J. Pharm. Pharmacol. 1999, 51: 1447–1453
Received May 4, 1999
Accepted July 7, 1999

Anti-inflammatory and Antinociceptive Effects in Rodents of the Essential Oil of *Croton cajucara* Benth

ELIETE J. B. BIGHETTI, CLÉLIA A. HIRUMA-LIMA*, JULIANO S. GRACIOSO AND ALBA R. M. SOUZA BRITO†

*Departimento de Farmacologia, Faculadade de Ciências Médicas, Kingston, *Departimento de Fisiologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas and †Institut Biologia e Saúde Pública, Fundação Universidade do Tocantins, Porto Nacional, TO, Brazil*

Abstract

The plant *Croton cajucara* Benth. (Euphorbiaceae) is widely used in Amazonian folk medicine for the treatment of a wide range of illnesses. In this investigation the analgesic and anti-inflammatory properties of the essential oil from the bark of *C. cajucara* Benth., administered orally, were determined in several standard rodent models of pain and inflammation.

We observed that pretreatment with essential oil significantly reduced the latency of sleeping time evoked by pentobarbital compared with the control group ($P < 0{\cdot}001$). Doses of 100 or 1000 mg kg^{-1} also increased the sleeping time induced by pentobarbital ($30{\cdot}9 \pm 3{\cdot}91$ and $52{\cdot}1 \pm 15{\cdot}6$ min, respectively) compared with the negative control ($12{\cdot}4 \pm 4{\cdot}27$ min). We investigated the antinociceptive effect of the essential oil in chemical (acetic acid) and thermal (hot-plate) models of nociception in mice. Dipyrone (200 mg kg^{-1}) and the highest doses of the essential oil (1000 mg kg^{-1}) significantly inhibited acetic acid-induced abdominal constriction in mice ($5{\cdot}00 \pm 1{\cdot}38$ and $6{\cdot}8 \pm 2{\cdot}1$ constrictions, respectively) compared with the negative control ($33{\cdot}1 \pm 2$). The same dose of essential oil also raised the pain thresholds of mice in the hot-plate test and significantly ($P < 0{\cdot}05$) increased the latency at all observation times. In acetic acid-induced abdominal constriction in mice pretreatment of the animals with naloxone (5 mg kg^{-1}) significantly reversed the analgesic effect of morphine and of the essential oil at the highest dose (1000 mg kg^{-1}).

The essential oil of *C. cajucara* was also investigated for its anti-inflammatory properties. At the lowest dose (100 mg kg^{-1}) the essential oil had anti-inflammatory effects in animal models of acute (carrageenin-induced paw oedema in mice) and chronic (cotton pellet granuloma) inflammation. The essential oil at doses of 50, 100 and 200 mg kg^{-1} significantly and dose-dependently inhibited carrageenan-induced oedema (49 ± 5; 37 ± 5; 34 ± 8 mg, respectively) compared with the negative control (74 ± 8 mg). The essential oil (100 mg kg^{-1}) also inhibited chronic inflammation by 38% whereas diclofenac inhibited it by 36%. However, the essential oil did not inhibit the migration of neutrophils into the peritoneal cavity.

These data show that the essential oil from *C. cajucara* contains compounds that had a significant antinociceptive effect when the oil was administered at the highest dose. This effect seems to be related to interaction with the opioid system. The essential oil also had a significant anti-inflammatory effect in acute and chronic inflammation models when administered at lower doses. This effect seems to be related to cyclooxygenase inhibition.

Croton cajucara Benth, a herb which grows exclusively in the Amazon forest, is used in folk medicine as an agent for the treatment of diabetes, diarrhoea, gastrointestinal disorders and liver diseases (Di Stasi et al 1989); the bark and leaves of the plant are commonly used in the form of tea or as powdered and dried pills (Souza Brito & Nunes 1997).

When our group reported the antiulcerogenic activity of dehydrocrotonin, a diterpene isolated

Correspondence: A. R. M. Souza Brito, Departamento de Fisiologia e Biofísica, I. B., Caixa Postal 6109, Universidade Estadual de Campinas, CEP13.083-970, Campinas, SP, Brazil.

from *C. cajucara* bark, in different ulcerogenic models using mice and rats (Souza Brito et al 1998), studies of dehydrocrotonin demonstrated its antinociceptive effect in the hot-plate and acetic acid-induced constriction tests (Carvalho et al 1996). Cajucarinolide and isocajucarinolide, two new clerodane diterpenes, have also been isolated from the bark of *C. cajucara*. These compounds have anti-inflammatory activity and inhibit bee venom phospholipase A_2 in-vitro (Ichihara et al 1992).

It was recently demonstrated that the essential oil from *C. cajucara* has important antiulcerogenic properties without significant acute toxicological effects (Hiruma-Lima et al 1999).

In this study, we have analysed another component of the bark, the essential oil, possibly involved in the anti-inflammatory and antinociceptive effects of this plant.

Materials and Methods

Preparation and analysis of the essential oil

The stem bark of *C. cajucara* Benth. was collected from our experimental plantation in Benfica, near Belém, Pará, Brazil. A voucher specimen (no. 247) was identified by Nelson A. Rosa and is deposited in the IAN Herbarium, Belém, Brazil. The air-dried and milled bark (20 kg) was subjected to steam distillation for 6 h. Preliminary GC–FID and GC–MS analysis performed with a Hewlett-Packard model 6890 chromatograph equipped with an HP-5 capillary column showed the essential oil to contain mainly sesquiterpenes. Complete analyses of the samples are in progress. The essential oil was emulsified in 12% Tween 80 before administration to the animals.

Animals

All experiments were performed on male albino Wistar rats, 180–230 g, and albino Swiss, 30–35 g, or Balb/C, 25–30 g, mice from the Central Animal House of the Universidade Estadual de Campinas (CEMIB/UNICAMP). The animals were fasted before all assays because the standard drugs or essential oil were always administered orally (p.o.), except for morphine, indomethacin and dexamethasone, which were injected subcutaneously (s.c.); pentobarbital sodium and naloxone were administered intraperitoneally (i.p.). The animals had free access to certified Nuvilab CR-a (Nuvital) and tap water and were kept in the animal house under standard conditions of 12 h dark–12 h light, humidity (55%) and temperature ($22 \pm 1^{\circ}$C). All experiments were performed in the morning, and according to current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann 1983). The Ethics Committee of Unicamp, Brazil, approved all experimental protocols.

Drugs

The substances and reagents were prepared immediately before use. Acetic acid, Giemsa, (Merck), pentobarbital sodium (Cristalia), dipyrone sodium (Hoechst) sodium diclofenac (Ciba-Geigy), dexamethasone (Prodome), Tween 80 (Synth) and ketamine chloride (Konig) were obtained commercially in Brazil. Indomethacin, carrageenan type IV, lipopolysaccharide, morphine hydrochloride and naloxone hydrochloride were from Sigma (MO). All drugs were diluted with physiological saline except for indomethacin which was diluted with sodium bicarbonate (5%). All reagents used were of a high grade of purity.

Pentobarbital-induced sleep

Thirty minutes after administration of different doses (50, 100 or 1000 mg kg^{-1}) of essential oil from *C. cajucara*, Swiss mice were given pentobarbital (40 mg kg^{-1}, i.p.). The latency to sleep (time between pentobarbital administration and loss of the righting reflex) and duration of sleeping time (time between loss and subsequent recovery of the righting reflex) were recorded for animals pretreated with 12% Tween 80 (vehicle) or with the drug (Pieretti et al 1991).

Abdominal constriction caused by intraperitoneal injection of acetic acid

The response to intraperitoneal injection of 0·6% acetic acid solution, contraction of the abdominal muscle and stretching of the hind limbs, was induced according to procedures described by Koster et al (1959). Animals (Swiss mice) were pretreated with the essential oil (100, 500 and 1000 mg kg^{-1}) and negative control animals received a similar volume of vehicle (10 mL kg^{-1}). Positive control mice received dipyrone (200 mg kg^{-1}, p.o.). The drugs were administered 30 min before injection of acetic acid. After the challenge, pairs of mice were placed in separate transparent boxes and the number of abdominal constrictions was counted over the period from 6 to 21 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions.

Hot-plate test

The hot-plate test was used to measure latencies according to the method described by Eddy & Leimback (1953), with minor modifications. In these experiments, the hot-plate apparatus (Ugo Basile, Model-DS 37) was maintained at $56 \pm 1^{\circ}C$. Animals (Swiss mice) were placed in a 24-cm diameter glass cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as latency. Latency was recorded for control mice (treated with vehicle) and for animals pretreated with morphine ($10\,mg\,kg^{-1}$, s.c.) used as positive control, or pretreated with essential oil ($1000\,mg\,kg^{-1}$, p.o.). All substances were administered 30 min before the beginning of the experiment. Animals were selected 24 h before on the basis of their reactivity to the test. Only the animals with a reaction within the range 3·9–6·9 s were selected. Negative control animals received a similar volume of 12% Tween 80 ($10\,mL\,kg^{-1}$, p.o.). All animals were observed before (0) and 30, 60 and 90 min after drug administration. A latency period of 30 s was defined as complete analgesia.

Investigation of the mechanism of the analgesic action of the essential oil

The possible participation of the opioid system in the antinociceptive effect of the essential oil from *C. cajucara* was investigated by the method described by Trentin et al (1997). To investigate this mechanism we also used the model of acetic acid-induced abdominal constriction in mice, with some modifications. Animals were pretreated with naloxone ($5\,mg\,kg^{-1}$, i.p.) 15 min before oral administration of the essential oil ($1000\,mg\,kg^{-1}$) and subcutaneous administration of morphine ($10\,mg\,kg^{-1}$). Control animals received a similar volume of vehicle ($10\,mL\,kg^{-1}$, p.o.).

Carrageenan-induced paw oedema in mice

The method used was similar to that described by Henriques et al (1987). Male mice were pretreated with indomethacin ($30\,mg\,kg^{-1}$, s.c.), as positive control, or with essential oil (50, 100 or $200\,mg\,kg^{-1}$, p.o.) 30 min before injection of 300 μg carrageenan (1% suspension in normal saline). The paw volume was determined 4 h after carrageenan injection. The increase in weight caused by the irritant was calculated by subtracting the weight of the untreated left paw from that of the treated right paw.

Granuloma cotton pellet in rats

Groups of Wistar rats were used to determine the effects of the essential oil ($100\,mg\,kg^{-1}$) on the proliferative phase of granuloma (the dry weight of the pellet 6 days after treatment) according to the method of Swingle & Shideman (1972). Dental cotton rolls (Johnson & Johnson) were cut into 20–30-mg pellets which were sterilized and implanted subcutaneously into the dorsal region of the rats. The animals were kept under ketamine anaesthesia. Different groups of animals were treated orally with $10\,mL\,kg^{-1}$ vehicle (control group); diclofenac ($5\,mg\,kg^{-1}$) was used as a standard reference drug. The animals were treated daily for 6 days. On the 7th day, they were killed by cervical dislocation and the granulomatous tissues were removed. The pellets were dried overnight at 60°C and the granulomas were weighed. The difference between the final and initial weights was regarded as the granulomatous tissue produced.

Migration of neutrophils to the peritoneal cavity

Groups of five male Balb/C mice were used to study the peritoneal exudate (Popper & Watnick 1974). The essential oil of *C. cajucara* was studied for its capacity to inhibit cellular migration to the peritoneal cavity. Drugs or vehicle were administered orally to groups of five Balb/C mice. LPS (200 ng/cavity) and Ca^{+2}-free phosphate-buffered saline (PBS) were injected into the peritoneal cavity 30 min later, and after 6 h the animals were killed. After gentle massage, peritoneal exudates were removed and total leucocytes determined in a Neubauer chamber; the differential leucocyte morphologies were also determined by microscopic counting of May–Giemsa-stained slides. The inhibitory effect of essential oil ($100\,mg\,kg^{-1}$) on neutrophil migration was compared with that of the negative (12% Tween 80) and positive (indomethacin, $20\,mg\,kg^{-1}$, s.c., and dexamethasone, $0{\cdot}5\,mg\,kg^{-1}$) controls, 1 h after intraperitoneal administration of the stimuli.

Statistical analysis

Results are presented as means ± s.d. or s.e.m. All data were analysed statistically by analysis of variance followed by the Dunnett pairwise test. A P value $< 0{\cdot}05$ was considered to be indicative of significance.

Results and Discussion

Previous chromatographic analysis has shown that the essential oil obtained from *Croton cajucara* bark contains no trace of dehydrocrotonin. Thus,

the anti-inflammatory and antinociceptive effect of the essential oil can be attributed solely to its own composition, mainly terpenes.

The potential analgesic and anti-inflammatory effects of the essential oil were investigated. In this study, pretreatment with essential oil significantly and dose-dependently increased the latency of sleeping time evoked by pentobarbital, as shown in Table 1.

All doses of the essential oil from *C. cajucara* significantly reduced the latency between pentobarbital administration and loss of the righting reflex. Pretreatment with doses of 100 or 1000 mg kg^{-1} increased the sleeping time induced by pentobarbital.

It is already known that many neurosedative drugs tend to increase sleeping time and reduce locomotor activity (Baldessarini 1990). Although this effect might result from a sedative and not necessarily analgesic property, this does not seem to be so for the essential oil of *C. cajucara*. In previous studies (Hiruma-Lima et al 1999) in which we evaluated the toxicity of the essential oil, we also observed that some significant alterations, such as reduced spontaneous activity, occurred only above an oral dose of 2·2 g kg^{-1}. Behavioural observations showed that the doses selected had no significant effect on the spontaneous activity of the animals.

To verify the possible analgesic effect of the essential oil we used two models of induced pain, chemical (abdominal constriction test) and thermal injury (hot-plate test). Our data showed that oral administration of the essential oil inhibited acetic acid-induced constriction in mice, as can be seen from Table 2.

Only the highest dose (1000 mg kg^{-1}) of the essential oil significantly reduced the number of constriction and stretching episodes induced by 0·6% acetic acid solution. The amount of protection by dipyrone, used as a positive control in this model, was 85% and the dose-dependent protective effect of the essential oil was 80% at the highest dose. When it was tested at 100 and 500 mg kg^{-1}, the essential oil had no significant protective effect against painful stimulation with acetic acid (17 and 38% inhibition, respectively).

Table 1. Effects of different oral doses of the essential oil from *C. cajucara* on sleep induced by intraperitoneal pentobarbital (40 mg kg^{-1}) in mice.

Treatment	n	Dose (mg kg^{-1})	Latency (min)	Duration (min)
Control	8	–	7·04 ± 1·35	12·4 ± 4·2
Oil	7	50	4·35 ± 0·77**	22·8 ± 9·6
Oil	6	100	4·41 ± 0·43**	30·9 ± 3·9*
Oil	8	1000	4·60 ± 0·14**	52·1 ± 15**

Values are means ± s.d. *$P < 0·005$, **$P < 0·001$ compared with control (analysis of variance, $F_{(3·25)}$ latency = 15·1; duration = 19·5 ($P < 0·05$); Dunnett's Test).

Table 2. Effect of the essential oil from *C. cajucara*, given orally, against acetic acid-induced abdominal constriction in mice.

Treatment	n	Dose (mg kg^{-1})	Number of abdominal constrictions	Protection (%)
Control	13	–	33·10 ± 2·04	–
Dipyrone	10	200	5·00 ± 1·38*	85
Oil	10	100	27·5 ± 1·5	17
Oil	8	500	20·6 ± 2·1	38
Oil	10	1000	6·80 ± 2·10*	80

Values are means ± s.e.m. *$P < 0·001$ compared with control (analysis of variance, $F_{(4,46)} = 32·0$ ($P < 0·05$); Dunnett's test).

It is known that constriction induced by acetic acid is a non-selective model, e.g. morphine-like or aspirin-like compounds inhibit the pain in this model. Collier et al (1968) postulated that acetic acid acts indirectly by inducing the release of endogenous mediators that stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs, to narcotics and to other centrally active drugs (Vaz et al 1996). Thus, the abdominal constriction elicited by acetic acid is considered a less selective antinociceptive model and has been used as a non-specific test to assess the potential analgesic activity of drugs. The data obtained suggest that the analgesic activity of the highest dose of essential oil might be partially related to inhibition of prostaglandin synthesis, considering that abdominal constriction is related to sensitization of nociceptive receptors by prostaglandins (Ferreira & Vane 1974).

In the second phase of this investigation we studied the effects of the essential oil in the hot-plate test. The efficacy of the essential oil against a thermal stimulus in the hot-plate test indicated a possible central analgesic property of the compounds present in the oil. These results are presented in Table 3.

Essential oil (1000 mg kg^{-1}) significantly increased the reaction time of mice to the stimuli, as observed for morphine (10 mg kg^{-1}) used as a reference drug in this model. Both substances had significant antinociceptive effects compared with control values. At this time we assumed possible action of the essential oil on opioid receptors. We used naloxone, a non-selective antagonist of opioid receptors, in an attempt to gain some insight into the mechanisms involved in the antinociceptive

Table 3. Effect of the essential oil from *C. cajucara* (1000 mg kg^{-1}), given orally, in the hot-plate test in mice.

Observation time (min)	Latency (s)		
	Control	Morphine	Oil
0	5 ± 0·93	5·0 ± 0·7	5·25 ± 0·71
30	5·12 ± 1·13	13·1 ± 2·3*	8·50 ± 1·19*
60	5·25 ± 1·03	13·3 ± 1·2**	8·75 ± 1·28**
90	6·25 ± 1·91	12·1 ± 2·9**	9·88 ± 1·64*

Values are the means ± s.d. (N = 8). *$P < 0·05$, **$P < 0·001$ compared with control (analysis of variance, $F_{(2,21)}$ 0 min = 0·26 ($P > 0·05$); 30 min = 48·6; 60 min = 88·6; 90 min = 14·3 ($P < 0·05$); Dunnett's test).

properties of the essential oil. The data shown in Table 4 indicate that the non-selective opioid antagonist naloxone consistently reversed the anti-nociception induced by the essential oil and morphine against acetic acid-induced pain. Our results show that the essential oil at a high dose probably interacts with the opioid system.

Because naloxone, a classical morphine-receptor antagonist (Faden 1988), modified the analgesia induced by treatment with the essential oil, it is most likely that the effect results from action on morphine receptors. Furthermore, the influence of this essential oil on the reaction time of mice submitted to the hot-plate test is consistent with the interpretation that its analgesic property is indeed of central origin, but only at the highest doses. However, a peripheral analgesic action of the oil has not been ruled out.

Because of the folk medicine use of *Croton cajucara* infusion against liver inflammation, and because of the anti-inflammatory property of dehydrocrotonin (Carvalho et al 1996), we decided to test the effect of the essential oil in some classic models of inflammation.

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair (Vane & Bolting 1995). It is also known that anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (Sertié et al 1990). This, associated with the complexity of the inflammatory process, makes the use of different experimental models essential when conducting pharmacological trials.

Indomethacin and diclofenac, like most non-steroidal anti-inflammatory compounds, inhibit the biosynthesis of prostaglandins and this effect might explain their anti-inflammatory activity on carrageenan-induced paw oedema (Higgs et al 1979). On the other hand, dexamethasone, a steroidal anti-inflammatory drug, inhibits the phospholipase A_2 enzyme which is responsible for release of arachidonic acid (Vane & Bolting 1995).

When assayed on the classic mice paw oedema induced by carrageenan, the essential oil reduced the paw swelling as effectively as did indomethacin. The anti-oedematogenic responses obtained as a result of administration of *C. cajucara* essential oil (50, 100 and 200 mg kg^{-1}), indomethacin (20 mg kg^{-1}) and vehicle on the carrageenan-induced hind paw oedema in mice are shown in Table 5.

The essential oil at doses of 50, 100 and 200 mg kg^{-1}, significantly inhibited carrageenan-induced oedema by 34, 50 and 54%, respectively, 4 h after carrageenan administration. These responses were dose-dependent. The maximum effect of the oil was obtained with the highest dose used, i.e. 200 mg kg^{-1}. Indomethacin significantly inhibited paw oedema in mice by 67·5%. These data reveal the existence of a marked peripheral analgesic property of the essential oil at low dose.

This effect confirmed the results obtained for the constrictions induced by acetic acid, which involve the synthesis of prostaglandins or other endogenous

Table 4. Effects of the essential oil from *C. cajucara* on acetic acid-induced abdominal constriction in mice pretreated with naloxone (5 mg kg^{-1}).

Treatment	n	Dose (mg kg^{-1})	Number of abdominal constrictions	Protection (%)
Control	13	–	33·1 ± 2·0	–
Morphine	7	10	0*	100
Naloxone + morphine	7	100	27·7 ± 1·7	16·3
Naloxone + oil	7	1000	24·6 ± 3·1	25·7

Values are means ± s.d. *$P < 0·001$ compared with control (analysis of variance, $F_{(3,30)} = 38·4$ ($P < 0·05$); Dunnett's test).

Table 5. Effects of the essential oil from *C. cajucara* and of indomethacin on carrageenan-induced hind paw oedema in mice.

Treatment	n	Dose (mg kg^{-1})	Paw weight (mg)	Inhibition (%)
Control	11	–	74 ± 8	–
Indomethacin	14	20	24 ± 7**	67·5
Oil	24	50	49 ± 5*	34
Oil	22	100	37 ± 5**	50
Oil	10	200	34 ± 8**	54

Values are means ± s.e.m. *$P < 0·005$, **$P < 0·001$ compared with control (analysis of variance, $F_{(4·76)} = 7·10$ ($P < 0·05$); Dunnett's test).

mediators of the inflammatory process in the nociceptive mechanism (Emin et al 1992).

It is known that the acute inflammatory response consists of three main vascular effects: vasodilatation and increased vascular flow; increased vascular permeability; and leukocyte migration to the injured tissues.

Histamine and 5-HT are usually responsible for eliciting the immediate response of inflammation in rats, whereas the kinins and prostaglandins mediate the more prolonged delayed-onset responses (Di Rosa et al 1971; Goetzel 1980).

The cotton pellet granuloma bioassay in rats is frequently used as an experimental model to study the effect of drugs on chronic inflammation. Swingle & Shideman (1972) demonstrated that there are three phases of inflammation after pellet implantation. The last phase is cell proliferation between the third and sixth day; this phase can be inhibited by anti-inflammatory steroids such as dexamethasone and also by non-steroidal anti-inflammatory drugs. Thus, we decided to investigate whether the essential oil could modify the last phase of the cotton pellet granuloma.

The effects of the essential oil and diclofenac on the cotton pellet granuloma are summarized in Table 6.

Table 6. Effects of the essential oil from *C. cajucara* and of diclofenac on cotton-pellet granulomas in rats.

Phase	Treatment	n	Dose ($mg\,kg^{-1}$)	Pellet increase (mg)	Inhibition (%)
Proliferative	Control	6	–	$26{\cdot}1 \pm 3{\cdot}9$	0
Proliferative	Diclofenac	6	5	$16{\cdot}8 \pm 2{\cdot}9$*	36
Proliferative	Oil	7	100	$16{\cdot}1 \pm 2{\cdot}8$*	38

Values are means ± s.d. *$P < 0{\cdot}001$ compared with control (analysis of variance, $F_{(2,15)} = 29{\cdot}1$ ($P < 0{\cdot}05$); Dunnett's test).

The essential oil significantly inhibited (by 38%) the proliferative phase of the granuloma, as also did diclofenac (36% inhibition). We suggest that the anti-inflammatory effect of the essential oil is also related to cyclooxygenase inhibition.

Finally, we studied the effects of essential oil and of two positive controls, dexamethasone and indomethacin, on induced neutrophil migration in mice. It has been suggested that the effect of drugs on neutrophil migration is a characteristic of anti-inflammatory agents whose mechanism of action is related not to inhibition of the arachidonic acid pathway but to inhibition of prostaglandin release by aspirin-like drugs (Flower 1990). The data obtained are shown in Table 7.

Neutrophil migration also was not significantly reduced in the peritonitis model. Dexamethasone, but not the essential oil or indomethacin, inhibited neutrophil migration into the peritoneal cavity by 88%. These data reveal that the anti-inflammatory effect of the essential oil is not similar to that of steroidal anti-inflammatory drugs. On the other hand, neither the anti-oedema nor the antiproliferative effects of the essential oil were correlated with an ability to reduce the number of neutrophils migrating into the peritoneal cavity.

These results, when taken together, lead us to conclude that the essential oil has both anti-inflammatory and analgesic activity. Our data provide a scientific basis for the utilization of this plant in folk medicine to treat some inflammatory processes. Studies are in progress to identify the components of the essential oil responsible for the effects described.

Acknowledgements

The authors are indebted to Dr Domingos Sávio Nunes for the essential oil of *Croton cajucara*. This study was supported by Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP, Fellowship, no. 97/00682-0, and grant no. 97/04365–9), Capes and CNPq.

Table 7. Effect of the essential oil from *C. cajucara* and indomethacin of dexamethasone on the white cells of peritoneal exudates in mice.

Treatment (before LPS)[a]	Dose ($mg\,kg^{-1}$)	Number of leukocytes ($\times 10^5$ mL)				
		Leucocytes	Inhibition (%)	Neutrophils	Inhibition (%)	Macrophages
Control	$10\,mL\,kg^{-1}$	13 ± 2	–	7 ± 1	–	$5{\cdot}0 \pm 0{\cdot}8$
Indomethacin	20	14 ± 3	–	6 ± 2	15	10 ± 3
Dexamethasone	0·5	7 ± 1	43	$0{\cdot}8 \pm 0{\cdot}1$*	88·5	$7{\cdot}0 \pm 0{\cdot}5$
Oil	100	14 ± 2	–	$4 \pm 0{\cdot}6$	42·8	9 ± 2

[a]200 ng/cavity LPS. Values are means ± s.e.m. of results from five animals. *$P < 0{\cdot}05$ compared with control (analysis of variance, $F_{(3,17)}$ leucocytes $= 3{\cdot}31$ ($P > 0{\cdot}05$); neutrophils $= 3{\cdot}18$ ($P < 0{\cdot}05$); macrophages $= 2{\cdot}67$ ($P < 0{\cdot}05$); Dunnett's test).

References

Baldessarini, R. J. (1990) Drugs and the treatment of psychiatric disorders. In: Gilman, A., Goodman, L. S., Rall, T. W., Murad, F. (eds) The Pharmacological Basis of Therapeutics, 8th edn, Macmillan, New York, pp 383–435

Carvalho, J. C., Silva, M. F., Maciel, M. A., Pinto, A., Nunes, D. S., Lima, R. M., Bastos, J. K., Sarti, S. J. (1996) Investigation of anti-inflammatory and antinociceptive activities *trans*-dehydrocrotonin, a 19-*nor*-clerodane diterpene from *Croton cajucara* Part 1. Planta Med. 62: 402–404

Collier, H. O. J., Dinnen, L. C., Johnson, C. A., Schneider, C. (1968) The abdominal constriction response and its suppression by analgesic drugs in mouse. Br. J. Pharmacol. Chemother. 32: 295–310

Di Rosa, M., Papadimitriou, J. M., Willoughby, D. A. (1971) A histopathological and pharmacological analysis of the mode of action of non-steroidal anti-inflammatory drugs. J. Pathol. 105: 329–356

Di Stasi, L. C., Santos, E. M. C., Moreira dos Santos, C., Hiruma, C. A. (1989) Plantas Medicinais da Amazônia, Ed. UNESP, São Paulo

Emim, J. A. S., Oliveira, A. B., Lapa, A. J. (1992) Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, duartin, and clanssequinone in rats and mice. J. Pharm. Pharmacol. 46: 118–122

Eddy, N. B., Leimback, D. (1953) Synthetic analgesics II. Dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther. 107: 385–393

Faden, A. I. (1988) Role of thyrotropin-releasing hormone and opiate receptor antagonists in limiting central nervous system injury. Adv. Neurol. 45: 531–546

Ferreira, S. H., Vane, J. R. (1974) New aspects of the mode of action of nonsteroid anti-inflammatory drugs. Ann. Ver. Pharmacol. 14: 57–73

Flower, R. J. (1990) Lipocortin. Prog. Clin. Biol. Res. 349: 11–25

Goetzel, E. J. (1980) Mediators of immediate hypersensitivity derived from arachidonic acid. N. Engl. J. Med. 303: 822–825

Henriques, M. G. M. O., Silva, P. M. R., Martins, M. A., Flores, C. A., Cunha, F. Q., Assreuy-Filho, J., Cordeiro, R. S. B. (1987) Mouse paw oedema. A new model for inflammation? Braz. J. Med. Biol. Res. 20: 243–249

Higgs, A. G., Flower, J. R., Vane, R. J. (1979) A new approach to anti-inflammatory drugs. Biochem. Pharmacol. 28: 1959–1961

Hiruma-Lima, C. A., Gracioso, J. S., Nunes, D. S., Souza Brito, A. R. M. (1999) Effects of an essential oil from the bark of *Croton cajucara* Benth. on experimental gastric ulcer models in rats and mice. J. Pharm. Pharmacol. 51: 1–7

Ichihara, Y., Takeya, J., Hitotsuyanagi, Y., Morita, H., Okuyama, S., Suganuma, M., Fujiki, F., Motidome, M., Itokawa, H. (1992) Cajucarinolide and Isocajucarinolide: anti-inflammatory diterpenes from *Croton cajucara*. Planta Med. 58: 549–551

Koster, R., Anderson, M., De Beer, E. J. (1959) Acetic acid for analgesic screening. Fed. Proc. 18: 418–420

Pieretti, S., Capasso, A., Di Giannuario, A., Loizzo, A., Sorrentino, L. (1991) The interaction of peripherally and centrally administered dexamethasone and RU-38486 on morphine analgesia in mice. Gen. Pharmacol. 22: 929–933

Popper, T. L., Watnick, A. S. (1974) In: Scherrer, R. A., Whitehouse, M. (Eds), Anti-inflammatory Agents—Chemistry and Pharmacology. Vol. 1, Academic Press, London, pp 245–290

Sertié, J. A., Basile, A. C., Panizza, S., Matida, A. K., Zelnik, R. (1990) Anti-inflammatory activity and sub-acute toxicity of artemetin. Planta Medica 56: 36–40

Souza Brito, A. R. M., Nunes, D. S. (1997) Ethnopharmacology and the sustainable development of new plant-derived drugs. Ciência e Cultura 49: 402–408

Souza Brito, A. R. M., Rodríguez, J. A., Hiruma-Lima, C. A., Haun, M., Nunes, D. S. (1998) Anti-ulcerogenic activity of *trans*-dehydrocrotonin from *Croton cajucara*. Planta Med. 64: 126–129

Swingle, K. F., Shideman, F. E. (1972) Phases of the inflammatory response to subcutaneous implantation of cotton pellet and their modification by certain anti-inflammatory drugs. J. Pharmacol. Exp. Ther. 183: 226–234

Trentin, A. P., Santos, A. R. S., Miguel, O. G., Pizzolatti, M. G., Yunes, R. A., Calixto, J. B. (1997) Mechanism involved in the antinociceptive effect in mice of the hydroalcoholic extract of *Siphocampylus verticillatus*. J. Pharm. Pharmacol. 49: 567–572

Vane, J. R., Bolting, R. M. (1995) New insights into the mode of action of anti-inflammatory drugs. Inflam. Res. 44: 1–10

Vaz, Z. R., Caechienl Filho, V., Yunes, R. A., Calixto, J. B. (1996) Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxybenzofuran, a novel xanthoxyine derivative, on chemical and thermal models of nociception in mice. J. Pharmacol. Exp. Ther. 278: 304–312

Zimmerman, M. (1983) Ethical guidelines for investigation of experimental pain in conscious animals. Pain 16: 109–110