Journal of Ethnopharmacology 146 (2013) 324–330

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

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

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form
7 December 2012
Accepted 27 December 2012
Available online 11 January 2013

Keywords:
Couroupita guianensis
Lecythidaceae
Nitric oxide
Cytokine
Anti-inflammatory activity

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.

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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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http://dx.doi.org/10.1016/j.jep.2012.12.053

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 pretreated 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).

2.8. Quantification of cytokines (IL-1β and TNF-α)

The cytokines levels in the exudates were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (B&D, USA). The results are expressed as pg/ml of each cytokine.

2.9. Cell culture

RAW 264.7 mouse monocyte-macrophages (ATCC TIB-71) were grown in plastic bottles in a RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM) and HEPES (15 mM) (from now named RPMI) in a humidified atmosphere containing 5% CO_2 and 95% air at 37 °C. When cultures formed a confluent monolayer cells were scrapped, centrifuged and put to adhere in 96 or 12 wells plate with RPMI at a density of 2×10^6 cell/ml (Raschke et al., 1978).

2.10. Cytotoxicity assay by the MTT method

The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to measure cell respiration as an indicator of cell viability (Denizot and Lang, 1986). Briefly, after 24 h incubation of RAW 264.7 adherent cells with or without ethanol extract, hexane or ethyl acetate fractions (1–300 μg/ml), supernatants were changed by 100 μl of RPMI medium containing 0.5 mg/ml MTT and cells incubated for 1 h at 37 °C in a 5% CO_2 atmosphere. After the medium was aspirated, 100 μl of DMSO was added to the cells to dissolve the formazan. The absorbance from each group was measured in a FlexStation microplate reader at 570 nm. The control groups consisted of cells with medium plus vehicle used to dissolve the fractions and was considered as 100% of viable cells. Results are expressed as percentage of viable cells when compared with control groups.

2.11. Nitric oxide-trapping capacity of Couroupita guianensis

To test the capacity of ethanol extract, hexane or ethyl acetate fractions in trapping nitric oxide (NO), we used a cell-free system as described by Matheus et al. (2006). SNAP (S-nitroso-N-acetyl DL-penicillamine) was used, as, when in solution, it liberates to the medium NO that transforms to nitrite (Field et al., 1978). The addition of a NO scavenger to the SNAP solution results in a decay in the supernatant nitrite accumulation. Using this protocol, each fraction (at 300 μg/ml) was incubated with 1 mM of SNAP. After 6 h of incubation, an aliquot of supernatant was removed to quantify the nitrite accumulated by Griess reaction (Green et al., 1982). Results are expressed as μM of nitrite calculated in comparison with the sodium nitrite standard curve.

2.12. Statistical analysis

All experimental groups for *in vivo* protocol were composed by 6–10 mice. For *in vitro* assays each group was done in triplicate. Each *in vitro* protocol was repeated at least 4 times. The results are presented as mean ± S.D. Statistical significance between groups was performed by the application of analyses of variance (ANOVA) followed by Bonferroni's test. *P* values less than 0.05 ($p<0.05$) were used as the significant level.

3. Results

3.1. Effect of ethanol extract and fractions of Couroupita guianensis Aubl. in formalin test

Previous results from our group demonstrated that ethanol extract and some fractions from *Couroupita guianensis* leaves significantly inhibited the acetic acid-induced contortions (Pinheiro et al., 2010). Taking this information into account, we decided to evaluate them in models of inflammation. In this regard, the first model used was the formalin-induced licking response. The intraplantar injection of formalin resulted in two-phase nociceptive behavior in mice. In the first phase (neurogenic phase), the response was immediate and lasted up to 5 min following formalin injection resulting in the licking time of 74.1 ± 97 s whereas the second phase (inflammatory phase) lasted 15–30 min and resulted in the licking time of 172.6 ± 46.0 s in control group (vehicle). The oral administration of ethanol extract or fractions (at dose of 10, 30 or 100 mg/kg) before formalin stimulus significantly decreased the licking behavior in mice in the neurogenic phase (1st phase). Ethanol extract inhibited in 23.5%, 53.8%, and 51.5% the licking time, while hexane fraction reduced in 26%, 40.8%, and 42% and ethyl acetate reduced in 22.3%, 60.9%, and 66%, at the doses of 10, 30, and 100 mg/kg, respectively (Fig. 1A). The positive control groups, acetylsalicylic acid (ASA) and morphine (at their ED_{50} dose) reduced the licking time of the 1st phase in 40.6% and 64.9%, respectively.

Fig. 1B shows the effect of *Couroupita guianensis* fractions in the 2nd phase of the formalin model. Ethanol extract only reduced the time that the animal spent licking the formalin-injected paw after pre-treatment with 100 mg/kg dose. Differently, the fractions reduced the time of licking in a dose dependent manner. Hexane reduced in 31.3%, 54.7%, and 61.3% and ethyl acetate reduced in 36.3%, 53.6%, and 74.9%, at the doses of 10, 30, and 100 mg/kg, respectively. In this phase the positive control groups ASA and morphine, reduced in 42.1% and 57.1% the response to formalin injection.

3.2. Effects of ethanol extract and fractions from Couroupita guianenis Aubl. on cell migration

The pre-treatment of mice with 30 and 100 mg/kg of ethanol extract significantly reduce the number of leukocytes that migrate to the peritoneal cavity after carrageenan injection. Otherwise, only the higher dose (100 mg/kg) of fractions were able to reduce the number of leukocytes. In the positive control group, composed by animals pre-treated with the anti-inflammatory drug dexamethasone (0.5 mg/kg), the reduction on cell migration was 63.6% ($14\pm3.5\times10^6$ cells/ml in control group versus $5.1\pm3\times10^6$ cells/ml in dexamethasone-treated group). It is interesting to note that at the higher dose, the ethanol extract and both fractions reduced by almost 100% the cell migration (97.2%, 92.1%, and 90% reduction with ethanol extract, hexane, and ethyl acetate, respectively) (Fig. 2).

3.3. Effects of ethanol extract and fractions of Couroupita guianenis Aubl. on protein extravased and nitric oxide production

Fig. 3 shows that all three doses of ethanol extract equally reduced the amount of protein extravased to the peritoneal cavity. A dose response curve between all pre-treated groups was not observed. Differently, when the animals were pre-treated with hexane or ethyl acetate fractions a typical dose response curve was observed. The lowest dose (10 mg/kg) did not influence the amount of protein extravased while the other two doses

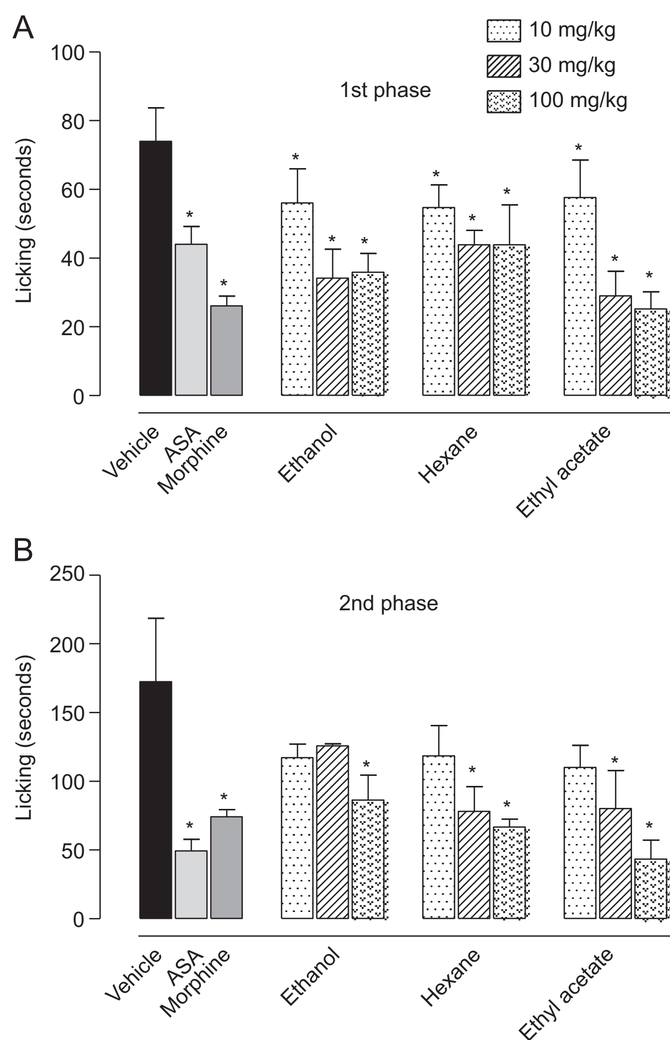

Fig. 1. Effects of *Coroupita guianensis* ethanol extract, hexane and ethyl acetate fractions on formalin-induced licking response in mice. Animals were pre-treated with different doses (10, 30 or 100 mg/kg, p.o.) of ethanol extract, hexane or ethyl acetate fractions, acetylsalicylic acid (ASA, 100 mg/kg, p.o.), morphine (5 mg/kg, s.c.) or vehicle. The results are presented as mean ± S.D. (n=6–10) of the time that the animal spent licking the formalin-injected paw. Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^{*}P<0.05$ when compared to vehicle-treated mice.

(30 and 100 mg/kg) significantly reduced the concentration of protein accumulated in the peritoneal cavity. As expected, in the positive control group composed by mice pre-treated with dexamethasone there were a reduction on protein levels of almost 50% (Fig. 3A).

Differently to the previous results, ethanol extract showed an increasing inhibitory effect on NO production its dose was increased. Hexane fraction also reduced the NO production despite the absence of a direct relation between the increments of its dose. Ethyl acetate fraction also reduced the levels of NO measured in the peritoneal cavity in dose dependent manner and dexamethasone reduced in more than 50% the nitrite accumulated (Fig. 3B).

3.4. *Effect of Couroupita guianenis Aublet on cytokine levels produced in peritoneal cavity*

Fig. 4A shows that the levels of TNF-α were reduced by pre-treatment of mice with crescent doses of ethanol extract or ethyl acetate, whereas hexane fraction did not reduce this cytokine

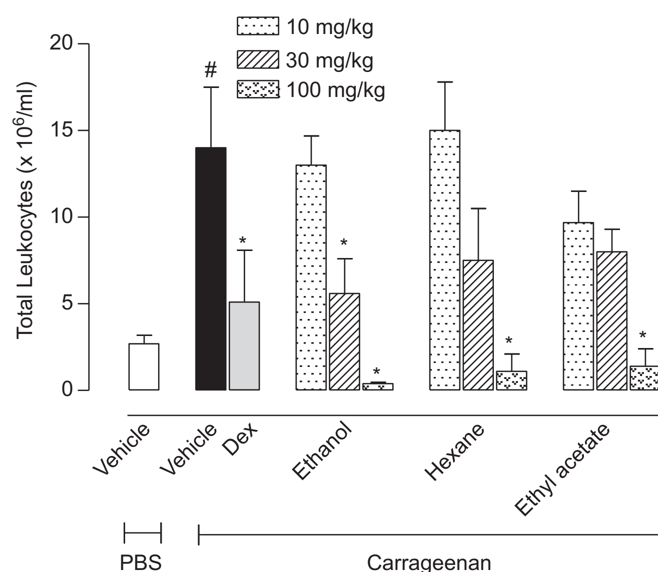

Fig. 2. Effects of *Coroupita guianensis* ethanol extract, hexane and ethyl acetate fractions on carrageenan-induced cell migration into the subcutaneous air pouch (SAP). Animals were pre-treated with different doses (10, 30 or 100 mg/kg, p.o.) of ethanol extract, hexane or ethyl acetate fractions, dexamethasone (DEX, 5 mg/kg, i.p.), or vehicle 1 h before carrageenan (1%, 1 ml) or PBS injection in the SAP. The results are presented as mean ± S.D. (n=6–10) of the number of leukocytes ($x\times10^{6}$ cells/ml). Statistical significance was calculated by ANOVA followed by Bonferroni's test. # $P<0.05$ when comparing carrageenan-injected group with PBS-injected group and $^{*}P<0.05$ when comparing ethanol extract, hexane or ethyl acetate fractions, dexamethasone-treated groups with vehicle-treated group.

level. On the other hand, IL-1β production was only reduced by hexane fraction pre-treatment. Ethanol extract and ethyl acetate fraction did not alter the levels of this cytokine (Fig. 4B).

3.5. *Effect of Couroupita guianensis Aublet in cell culture*

In order to investigate if the inhibitory effect of *Couroupita guianensis* on NO production observed in the peritoneal cavity could be due to NO sequestration, a "cell-free" system was used with s-nitroso n-acetyl DL-penicillamine (SNAP) as a NO donor in the presence or absence of ethanol extract or fractions. Incubation of crescent doses of *Couroupita guianensis* fractions with 1 mM SNAP did not resulted in a significant reduction on the levels of NO produced by SNAP when comparing with the NO donor alone (Table 1).

To investigate whether the inhibition observed in NO levels *in vivo* would also be seen *in vitro*, RAW 264.7 cells were activated with LPS in the presence or absence of ethanol extract or fractions and the levels of nitrite accumulated in the medium was measured after 24 h. Table 2 shows that after treatment with LPS the cells produced a large amount of NO, quantified as it stable metabolite (nitrite). Ethanol extract and its fractions significantly reduced the levels of NO accumulated in cells supernatant in a dose dependent manner (Table 2). To exclude the possibility of any cytotoxic effect responsible for this reduction, cell viability was measured by MTT assay. Neither ethanol extract from *Couroupita guianensis* nor fractions (0–300 μg/ml) decreased cell viability after 24 h incubation (data not shown).

4. Discussion

Studies conducted in our laboratory have shown that leaves from *Couroupita guianensis* develop antinociceptive effect in several

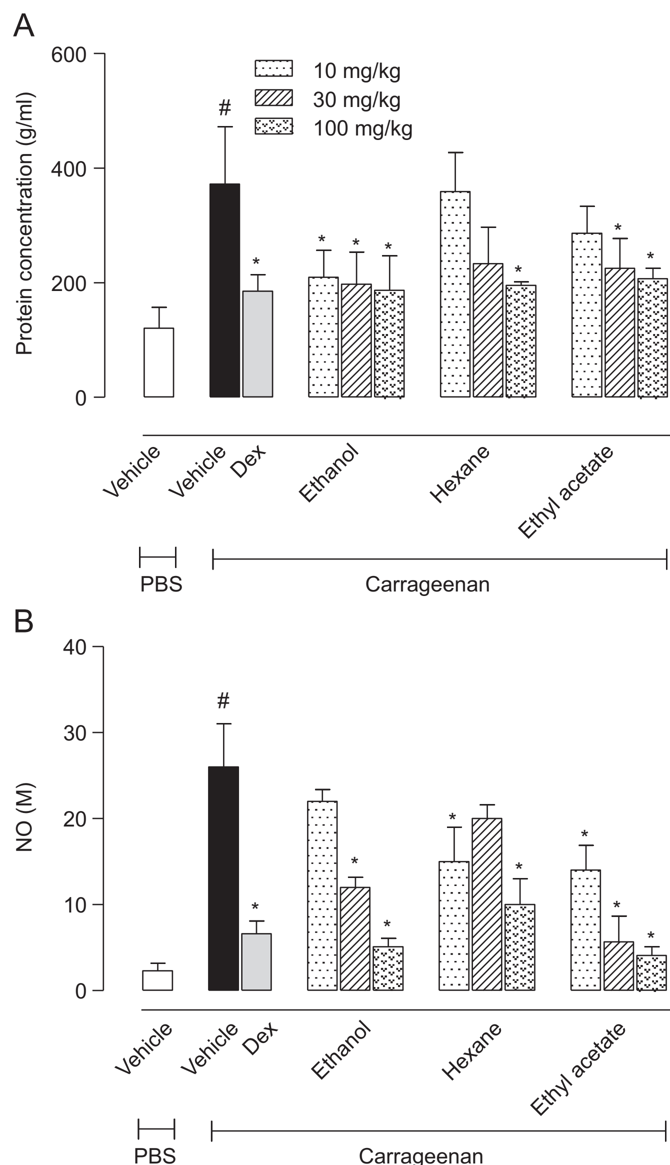

Fig. 3. Effects of *Coroupita guianensis* ethanol extract, hexane and ethyl acetate fractions on protein extravasation (A) or nitric oxide (NO) production (B) in the subcutaneous air pouch (SAP). Animals were pre-treated with different doses (10, 30 or 100 mg/kg, p.o.) of ethanol extract, hexane or ethyl acetate fractions, dexamethasone (DEX, 5 mg/kg, i.p.), or vehicle 1 h before carrageenan (1%, 1 ml) or PBS injection in the SAP. The results are presented as mean ± S.D. (n=6–10) of protein (in μg/ml) or NO (in μM) accumulated the SAP. Statistical significance was calculated by ANOVA followed by Bonferroni's test. # $P<0.05$ when comparing carrageenan-injected group with PBS-injected group and $^{*}P<0.05$ when comparing ethanol extract, hexane or ethyl acetate fractions, dexamethasone-treated groups with vehicle-treated group.

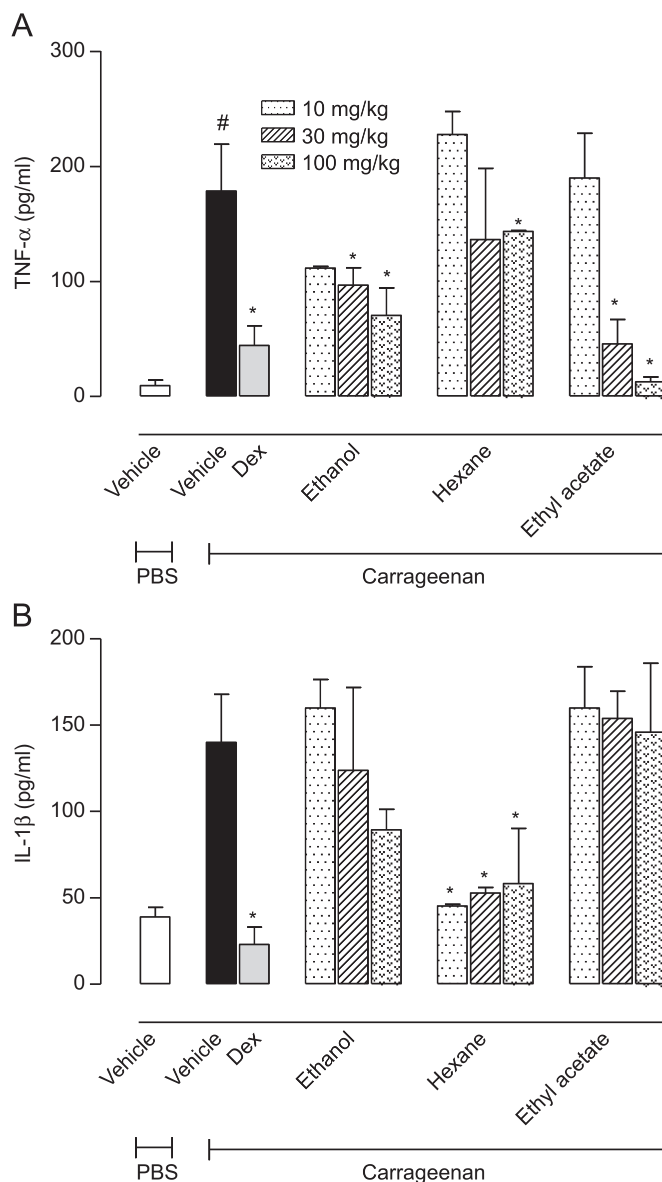

Fig. 4. Effects of *Coroupita guianensis* ethanol extract, hexane and ethyl acetate fractions on protein extravasation (A) or nitric oxide (NO) production (B) in the subcutaneous air pouch (SAP). Animals were pre-treated with different doses (10, 30 or 100 mg/kg, p.o.) of ethanol extract, hexane or ethyl acetate fractions, dexamethasone (DEX, 5 mg/kg, i.p.), or vehicle 1 h before carrageenan (1%, 1 ml) or PBS injection in the SAP. The results are presented as mean ± S.D. (n=6–10) of protein (in μg/ml) or NO (in μM) accumulated the SAP. Statistical significance was calculated by ANOVA followed by Bonferroni's test. # $P<0.05$ when comparing carrageenan-injected group with PBS-injected group and $^{*}P<0.05$ when comparing ethanol extract, hexane or ethyl acetate fractions, dexamethasone-treated groups with vehicle-treated group.

models, such as acetic acid-induced contortions, tail flick, and hot plate (Pinheiro et al., 2010).

The formalin injection in the paw evokes a biphasic response and is commonly employed as a model of acute and tonic peripheral pains to test for analgesic and anti-inflammatory drugs. The first phase occurs in response to direct stimulation of nociceptors by bradykinin, serotonin, and histamine liberated locally (Chapman and Dickenson, 1992; De Campos et al., 1996; Parada et al., 2001). The second phase is due to inflammatory pain mediated by a combination of peripheral input and spinal cord sensitization and that can be inhibited by nonsteroidal anti-inflammatory drugs (Hunskaar and Hole, 1987; Tsølsen et al., 1992). Our results demonstrated that the fractions from *Couroupita guianensis* significantly reduced both phases. The reductions on the first phase is in accordance with our previous study where we demonstrated that the same fractions developed a central antinociceptive effect and also reduced the acetic acid-induced contortions (Pinheiro et al., 2010). The inhibitory effect observed in the second phase suggests a possible inhibition of inflammatory mediators released in the mouse paw and also corroborates with the inhibitory effect of the fractions on the acetic acid-induced writhing response.

The carrageenan-induced acute peritonitis is a well-known and commonly used model in the investigation of acute inflammatory processes leading to the evaluation of leukocyte migration levels by counting the total number of cells that reached the

Table 1
NO scavenger activity of ethanol extract, ethyl acetate or hexane fractions from *Coroupita guianensis* leaves.

Group[a]	Dose (μg/ml)	NO (μM)
SNAP (1 mM)	–	138.3 ± 21.9
Ethanol extract	30	144.7 ± 18.4
	100	131.6 ± 19.9
	300	141.1 ± 10.8
Hexane fraction	30	130.5 ± 19.9
	100	133.8 ± 20.6
	300	141.0 ± 15.8
Ethyl acetate fraction	30	135.5 ± 13.8
	100	133.7 ± 15.2
	300	142.9 ± 8.9

[a] Each group was done in triplicate and each protocol was repeated at least 4 times. The results are presented as mean ± S.D. Statistical significance between groups was performed by the application of analyses of variance (ANOVA) followed by Bonferroni's test. *P*-values less than 0.05 ($p < 0.05$) were used as the significant level when comparing with LPS-treated group.

Table 2
Effect of ethanol extract, ethyl acetate or hexane fractions from *Coroupita guianensis* leaves on NO production by RAW 264.7 cells.

Group[a]	Dose (μg/ml)	NO (μM)
Vehicle	–	13.8 ± 2.9
LPS (1 μg/ml)	–	79.9 ± 6.3
Ethanol extract	30	68.7 ± 7.7[a]
	100	59.1 ± 6.8[a]
	300	48.7 ± 9.7[a]
Hexane fraction	30	77.6 ± 10.4
	100	62.2 ± 8.3[a]
	300	60.3 ± 7.7
Ethyl acetate fraction	30	66.4 ± 9.8[a]
	100	61.7 ± 5.8[a]
	300	56.3 ± 9.9[a]

[a] Each group was done in triplicate and each protocol was repeated at least 4 times. The results are presented as mean ± S.D. Statistical significance between groups was performed by the application of analyses of variance (ANOVA) followed by Bonferroni's test. *P*-values less than 0.05 ($*p < 0.05$) were used as the significant level when comparing with LPS-treated group.

cavity during the process of acute inflammation (Scognamillo-Szabó et al., 2004). The mechanism of action by which carrageenan induces the inflammatory processes is a synergism between several inflammatory mediators, such as bradykinin, serotonin, histamine, prostaglandins, leukotriene B4, and other chemotactic agents (Foster et al., 1986). Our results suggest that the actions of the *Couroupita guianensis* extract and fractions may be related, at least in part, to the inhibition of some of the several mediators liberated in the peritoneal cavity. Complementing the data observed in the acetic acid-induced contortions (Pinheiro et al., 2010) and in the first phase of formalin model, we could suggest that some inhibitory effects observed in this model could also be due to an effect on histamine, bradykinin or serotonin systems.

The inflammatory processes evoked by carrageenan also involves an increase in vascular permeability, with the consequent increase in protein leakage and the accumulation of nitric oxide (NO) and cytokines in the exudate (Salvemini et al., 1996; Loram et al., 2007). In our study *Couroupita guianensis* fractions significantly reduced both protein leakage and NO accumulated in the cavity, complementing the anti-inflammatory results obtained in other models.

Several inflammatory cytokines, particularly TNF- α, IL-1 ß are known to play key roles in the induction and perpetuation of inflammation. In this regard we assess the effect of the fractions under study in the concentrations of those cytokines accumulated in the inflammatory exudates of the peritoneal cavity after carrageenan injection. Our results showed that ethanol extract and ethyl acetate fraction decreased the concentrations of TNF-α, whereas hexane fraction reduced the levels of IL-1β in the inflammatory exudates. The reduction in IL-1ß and TNF-α level could also be responsible for inhibition of leukocyte migration. These results are suggestive that the *Couroupita guianensis* fractions may be acting as modulators of the immune system by decreasing cell migration, exudation, and the production of pro-inflammatory cytokines.

Antioxidant substances with important activity have also been described in other plants (Dreikorn, 2002; Mahady, 2002; Banerjee et al., 2003). In view of this we tested the possibility of our fractions in reduce NO levels through NO scavenger activity *in vivo* or *in vitro*. Our study demonstrated that none of fractions tested develop NO scavenger activity, suggesting that the inhibitory effects observed both *in vivo* and *in vitro* were not related with NO scavenger effect but probably due to a direct effect on NO production by peritoneal cells. One of the problems in using plant extracts and fractions is the possible cytotoxicity resulting from the residues of the solvents used in the preparation or from other toxic substances present in the fractions. For this reason, we decided to test ethanol extract and fractions in a cell viability assay (MTT assay). None of them reduced cell viability suggesting that, at the concentrations used, there was no toxic effect.

The mechanism of the anti-inflammatory activities from *Couroupita guianensis* may be explained by the presence of α-amirin, β-amirin, β-sitosterol, tannins (Row et al., 1966), and ketosteroids (Anjaneyulu and Rao, 1998). The anti-inflammatory effect observed in this work and the antinociceptive activities previously demonstrated (Pinheiro et al., 2010) for this species can be assigned by the existing set of components acting simultaneously on the plant and amplifying the individual effect therein in a complimentary manner.

It was also observed that the fractions, which were expected to contain a concentrated amount of possible active compounds, were not significantly more active than the ethanol extract. One possible explanation could be the fact that the ethanol extract contains more chemical constituents belonging to different classes of compounds. These substances could act in a synergic or antagonic manner. The hexane fraction is composed mainly by terpenes, steroids and lipids and the ethyl acetate fraction in composed mainly by flavonoids, coumarines and alkaloids. Although it is well known that these substances can present biological effect when isolated, it could be that the amount of each one present in the fraction is not enough to develop a significant effect or it may be that these substances when combined in the crude ethanol extract act synergistically amplifying the final effect.

Although the active doses of the plant extract were higher than those of the reference drug, it should be noted that an extract has different chemical compounds, it is composed by several substances not being a pure one like the standards.

In conclusion, this work demonstrates that fractions from the leaves of *Couroupita guianensis* exhibit anti-inflammatory activity even when used by the oral route. This study thus justifies its medicinal uses in the traditional medicine in rural settings of Brazil.

Acknowledgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant support and fellowship to PDF), Fundação Carlos Chagas Filho de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ, grant support to PDF), and Conselho de Administração de Pessoal de Ensino Superior (CAPES, fellowship

to MMGP). FB wishes to acknowledge the High Education Authority's Programme for Research in Third-Level Institutions Cycle 5's funding support for TBSI.

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