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Beneficial effects of the ethanol extract of *Caesalpinia pyramidalis* on the inflammatory response and abdominal hyperalgesia in rats with acute pancreatitis

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ABSTRACT

Ethnopharmacological relevance: *Caesalpinia pyramidalis* Tul. (Fabaceae) is a plant found in the Northeast of Brazil that is popularly used to treat inflammation. Acute pancreatitis (AP) is an inflammatory disease for which abdominal pain is a relevant symptom. As there is no specific therapy for AP, we investigated the effect of the ethanol extract from the inner bark of *C. pyramidalis* (EECp) on the AP induced by common bile duct obstruction (CBDO) in rats.

Material and methods: AP was induced in male Wistar rats (200–250g, $n=6$–8) through laparotomy and subsequent CBDO. Animals were euthanized after 6 (G6h) or 24 h (G24h) of induction. In the G6h protocol, animals were pretreated with EECp (100–400 mg/kg, p.o.) or vehicle (Tween 80; 0.2%) 1 h before CBDO or sham surgery. For the G24h protocol, rats were pretreated with EECp (400 mg/kg, 1 h before CBDO or 1 h before and 12 h after CBDO) or vehicle. The following parameters were measured: inflammatory/oxidative (myeloperoxidase activity and malondialdehyde formation in the pancreas and lung, leukocyte counts in the blood and serum nitrate/nitrite), enzymatic (serum amylase and lipase levels) and nociceptive (abdominal hyperalgesia).

Results: Induction of AP by CBDO significantly increased all the parameters evaluated in both G6h and G24h protocols when compared with the respective sham group. In the G6h protocol, the EECp pretreatment (400 mg/kg) significantly reduced all these parameters, besides completely inhibiting abdominal hyperalgesia. The same profile of reduction was observed from two administrations of EECp in the G24h protocol, while one single dose of EECp was able to significantly reduce pancreatic MDA, serum lipase levels, leukocyte counts in the blood and abdominal hyperalgesia without affecting the other parameters in the G24h protocol. Furthermore, rutin was found in the EECp.

Conclusions: Our results demonstrated that EECp decreases inflammation, lipoperoxidation and hyperalgesia in CBDO-induced AP, making it of interest in future approaches to treat this condition.

1. Introduction

Acute pancreatitis is an inflammatory disease caused mainly by gallstones or abuse of alcohol (Wang et al., 2009). This disease has an incidence varying from 5 to 80 per 100,000 inhabitants (Abela and Carter, 2010) and a mortality rate that can reach 5%, depending on the severity (Pandol et al., 2007). The pathophysiology of acute pancreatitis is not completely understood and involves many events and mediators that, along with the rapid onset of this disease, frequently make the clinical management of acute pancreatitis complicated (Bang et al., 2008). Although the etiology of acute pancreatitis is still controversial, it is generally accepted that an important initial event is the activation of tripsinogen in the pancreatic acini (Frossard et al., 2008), followed by activation of other enzymes (e.g., elastase and phospholipase A_2) and intrapancreatic inflammation caused by the production of chemokines and leukocyte recruitment, besides pancreatic edema (Bhatia et al., 2005). This process can spread out through the activated pancreatic enzymes and mediators that reach the circulation and consequently other tissues, leading to systemic inflammation (Bhatia et al., 2000; Al Mofleh, 2008; Dios, 2010).

Abbreviations: ANOVA, analysis of variance; AP, acute pancreatitis; CBDO, common bile duct obstruction; Dexa, dexamethasone; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EECp, ethanol extract of the inner bark of *Caesapinia pyramidalis*; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; NO_x^-, nitrate/nitrite; SEM, standard error of mean; TBARS, thiobarbituric acid reactive substances

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Another key feature of acute pancreatitis is abdominal pain, which is one of the main complaints of patients (Liddle and Nathan, 2004; Ceyhan et al., 2008) and is perceived as an epigastric discomfort that can radiate to other regions (Vera-Portocarrero and Westlund, 2005; Frossard et al., 2008).

The treatment of acute pancreatitis is still difficult and usually involves supportive therapy, prevention of infection, dissemination of the inflammatory process and pain releif (Van Esch et al., 2006; Bang et al., 2008). There is no specific pharmacological therapy for this condition and a great need for new alternatives to treat acute pancreatitis are undoubtedly of interest. Regarding this concern, natural products have been throughout the decades a source of substances with pharmacological potential, and in Brazil the "caatinga" ecosystem is largely known for its biodiversity (Desmarchelier et al., 1999; Albuquerque et al., 2007; Agra et al., 2008).

In this way, *Caesalpinia pyramidalis* Tul. (Fabaceae) is an endemic tree of the Northeast region of Brazil that is known popularly as "catingueira" (Silva and Matos, 1998). Parts of this plant, especially the inner bark or leaves, are traditionally used because of their anti-inflammatory, dyspeptic, digestive, diuretic, antipyretic, and expectorant effects (Silva and Matos, 1998; Mendes et al., 2000; Bahia et al., 2005; Agra et al., 2007). A common use of this plant is made by macerating a handful of its stem bark in a liter of wine or sugarcane brandy (locally known as "cachaça"), which is consumed before meals, two times a day, for treating dysentery, diarrhea, stomachache and other gastrointestinal diseases (Agra et al., 2008).

A previous study has shown that the ethanol extract of the inner bark of this plant possesses anti-inflammatory and anti-nociceptive activities by using models as carrageenan-induced edema and peritonitis in rats and mice, respectively, as well as acetic acid-induced abdominal writhing and formalin-induced nociception in mice (Santos et al., 2011). Altogether, these findings suggest that *C. pyramidalis* has a potential for treating inflammatory and painful conditions, particularly those involving the gastrointestinal tract; it also has a potential for treating acute pancreatitis.

In this study, we aimed to investigate the effect of the ethanol extract from the inner bark of *C. pyramidalis* (EECp) in a model of acute pancreatitis in rats. The use of EECp for inflammatory and gastrointestinal disorders in popular medicine and previous data confirming the anti-inflammatory and antinociceptive properties of this extract give basis to this hypothesis.

2. Material and methods

2.1. Drugs and reagents

Rutin, apigenin, quercetin, baicalein, dexamethasone, hexadecyltrimethylammonium bromide, *o*-dianisidine hydrochloride, nitrate reductase and cofactors, Griess reagent and Türk solution were purchased from Sigma (USA). Morphine and diazepam were obtained from União Química (Brazil), and isoflurane (Isoforine®) was obtained from Cristália, Itapira, SP (Brazil). Other reagents were obtained from Merck.

2.2. Plant material and preparation of the inner bark ethanol extract

The inner bark of *Caesalpinia pyramidalis* was collected at the Xingó Village, Canindé de São Francisco, Sergipe State, Brazil (09°66′00″ S, 37°78′94″ W). A specimen was identified by the botanist Dr. Ana Paula Nascimento Prata, Department of Biology at the Federal University of Sergipe, and deposited in the Herbarium of this institution (Marechal Rondon Av., São Cristóvão, Sergipe, 49100–000, Brazil) under the registration number ASE 13,164. The inner bark was dried at 40 °C with forced air for 2 day and subsequently powdered (2840*g*) and extracted by maceration at room temperature with 90% ethanol for 5 day. The extract was filtered in vacuum, and the solvent was removed using a rotary evaporator (45 °C). The percentage of EECp yield was 2.6% (73.8*g*).

2.3. High performance liquid chromatography-diode array detection analysis

High performance liquid chromatography was performed with a Shimadzu Liquid Chromatograph (Prominence model, Kyoto, Japan) consisting of a vacuum degasser DGU-20A3 model, SIL-10A autosampler, two high pressure pumps LC-6A, and an SPDM20Avp photodiode array detector system coupled with a CBM 20A interface. Data collection was carried out using LC Solution software. Analysis was performed in an analytical Phenomenex LUNA® C18 column (250 × 4.6 mm i.d., 5 μm of particle diameter, Torrance, CA, USA). Separation of compounds was done by reverse mode gradient elution. The flow rate was 1.0 mL/min, the volume injected was 20 μL, and a mobile phase consisting of 0.5% aqueous formic acid (v/v, A) and acetonitrile (B) was used in the following proportion: 30–63.3% (B) for 30 min, 40–100% for 5 min and 100% for 5 min. The photodiode array detector was set at 254 nm for acquiring the chromatograms.

Samples of EECp or standards (rutin, apigenin, quercetin or baicalein) were dissolved in methanol at concentrations of 5 and 0.1 mg/mL, respectively, and submitted to filtration through a cellulose membrane (pore diameter of 0.45 μm).

2.4. Animals

Male Wistar rats (220–270*g*) were obtained from the Animal Center of the Federal University of Sergipe. Animals were maintained at 21 ± 2 °C with free access to food (Purina®) and filtered water under a 12:12 h light/dark cycle. The animals were deprived of food for 8 h before the experiment, but had free access to water. All experimental procedures were conducted in accordance with the guidelines of the Brazilian College of Animal Experimentation and were approved by the Ethics Committee for Animal Use in Research at the Federal University of Sergipe (protocol numbers 06/10 and 28/11), which was conducted in accordance with the internationally accepted principles for laboratory animal use and care. At the conclusion of the experiments, animals were euthanized by overdose of isoflurane followed by exsanguination.

2.5. Experimental procedure

Under anesthesia by inhalatory isoflurane (2–3%), acute pancreatitis was induced by common bile duct obstruction (CBDO), as previously described by Samuel et al. (1994). This consisted of a midline laparotomy through which the pancreas was exteriorized by the duodenal loop in order to perform the common bile duct ligation. For this purpose, two suture threads were wrapped around the common bile duct, which was sutured in two positions with a distance of at least 0.5 cm between them, followed by a small incision to assure that the duct flow was obstructed. Concomitantly, groups of animals underwent anesthesia, laparotomy and exteriorization of the pancreas without obstruction of the common bile duct as an experimental control (sham group). The animals were euthanized after 6 or 24 h of the CBDO, depending on the experimental protocol.

2.6. Experimental design

Nine experimental groups were used in this study. In the 6 h protocol, 5 groups were considered: the group pretreated with

vehicle (0.2% Tween 80 in NaCl 0.9%, 10 mL/kg, p.o.) 1 h before undergoing only laparotomy and duct exposure (vehicle+sham group; $n=6$); the group pretreated with vehicle (1 h before) and submitted to CBDO (vehicle+CBDO group; $n=8$); and three groups that were pretreated with EECp (100, 200 or 400 mg/kg, p.o.) and submitted to CBDO (EECp+CBDO groups; $n=8$ each). The doses used in this study were based on a previous study from our laboratory (Santos et al., 2011).

In the 24 h protocol, the animals were divided into 4 groups, as follows. (i) vehicle+sham group: animals were pretreated with vehicle (1 h before), submitted to laparotomy without duct obstruction, and then received another administration of vehicle (12 h after, p.o.; $n=6$); (ii) vehicle+CBDO group: animals were pretreated with vehicle (1 h before, p.o.), submitted to CBDO, and then received another administration of vehicle (12 h after, p.o.; $n=8$); (iii) EECp (1 dose)+CBDO group: animals were pretreated with EECp (400 mg/kg, 1 h before, p.o.), submitted to CBDO, and subsequently administered vehicle (12 h after, p.o.; $n=6$); and (iv) EECp (2 doses)+CBDO group: animals were pretreated with EECp (400 mg/kg, 1 h before, p.o.), submitted to CBDO, and subsequently administered EECp again (400 mg/kg, 12 h after, p.o.; $n=6$). In these experiments, all rats were euthanized 24 h after CBDO.

An additional group treated with morphine was considered in the 24 h protocol, only for the measurement of abdominal hyperalgesia (positive control). This group ($n=6$) received morphine at 5 mg/kg, i.p., 30 min before each measurement performed at 6, 12 and 24 h (Vera-Portocarrero et al., 2003). As a control of the inflammatory parameters, the groups of animals were pretreated with dexamethasone (10 mg/kg, s.c.; $n=6$) 1 h before CBDO in the 6 h protocol, or 1 h before and 12 h after CBDO in the 24 h protocol ($n=6$).

After 6 or 24 h intervals, the animals were euthanized and samples of pancreas, lung, liver and kidney were stored at −20 °C. After collection, the blood was centrifuged at $1000 \times g$ for 15 min at 4 °C to separate plasma that was then stored at −20 °C.

2.7. Measurement of abdominal hyperalgesia

To evaluate the mechanical hyperalgesia in the rat abdominal region, we used the electronic von Frey (Insight, Ribeirão Preto, São Paulo, Brazil), according to previous description (Camargo et al., 2011). The animals were transferred to individual cages and acclimatized over 3 day by spending 30 min each day. On the day of the test, mechanical stimuli were applied to the anterior-lateral region of the abdomen of the animals, in triplicate, prior to any manipulation and immediately before euthanasia for the 6 h protocol or after 6, 12 or 24 h of CBDO for the 24 h protocol. At each time point, an increasing stimulus (in *g*) was applied to the abdominal region of the rats, with an interval of at least 1 min, until any withdrawal behavior was observed, at which time the threshold force value setting in the equipment (from 0.1 to 1000*g*) was registered. The test was performed "blind". Data were expressed as variation (Δ) by subtracting the mean obtained from the three measurements, taken at the referred to time point after pancreatitis induction, from the mean basal value recorded for each animal (prior to pancreatitis).

2.8. Determination of biochemical and inflammatory parameters

For myeloperoxidase activity (MPO) determination, pancreas and lung samples were collected, weighed and homogenized with potassium phosphate buffer (50 mM, pH 6.0 containing 0.5% of hexadecyltrimethylammonium bromide), and 1-mL aliquots of the homogenates were centrifuged (2 min, $8000 \times g$, 4 °C). In a 96-well plate, aliquots of supernatant were incubated with a solution of *o*-dianisidine hydrochloride (0.167 mg/mL containing 0.005% H_2O_2). The MPO activity was measured kinetically in a microliter plate scanner (Labsystem Multiskan) at 460 nm and intervals of 15 s over a period of 5 min. Results were expressed as units of MPO per mg tissue (UMPO/mg tissue). An UMPO was considered as the amount of enzyme that degrades 1 mmol of hydrogen peroxide/min (Bradley et al., 1982).

For determination of thiobarbituric acid reactive substances (TBARS), samples of pancreas, lung, liver and kidney were weighed and homogenized in potassium phosphate buffer (50 mM, pH 7.4) containing butylated hydroxytoluene (12.6 mM). Then, aliquots of the homogenate (in duplicate) were incubated (90 °C, 45 min) with thiobarbituric acid (0.37%) in an acidic solution (trichloroacetic acid at 15% and hydrochloric acid at 0.25 N). At the end of incubation, the homogenates were centrifuged (5 min, $8000 \times g$), and aliquots from the supernatants were extracted with *n*-butanol, followed by stirring in a vortex for 30 s and further centrifugation (2 min, $8000 \times g$). The supernatant absorbance was measured at 535 nm in a microplate reader (corrected by the values of absorbance at 572 nm). The results were calculated using a molar extinction coefficient of $1.55 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmol of malondialdehyde (MDA) formed per mg of tissue (Bose et al., 1989).

The levels of amylase and lipase in serum were assessed for each experimental group by using specific commercial kits for kinetic determination of amylase (Katal Biotechnology, Belo Horizonte, MG, Brazil) and a colorimetric kit for determination of lipase (Human do Brasil, São Paulo, SP, Brazil) according to the manufacturer's instructions.

The edema index was calculated as the ratio of wet weight and dry weight of the samples of pancreatic tissue. For this purpose, the samples were dried at 90 °C for 12 h, and tissue weight was measured before and after this procedure.

Total and differential leukocyte counts were performed in aliquots of 20 μL of peripheral blood taken from the tail vein of anesthetized rats immediately before euthanasia. The total leukocyte count was performed in a Neubauer chamber, and the differential count was conducted under a light microscope with immersion oil objective in cytocentrifuged smears colored with Giemsa Newprov®, where 100 cells per slide were counted, based on normal morphological criteria. Results were expressed as number of leukocytes/mL of peripheral blood.

Total nitrate/nitrite (NO_x^-) concentration was determined in serum submitted to ultrafiltration (10 kDa; Microcon centrifugal filter units) using the Griess reaction for nitrite, after the nitrate reductase-catalyzed reduction of nitrate to nitrite, according to Grisham et al. (1996).

2.9. Spontaneous locomotor activity

Rats were orally treated with vehicle or EECp (400 mg/kg) 1 h before being introduced to the open field apparatus, or with diazepam (1.5 mg/kg, i.p.) 30 min before. The spontaneous locomotor activity of the animals was assessed in a circular open field, 60 cm in diameter, over a period of 4 min (Capaz et al., 1981). This procedure was repeated 6 h after the first measurement (total interval of 7 h between the administration of EECp and the second measurement), and the values were expressed as the number of field crossings during the 4 min.

2.10. Statistical analysis

Results were expressed as mean ± standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test by using GraphPad Prism software (version 4.0). In the time course data for abdominal

hyperalgesia (Fig. 4 B), two-way ANOVA was used to compare the groups and treatments over all times, and if a significant time *vs.* treatment interaction was found, one-way ANOVA followed by Tukey's test was performed for each time to distinguish the effect of the specific treatment. $p < 0.05$ was considered significant.

3. Results

3.1. Effect of EECp on serum amylase and lipase concentrations

As expected, the levels of serum amylase and lipase concentrations were augmented after 6 h ($p < 0.001$ each) or 24 h ($p < 0.001$ each) of the CBDO when compared with the sham group (Fig. 1). Previous administration of EECp at 200 or 400 mg/kg, but not at 100 mg/kg, significantly decreased the serum amylase ($p < 0.001$ or $p < 0.01$, respectively) after 6 h of CBDO when compared with the vehicle group (Fig. 1A). In these same groups of animals, EECp (100–400 mg/kg) also significantly reduced the serum lipase ($p < 0.05$, $p < 0.01$ or $p < 0.01$ for 100, 200 or 400 mg/kg, respectively; Fig. 1B). Twenty-four hour after the CBDO, the previous treatment with 400 mg/kg of EECp reduced the amylase ($p < 0.05$) and lipase levels ($p < 0.01$) when compared with the vehicle-treated group. In addition, two administrations of EECp significantly reduced both serum amylase and lipase ($p < 0.01$) levels when compared with the respective vehicle-treated group (Fig. 1C and D). Treatment with dexamethasone reduced the circulating levels of amylase and lipase in both the 6 and 24 h protocols (Fig. 1).

3.2. Effect of EECp on MPO activity in pancreatic and lung tissue

The CBDO-induced pancreatic inflammation was characterized by a marked increase of pancreatic MPO activity after 6 ($p < 0.01$) or 24 h ($p < 0.001$) when compared with the respective sham groups (Fig. 2A and B). The previous treatment with EECp at 400 mg/kg reverted this effect ($p < 0.01$) after 6 h of obstruction, which was not observed at lower doses of this extract when compared with animals pretreated with vehicle (Fig. 2A). Also, two administrations of EECp (−1 and 12 h) significantly reduced ($p < 0.01$) the pancreatic MPO activity after 24 h of induction. Single pretreatment with EECp (−1 h) did not modify this activity (Fig. 2B). The treatment with dexamethasone (10 mg/kg) inhibited the pancreatic MPO activity ($p < 0.01$) in both 6 ($p < 0.05$) and 24 h ($p < 0.01$) protocol.

The induction of pancreatitis also significantly increased MPO activity in the lung tissue at both 6 ($p < 0.01$) and 24 h ($p < 0.001$) when compared with the respective sham groups (Fig. 2C and D). After 6 h of obstruction, the pretreatment with EECp (−1 h), at doses varying from 100 to 400 mg/kg, significantly reduced the lung MPO activity (Fig. 2C). Neither one nor two administrations of EECp were able to alter MPO activity in lung tissue after 24 h of the induction of pancreatitis (Fig. 2D). The treatment with

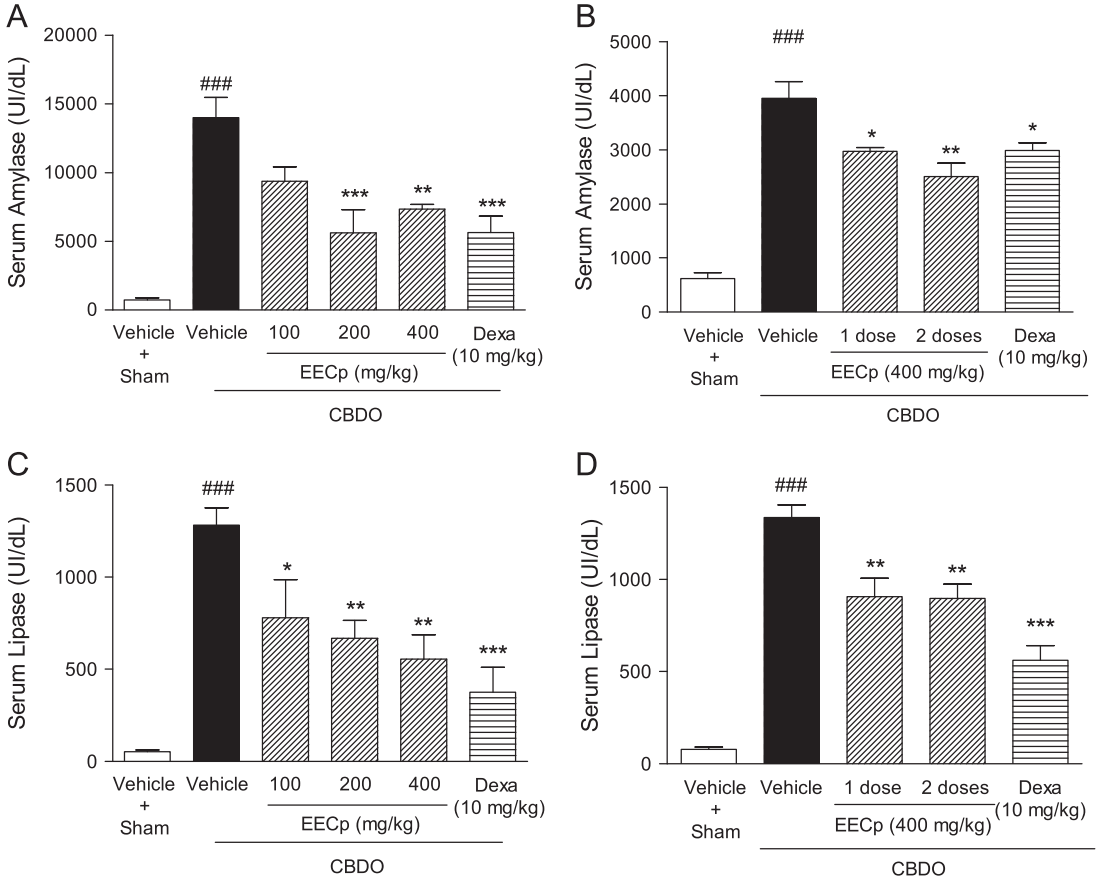

Fig. 1. The ethanol extract of the inner bark of *Caesalpinia pyramidalis* (EECp) reduces serum amylase (A and B) and lipase (C and D) concentrations during common bile duct obstruction (CBDO)-induced pancreatitis. In panels A and C, rats were pretreated with EECp (100, 200 or 400 mg/kg; −1 h), dexamethasone (Dexa; 10 mg/kg; −1 h) or vehicle, submitted to CBDO or sham surgery, and sacrificed 6 h after the obstruction. In panels B and D, EECp (400 mg/kg) was administrated to rats only 1 h before (1 dose) CBDO or 1 h before and 12 h after (2 doses) CBDO. In this case, when rats did not receive EECp, they were given vehicle instead. Dexa (10 mg/kg) was also administered 1 h before and 12 h after CBDO. Rats were sacrificed 24 h after the obstruction. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ or $^{\#\#\#}p < 0.001$ *vs.* vehicle + sham; $*p < 0.05$, $**p < 0.01$ or $***p < 0.001$ *vs.* vehicle + CBDO (one-way ANOVA followed by Tukey's test); $n = 6–8$.

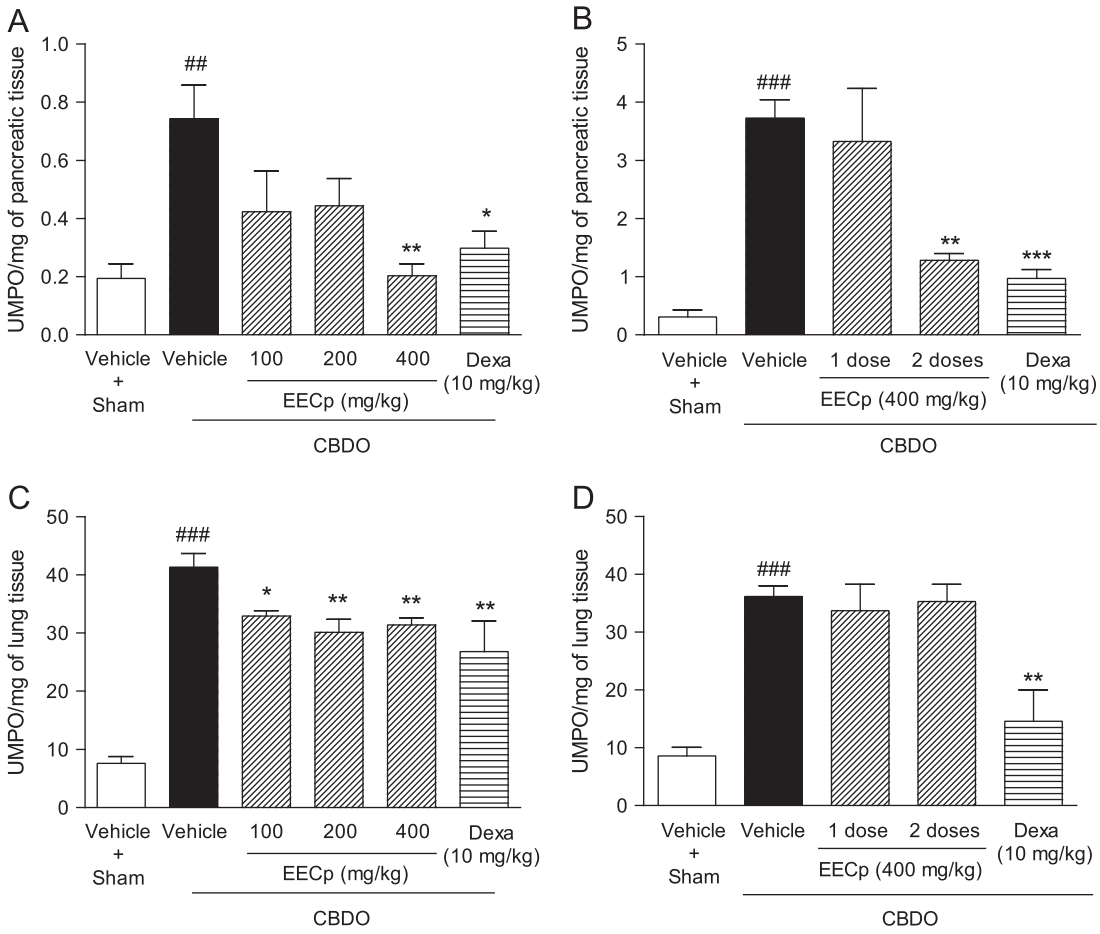

Fig. 2. Effect of the ethanol extract of the inner bark of *Caesalpinia pyramidalis* (EECp) on pancreatic and lung myeloperoxidase (MPO) activity. In panels A and C, rats were pretreated with EECp (100, 200 or 400 mg/kg; −1 h), dexamethasone (Dexa, 10 mg/kg; −1 h) or vehicle, submitted to common bile duct obstruction (CBDO) or sham surgery, and sacrificed 6 h after the obstruction. In panels B and D, EECp (400 mg/kg) was administrated to rats only 1 h before (1 dose) CBDO or 1 h before and 12 h after (2 doses) CBDO. In this case, when rats did not receive EECp, they were given vehicle instead. Dexa (10 mg/kg) was also administered 1 h before and 12 h after CBDO. Rats were sacrificed 24 h after the obstruction. MPO activity was measured in the pancreatic (A and B) and lung (C and D) tissues (n=6–8 rats). $^{\#\#}p<0.01$ or $^{\#\#\#}p<0.001$ *vs.* vehicle+sham; $^{*}p<0.05$,$^{**}p<0.01$ or $^{***}p<0.001$ *vs.* vehicle+CBDO (one-way ANOVA followed by Tukey's test).

dexamethasone (10 mg/kg) significantly reduced lung MPO activity ($p<0.01$) in both the 6 and 24 h protocols.

3.3. Effect of EECp on pancreatic edema

There was no significant alteration of pancreatic edema after 6 h of vehicle treatment plus CBDO (4.6 ± 0.3) when compared with the sham group (3.7 ± 0.3), and this was not modified by the previous treatment with any dose of EECp used (3.8 ± 0.3, 3.9 ± 0.4 and 4.6 ± 0.1 for 100, 200 and 400 mg/kg, respectively) or dexamethasone (4.5 ± 0.3). In contrast, after 24 h of the induction of pancreatitis, a significant increase in pancreatic edema was observed (5.9 ± 0.2, $p<0.01$) when compared with the sham group (4.4 ± 0.2), which was reduced by two administrations of EECp at 400 mg/kg (4.5 ± 0.1, $p<0.01$), but not by one single administration of this extract (5.1 ± 0.2). Dexamethasone at 10 mg/kg (2 doses) completely inhibited pancreatic edema (3.7 ± 0.2, $p<0.001$).

3.4. Effect of EECp on circulating leukocyte counts

Associated with the induction of pancreatitis and investigation of pancreatic and lung effects, the total and differential leukocyte counts in the peripheral blood were evaluated as an additional marker of systemic inflammation. In the experiments performed 6 h after CBDO, we found a clear elevation of total leukocyte counts ($p<0.001$), which was caused by either increases in the polymorphonuclear cell number (mostly neutrophils, $p<0.001$) or in the mononuclear cell number (monocytes and lymphocytes, $p<0.001$) when compared with the respective sham group, as shown in Table 1. All doses of EECp (100–400 mg/kg) used in the experiments conducted 6 h after CBDO, as well as any scheme of treatment (one or two administrations) used in the experiments conducted 24 h after CBDO, reduced the total and differential leukocyte counts ($p<0.001$; Table 1), as did dexamethasone, when compared with the respective vehicle-treated group.

Experiments were performed in 6 or 24 h intervals of induction. In the 6 h interval, groups of animals were treated with EECp (100, 200 or 400 mg/kg), dexamethasone (Dexa, 10 mg/kg) or vehicle (Tween 80, 0.2% in saline) 1 h before they were submitted to CBDO or sham surgery and sacrificed 6 h after the obstruction. In the 24 h interval, EECp (400 mg/kg) was administrated to rats only 1 h before (1 dose) CBDO or 1 h before and 12 h after (2 doses) CBDO. In this case, when rats did not receive EECp, they were given vehicle instead. Dexa (10 mg/kg) was also administered 1 h before and 12 h after CBDO. Rats were sacrificed 24 h after the obstruction. Data are expressed as mean ± SEM for n=6–8; one-way ANOVA followed by Tukey's post test. $^{a}p<0.001$ *vs.* the respective sham+vehicle group or $^{b}p<0.001$ *vs.* the respective CBDO+vehicle group.

Table 1
The ethanol extract of *Caesalpinia pyramidalis* (EECp) reduces the total and differential leukocyte counts in the peripheral blood of rats submitted to common bile duct obstruction (CBDO)-induced acute pancreatitis.

	Treatment	Total leukocyte ($\times 10^6$/mL)	Polymorphonuclear cells ($\times 10^6$/mL)	Mononuclear cells ($\times 10^6$/mL)
6 h	Sham+Vehicle	1.3 ± 0.5	0.7 ± 0.3	0.6 ± 0.2
	CBDO+Vehicle	6.8 ± 0.1^a	4.9 ± 0.3^a	1.9 ± 0.3^a
	CBDO+EECp (100 mg/kg)	2.3 ± 0.1^b	0.5 ± 0.1^b	0.8 ± 0.1^b
	CBDO+EECp (200 mg/kg)	2.5 ± 0.1^b	1.6 ± 0.1^b	0.9 ± 0.1^b
	CBDO+EECp (400 mg/kg)	2.6 ± 0.2^b	1.7 ± 0.3^b	0.9 ± 0.2^b
	CBDO+Dexa (10 mg/kg)	3.8 ± 0.5^b	2.2 ± 0.4^b	1.7 ± 0.2
24 h	Sham+Vehicle	2.6 ± 0.4	1.5 ± 0.5	1.1 ± 0.1
	CBDO+Vehicle	10.3 ± 0.8^a	4.8 ± 0.6^a	5.5 ± 1.3^a
	CBDO+EECp (400 mg/kg, 1 dose)	6.0 ± 1.9^b	3.6 ± 1.4^b	2.4 ± 0.6^b
	CBDO+EECp (400 mg/kg, 2 doses)	5.1 ± 1.1^b	2.5 ± 0.4^b	2.6 ± 0.8^b
	CBDO+Dexa (10 mg/kg)	2.3 ± 0.3^b	1.4 ± 0.4^b	0.9 ± 0.1^b

Experiments were performed in 6 or 24 h intervals of induction. In the 6 h interval, groups of animals were treated with EECp (100, 200 or 400 mg/kg), dexamethasone (Dexa, 10 mg/kg) or vehicle (Tween 80, 0.2% in saline) 1 h before they were submitted to CBDO or sham surgery and sacrificed 6 h after the obstruction. In the 24 h interval, EECp (400 mg/kg) was administrated to rats only 1 h before (1 dose) CBDO or 1 h before and 12 h after (2 doses) CBDO. In this case, when rats did not receive EECp, they were given vehicle instead. Dexa (10 mg/kg) was also administered 1 h before and 12 h after CBDO. Rats were sacrificed 24 h after the obstruction. Data are expressed as mean ± SEM for $n=6$–8; one-way ANOVA followed by Tukey's post test.

[a] $p < 0.001$ *vs.* the respective sham+vehicle group or
[b] $p < 0.001$ *vs.* the respective CBDO+vehicle group.

3.5. Effect of EECp on TBARS formation

Besides the increase in MPO activity, CBDO also induced lipid peroxidation, detected through the increase of the formation of MDA in both pancreas and lung tissues after 6 ($p < 0.001$) or 24 h ($p < 0.001$; Table 2) when compared with the respective sham groups. The administration of EECp (400 mg/kg) significantly decreased MDA formation in both the pancreas ($p < 0.05$) and lung tissues ($p < 0.001$) after 6 h of CBDO when compared with the vehicle-treated group (Table 2). After 24 h of CBDO, both the one- and two-treatment schemes with EECp (400 mg/kg) diminished MDA formation in the pancreatic tissue ($p < 0.001$; Table 2); however, MDA formation was significantly decreased ($p < 0.05$) in lung tissue only after two administrations of EECp (400 mg/kg; Table 2) when compared with the vehicle-treated group. Dexamethasone pretreatment did not alter the MDA formation in these tissues (data not shown).

MDA formation in the liver and kidney was also investigated as a marker of oxidative injury in these tissues. Neither the CBDO induction nor the administration of EECp at any dose or scheme of treatment used altered the MDA formation in these tissues (data not shown).

Experiments were performed at 6 or 24 h intervals from induction. In the 6 h protocol, the groups of animals were treated with EECp (100, 200 or 400 mg/kg) or vehicle (Tween 80, 0.2% in saline) 1 h before they were submitted to CBDO or sham surgery, and then sacrificed 6 h after the obstruction. In the 24 h protocol, EECp (400 mg/kg) was administrated to the rats only 1 h before CBDO (1 dose) or 1 h before and 12 h after CBDO (2 doses). In this case, when the rats did not receive EECp, they were given vehicle instead. The rats were sacrificed 24 h after the obstruction. Data are expressed as mean ± SEM for $n=6$–8; one-way ANOVA followed by Tukey's post test. $^a p < 0.001$ *vs.* the respective sham+vehicle group, $^b p < 0.05$ *vs.* the respective CBDO+vehicle group, or $^c p < 0.001$ *vs.* the respective CBDO+vehicle group.

3.6. Effect of EECp on serum NO_x^- concentration

Total serum NO_x^- concentration was significantly increased ($p < 0.01$) in animals that were submitted to CBDO in the 6 h protocol when compared with the sham group. The pretreatment with EECp at 400 mg/kg, but not at lower doses, significantly decreased this effect ($p < 0.05$ *vs.* the vehicle-treated group; Fig. 3A), as did dexamethasone ($p < 0.05$). Twenty-four h after CBDO, the serum NO_x^- concentration was also increased in animals with pancreatitis ($p < 0.01$ *vs.* the sham group), which was significantly decreased ($p < 0.05$) by the treatment with two doses of EECp, but not with one single treatment (Fig. 3B). Treatment with dexamethasone also decreased this concentration ($p < 0.05$, Fig. 3B).

Table 2
The ethanol extract from the inner bark of *Caesalpinia pyramidalis* (EECp) decreases malondialdehyde (MDA) formation in pancreatic and lung tissues of animals with common bile duct obstruction (CBDO)-induced pancreatitis.

	Treatment	Pancreatic MDA (pmol/mg of tissue)	Lung MDA (pmol/mg of tissue)
6 h	Sham+Vehicle	26 ± 8	23 ± 6
	CBDO+Vehicle	113 ± 15^a	111 ± 10^a
	CBDO+EECp (100 mg/kg)	80 ± 17	82 ± 8
	CBDO+EECp (200 mg/kg)	88 ± 6	72 ± 12
	CBDO+EECp (400 mg/kg)	55 ± 5^b	55 ± 4^c
24 h	Sham+Vehicle	17 ± 2	16 ± 2
	CBDO+Vehicle	100 ± 12^a	108 ± 17^a
	CBDO+EECp (400 mg/kg, 1 dose)	32 ± 6^c	34 ± 9^c
	CBDO+EECp (400 mg/kg, 2 doses)	24 ± 5^c	17 ± 1^c

Experiments were performed at 6 or 24 h intervals from induction. In the 6 h protocol, the groups of animals were treated with EECp (100, 200 or 400 mg/kg) or vehicle (Tween 80, 0.2% in saline) 1 h before they were submitted to CBDO or sham surgery, and then sacrificed 6 h after the obstruction. In the 24 h protocol, EECp (400 mg/kg) was administrated to the rats only 1 h before CBDO (1 dose) or 1 h before and 12 h after CBDO (2 doses). In this case, when the rats did not receive EECp, they were given vehicle instead. The rats were sacrificed 24 h after the obstruction. Data are expressed as mean ± SEM for $n=6$–8; one-way ANOVA followed by Tukey's post test.

[a] $p < 0.001$ *vs.* the respective sham+vehicle group.
[b] $p < 0.05$ *vs.* the respective CBDO+vehicle group, or.
[c] $p < 0.001$ *vs.* the respective CBDO+vehicle group.

3.7. Effect of EECp on mechanical abdominal hyperalgesia

CBDO also induced a marked abdominal hyperalgesia in rats (Fig. 4) that was detected through the decreased intensity of

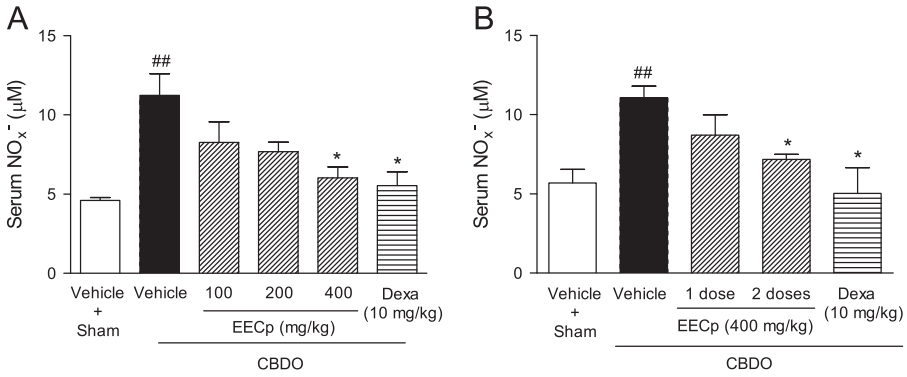

Fig. 3. The ethanol extract of the inner bark of *Caesalpinia pyramidalis* (EECp) reduces total nitrate/nitrite (NO_x^-) concentration during common bile duct obstruction (CBDO)-induced pancreatitis. In panel A, rats were pretreated with EECp (100, 200 or 400 mg/kg; −1 h), dexamethasone (Dexa, 10 mg/kg; −1 h) or vehicle, submitted to CBDO or sham surgery, and sacrificed 6 h after the obstruction. In panel B, EECp (400 mg/kg) was administrated to rats only 1 h before (1 dose) CBDO or 1 h before and 12 h after (2 doses) CBDO. In this case, when rats did not receive EECp, they were given vehicle instead. Dexa (10 mg/kg) was also administered 1 h before and 12 h after CBDO. The rats were sacrificed 24 h after the obstruction. $^{\#\#}p<0.01$ *vs.* vehicle+sham; $^{*}p<0.05$ *vs.* vehicle+CBDO (one-way ANOVA followed by Tukey's test); n=6–8.

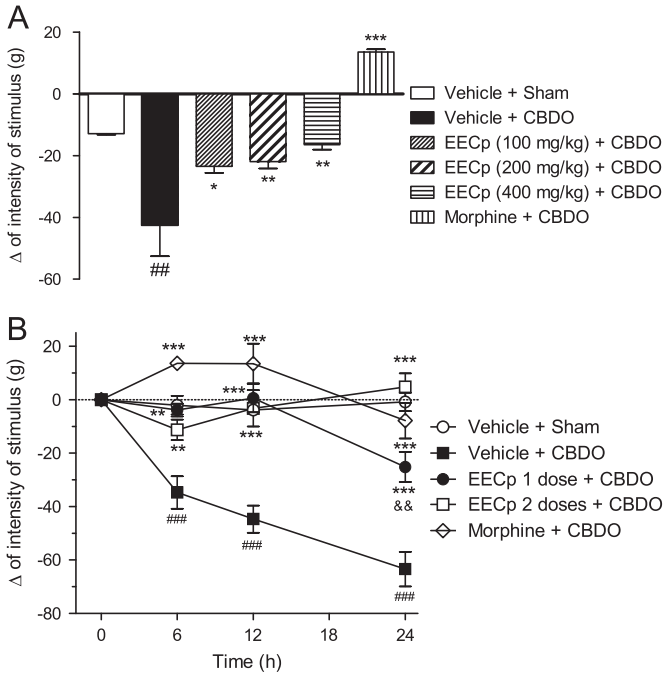

Fig. 4. The ethanol extract from the inner bark of *Caesalpinia pyramidalis* (EECp) inhibits abdominal hyperalgesia in common bile duct obstruction (CBDO)-induced pancreatitis in rats. In panel A, rats were pretreated with EECp (100, 200 or 400 mg/kg; −1 h), morphine (−0.5 h) or vehicle (Tween 80, 0.2% in saline), submitted to CBDO or sham surgery, and then sacrificed 6 h after the obstruction. In panel B, EECp (400 mg/kg) was administrated to rats only 1 h before CBDO (1 dose) or 1 h before and 12 h after CBDO (2 doses). In this case, when rats did not receive EECp, they were given vehicle instead. Morphine was given 0.5 h before each measurement. The rats were sacrificed 24 h after the obstruction. The threshold forces to cause withdrawal behavior were measured before (−1 h) and at 6 h (panel A), or at 6, 12 and 24 h (panel B), and the data are expressed as the variation observed (g) for the intensity of stimulus for n=6–8. $^{\#\#}p<0.01$ or $^{\#\#\#}p<0.001$ *vs.* the respective vehicle+sham; $^{*}p<0.05$, $^{**}p<0.01$, or $^{***}p<0.001$ *vs.* the respective vehicle+CBDO. Panel A: one-way ANOVA followed by Tukey's test; Panel B: two-way ANOVA ($p<0.001$ and F=10.91) and one-way ANOVA followed by Tukey's test.

stimulus needed to cause a withdrawal behavior in the rats after 6 ($p<0.01$), 12 ($p<0.001$) or 24 h when compared with the respective sham group. Two-way ANOVA indicated a significant interaction between the time and treatment in this experiment ($p<0.001$; F=10.91). The sham procedure did not alter the withdrawal behavior of the rats (Fig. 4B).

Fig. 4A demonstrated that pretreatment with EECp clearly reduced the abdominal hyperalgesia of rats submitted to CBDO at 100 ($p<0.05$), 200 or 400 mg/kg ($p<0.01$) after 6 h of induction when compared with the sham group. The experiments performed at 24 h after CBDO have shown that a single previous treatment of animals with EECp (400 mg/kg) maintained complete reduction of abdominal hyperalgesia for the following 12 h ($p<0.001$ at each time evaluated, Fig. 4B) when compared with the vehicle-treated group. Twenty-four hour after CBDO, animals pretreated with this single dose of EECp still presented a significant reduction in hyperalgesia, although the variation of the intensity of stimulus was significantly different from the sham group ($p<0.001$). The administration of the second dose of EECp (400 mg/kg) after 12 h of CBDO completely reduced the variation of stimulus intensity applied to the abdominal region ($p<0.001$ when compared with the groups that received only vehicle or $p<0.01$ when compared with the group that received the single dose of EECp 1 h before CBDO). Treatment with morphine significantly reduced hyperalgesia ($p<0.001$ for 6, 12 or 24 h) when compared with the vehicle-treated group (Fig. 4).

3.8. Lack of effect of EECp on the locomotor activity of rats

One hour after the oral treatment of rats with EECp (400 mg/kg), there was no change in the number of crossings in the open field apparatus (29.0 ± 3.3) when compared with the vehicle-treated group (32.8 ± 0.9). In the same way, after 7 h of EECp administration (6 h after the first measurement), this extract did not alter the number of crossings (16.8 ± 1.7) when compared with the vehicle-treated group (21.7 ± 4.9). In contrast, the treatment of animals with diazepam (1.5 mg/kg) significantly decreased the number of field crossings by the animals after 30 min (12.0 ± 3.5; $p<0.001$) or 7 h (3.0 ± 1.5; $p<0.01$) when compared with the groups treated with vehicle at 1 h or 7 h after, respectively.

3.9. HPLC chromatographic analysis of EECp

EECp was submitted to HPLC chromatographic analysis for the presence of flavonoids because of the description in the literature of high quantities of flavonoids in the inner bark and/or leaves of this plant (Silva et al., 2011). Fig. 5 shows a typical HPLC chromatogram presenting at least 5 important peaks. The presence of rutin, apigenin, baicalein and quercetin was investigated by comparing the retention time of EECp and the flavonoids' standard solutions, but only the retention time of the rutin

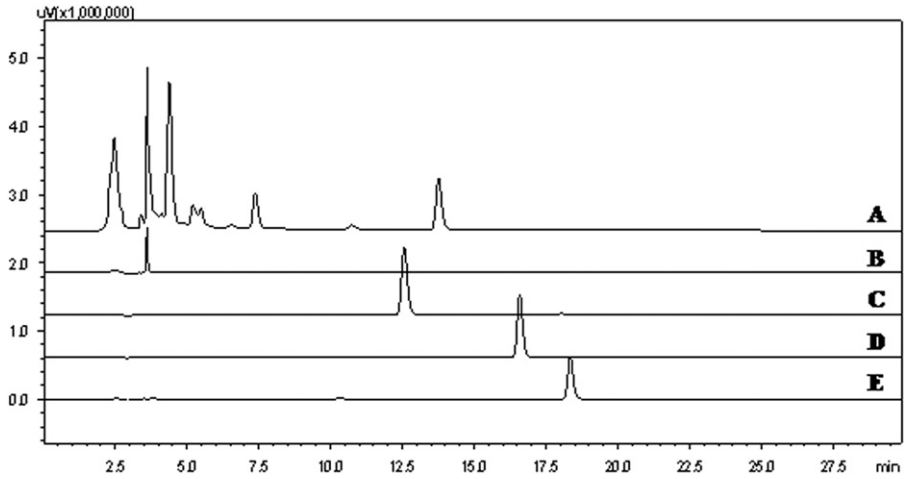

Fig. 5. Chromatograms of the ethanol extract of the inner bark of *Caesalpinia pyramidalis* (A) and authentic standard solution of rutin (B), quercetin (C), apigenin (D) and baicalein (E) subjected to high performance liquid chromatography-diode array detection analysis set at 254 nm.

standard matched with one peak found in the EECp (Fig. 5, line B). This was confirmed by the fortification of the EECp with rutin and the subsequent analysis, which showed a superposition of the retention times of the peaks (not shown), indicating that rutin is present in the EECp.

4. Discussion

Treating acute pancreatitis is still a challenge to clinical management because this disease is of rapid onset, the pancreas is relatively inaccessible to physical examination, and in many cases systemic inflammation adds complications to the patient's health (Pandol et al., 2007). The pharmacological treatment of this disease requires new options in order to be improved, especially for preventing systemic inflammation and treating abdominal pain that are important characteristics of pancreatitis (Ceyhan et al., 2008).

In this way, ethnopharmacological knowledge can be useful in guiding the search for new compounds to treat pancreatitis. In this study, we investigated the effect of the ethanol extract of a plant derived from the Brazilian "caatinga" ecosystem. Parts of *C. pyramidalis* are usually utilized by the population as a decoct to treat various conditions, including gastrointestinal disorders (Silva and Matos, 1998; Mendes et al., 2000; Bahia et al., 2005; Agra et al., 2007; Agra et al., 2008), and the pharmacological validation of this use has been published elsewhere (Santos et al., 2011). The results obtained from the present study further confirm its traditional use by indicating that EECp exerts important modulatory effects on acute pancreatitis in rats, suggesting an innovative value for this extract and enabling future studies on its components' activities.

We chose CBDO as the model to induce pancreatitis in rats because it mimics the pancreatitis induced in humans by lithiasis, which is the main etiologic factor of this disease (Meyerholz et al., 2008; Samuel et al., 2005). Thus, by using CBDO-induced pancreatitis, many signs commonly observed in patients can be investigated. During the present study, pancreatitis induced by CBDO was characterized by increased levels of pancreatic enzymes in the blood, abdominal hyperalgesia, and pancreatic neutrophil infiltration, edema and lipid peroxidation. We used two experimental sets to investigate the effects of EECp in the early (6 h) or late (24 h) periods after induction of pancreatitis. This is justified based on the fact that in this model of pancreatitis the source of the injury is induced surgically and is not reversible, which makes the local injury and systemic complications worse at later times. After preliminary experiments, we found that a single previous administration of EECp could be enough to reduce some of the inflammatory/nociceptive parameters after 6 h of CBDO. After performing experiments with the three doses of EECp (100, 200 or 400 mg/kg), the 400 mg/kg dose was selected to carry out the 24 h interval experiments. In these experiments, both a single previous treatment (-1 h) and a previous treatment followed by another after 12 h of induction were compared to analyze the inflammatory and nociceptive signs.

One of the hallmarks of pancreatitis is the elevation of amylase and/or lipase in the blood (Forsmark and Baillie, 2007). In the present study, CBDO increased both amylase and lipase levels after 6 or 24 h of induction, which is in agreement with other studies (Hirata et al., 2002; Barrett et al., 2008). It is interesting that, while concentrations of amylase were lower at 24 h than at 6 h, the concentrations of lipase were maintained high throughout both time periods considered. The increase in serum levels of amylase usually precedes the increase in lipase levels, as well as it decreases faster than lipase (Orebaugh, 1994; Shields et al., 2006). Treatment with EECp was able to reduce both serum amylase and lipase after 6 or 24 h of induction, in a similar way that dexamethasone did, which indicates the potential of EECp to reduce pancreatitis.

As a consequence of pancreatic enzyme activation in the acini, local inflammatory response is developed, with characteristics including edema and neutrophil infiltration. In the present study, MPO activity was increased in pancreatic tissue after 6 or 24 h of pancreatitis induction, which were reduced by EECp. This enzyme activity is a common marker of neutrophil infiltration into the tissues, as MPO is found in the azurophilic granules of neutrophils (Bradley et al., 1982). This finding is consistent with the decrease of neutrophil infiltration and the anti-inflammatory effect of EECp. In addition, pancreatic edema was detected only at the 24 h time point, and the treatment with EECp was able to decrease this effect, reinforcing its antiedematogenic activity, as previously described (Santos et al., 2011).

Lung injury is the main secondary complication of acute pancreatitis and the primary cause of death in patients (McKay and Butter, 2003; Wang et al., 2009; Zhou et al., 2010). Lung neutrophil infiltration was partially decreased by EECp after 6 h of CBDO. However, after 24 h of CBDO, neither one single nor two administrations of EECp were enough to reduce lung neutrophil infiltration. There is no reasonable explanation for this fact, but we must take into consideration that the pancreatic lesion is

severe and persistent in this model. This lesion substantially elevated the levels of pancreatic enzymes evaluated (and possibly increased the levels of other enzymes like tripsin, phospholipase A_2 and elastase), which are believed to trigger lung injury (Al Mofleh, 2008). As the effect of EECp on the levels of amylase and lipase, after 24 h, was less effective than that detected after 6 h of CBDO, it is possible to speculate on an association between these two observations. Even though there are these considerations, the results clearly demonstrated that EECp reduces pancreatic neutrophil infiltration, which may be of value for the treatment of pancreatitis.

In addition, the increase of total leukocyte counts in the peripheral blood in CBDO-induced pancreatitis was used as a general sign of systemic inflammation and was caused by the increases in both polymorphonuclear and mononuclear cells, as previously observed by others (Dios et al., 2002; Yubero et al., 2009; Ramudo et al., 2010). The leukocytosis induced by CBDO was reduced by the treatment with EECp at all doses (100–400 mg/kg) and schemes of treatment used here (one or two administrations). This potential of EECp to decrease leukocyte recruitment agrees with a study by Santos et al. (2011). These authors reported that a previous treatment of rats with EECp decreased paw edema and MPO activity induced by subplantar injection of carrageenan. Also, they found that EECp reduced leukocyte recruitment to the mouse peritoneal cavity induced by carrageenan. These findings are in accordance with the data from the present study, in the model of CBDO-induced pancreatitis in rats. The main difference between our study and the study by Santos et al. (2011) is the fact that we used a model of inflammation that brings a higher clinical value to the effect observed, since pancreatitis can cause systemic inflammation that resembles a condition observed in humans.

In the study by Santos et al. (2011), the authors suggested that the anti-inflammatory effects of EECp could be related to the *in vitro* antioxidant activity of this extract. In our study, this was also taken into consideration, and the effect of EECp on *in vivo* lipid peroxidation was investigated. In CBDO-induced pancreatitis, the inflammatory process might produce free radicals that in turn cause lipoperoxidation, detectable through the formation of the TBARS that generate MDA as the major aldehyde, a fact that was observed in the pancreas and lung of animals in the present study, concordant with previous findings (Sevillano et al., 2003; Shi et al., 2005). In this regard, EECp decreased MDA formation in the pancreatic and lung tissues. In the 6 h protocol, only the high dose of EECp (400 mg/kg) was able to decrease MDA formation after 6 h of pancreatitis induction, but maintained this effect until 24 h after CBDO, which was also observed for two administrations of this ethanol extract. This indicates that EECp provided protection against the lipoperoxidation caused by CBDO, an observation that completely agrees with prior findings that the aqueous (Alviano et al., 2008) or ethanolic (Silva et al., 2011) extracts of *C. pyramidalis* inner bark induce 2,2-diphenyl-1-picrylhydrazyl (DPPH) consumption *in vitro*, a widely used antioxidant assay. In agreement with the antioxidant and anti-inflammatory effects of EECp, our results show that the production of NO was decreased in animals treated with this extract, as NO metabolites were detected at lower concentrations in the serum of these animals.

The inflammatory process and oxidative stress caused by pancreatitis in rats closely resemble this condition in humans, and EECp effectively decreased most of these alterations. However, another feature of clinical relevance in acute pancreatitis is abdominal pain (Vera-Portocarrero and Westlund, 2005; Frossard et al., 2008). Studying abdominal pain in CBDO-induced pancreatitis can be a difficult task because this model of induction involves a laparotomy. In spite of this complexity, a recent study has shown that in the model of pancreatitis induced by common bile duct injection of secretory phospholipase A_2, which also involves laparotomy, it is possible to detect an early abdominal hyperalgesia after 4 h of the induction (Camargo et al., 2011). It is interesting that these authors reported that the stimulation of the abdominal region of rats that had received ductal saline injection did not alter the intensity of stimulus necessary to induce a withdrawal behavior. We observed a similar result in the present study, where the sham group did not show any significant alteration of the withdrawal threshold. In contrast, animals submitted to CBDO showed a time-dependent decrease of the intensity of stimulus applied to the abdominal region during the period of evaluation (0–24 h). It is worth pointing out that this is the first description of abdominal hyperalgesia for CBDO-induced pancreatitis in literature. A previous single administration of EECp (100–400 mg/kg) was able to inhibit abdominal hyperalgesia after 6 h of CBDO. Moreover, one single dose of 400 mg/kg completely inhibited hyperalgesia after 12 h of obstruction and partially reduced this phenomenon after 24 h. A second administration of EECp after 12 h of CBDO totally reversed abdominal hyperalgesia at the 24 h time point, an effect that was analogous to morphine.

It is worth mentioning that EECp induced an anti-hyperalgesic effect even at the lower dose used, which did not adequately reduce MPO activity in the pancreatic tissue. In the same way, even with the lack of effect on pancreatic MPO activity after 24 h of CBDO in animals pretreated with one single dose of EECp, hyperalgesia was still reduced by this pretreatment. These observations indicate that EECp seems to have a more pronounced anti-hyperalgesic than anti-inflammatory effect, or may have some components able to affect nociceptive pathways. The mechanisms underlying this effect are not understood, and further experiments are necessary to elucidate these facts.

Furthermore, this anti-hyperalgesic effect of EECp is reinforced by the findings of Santos et al. (2011). We observed that treatment with EECp reduced the nociception induced by acetic acid injection in the mouse abdominal region or formalin injection in the mouse paw, which is consistent with an analgesic component in this extract. As these effects could be affected by a possible central action of EECp, experiments were performed with rats treated or not with EECp in the open field. Since no alteration of the locomotor activity of rats was found with this apparatus, this suggests that EECp does not cause central effects that could interfere in the measurement of abdominal hyperalgesia, reinforcing the results about the anti-hyperalgesic effects of EECp. This observation is in conformity with the findings that EECp administration to mice, in the same dose range used in the present study, did not change the performance of mice in the Rota-rod test (Santos et al., 2011).

These actions of EECp might be caused by its components. In this way, there are a number of compounds described in this plant, and many of them are phenolic compounds, especially flavonoids (Mendes et al., 2000; Bahia et al., 2005, 2010; Silva et al., 2011). The components of EECp are still unknown, and the present study has suggested that rutin - but not apigenin (found in the leaves of *Caesapinia pyramidalis*; Bahia et al., 2005), quercetin or baicalein—is likely to be present in EECp. Future studies are necessary to completely identify rutin and any other components of this extract that may be of value in understanding the effects of EECp. In the case of rutin, it was demonstrated that this flavonoid possesses anti-inflammatory, antioxidant and anti-nociceptive effects that could account for the amelioration of acute pancreatitis in rats, as observed in the present study. For instance, rutin decreased paw edema induced by carrageenan and arthritis induced by adjuvant plus carrageenan in rats (Guardia et al., 2001; Rotelli et al., 2003). It also reduced nociception induced by glutamate in mice paws (Lapa et al., 2009), renal inflammation induced by cysplatin in rats (Arjumand et al., 2011)

and cystitis induced by cyclophosphamide in mice (Boeira et al., 2011). These studies reinforce the possibility of rutin's role in the effects observed in CBDO-induced pancreatitis in rats.

Conclusions

In summary, the present study demonstrated that EECp decreased abdominal hyperalgesia and the local and systemic inflammation that occur during CBDO-induced pancreatitis, which further confirms the anti-inflammatory and antinociceptive activities of this extract. This ethanol extract is of great interest as a source of novel molecules for developing strategies more appropriate in treating pancreatitis. Future studies are necessary in order to better identify the components of EECp and the mechanisms underlying these activities.

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