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Analgesic and anti-inflammatory effects of essential oils of Eucalyptus

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Abstract

Many species of the genus *Eucalyptus* from the Myrtaceae family are used in Brazilian folk medicine for the treatment of various medical conditions such as cold, flue, fever, and bronchial infections. In the current investigation, we evaluated the analgesic and anti-inflammatory effects of essential oil extracts from three species of *Eucalyptus* employing various standard experimental test models. Using acetic acid-induced writhes in mice and hot plate thermal stimulation in rats, it was shown that the essential oils of *Eucalyptus citriodora* (EC), *Eucalyptus tereticornis* (ET), and *Eucalyptus globulus* (EG) induced analgesic effects in both models, suggesting peripheral and central actions. In addition, essential oil extracts from the three *Eucalyptus* species produced anti-inflammatory effects, as demonstrated by inhibition of rat paw edema induced by carrageenan and dextran, neutrophil migration into rat peritoneal cavities induced by carrageenan, and vascular permeability induced by carrageenan and histamine. However, no consistent results were observed for some of the parameters evaluated, both in terms of activities and dose–response relationships, reflecting the complex nature of the oil extracts and/or the assay systems used. Taken together, the data suggest that essential oil extracts of EC, ET, and EG possess central and peripheral analgesic effects as well as neutrophil-dependent and independent anti-inflammatory activities. These initial observations provide support for the reported use of the eucalyptus plant in Brazilian folk medicine. Further investigation is warranted for possible development of new classes of analgesic and anti-inflammatory drugs from components of the essential oils of the *Eucalyptus* species.

Keywords: Analgesic; Anti-inflammatory; Essential oils; Eucalyptus

1. Introduction

Many species of the genus *Eucalyptus* from the Myrtaceae family are used in Brazilian folk medicine for a variety of medical conditions. For instance, hot water extracts of dried leaves of *Eucalyptus citriodora* (EC) Hook are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies for the symptoms of respiratory infections, such as cold, flue, and sinus congestion. Essential oils from *Eucalyptus* species are also widely used in modern cosmetics, food, and pharmaceutical industries (Gomes-Carneiro et al., 1998). In this regard, monoterpenoid components of the aromatic constituents of the oils are commercially available for the treatment of the common cold and other symptoms of respiratory infections (Trigg, 1996; Cockcroft et al., 1998; Juergens et al., 1998a). Phytochemical analysis has shown that the profile of the monoterpenoids varies among the *Eucalyptus* species, with potential variations in medicinal properties. *Eucalyptus citriodora* has been shown to contain 60% of the monoterpenoid, citronellal, whereas *Eucalyptus tereticornis* (ET) and *Eucalyptus globulus* (EG) contain 60–90% of eucalyptol (1,8-cineole), another major monoterpenoid (Juergens et al., 1998a). While citronellal is effective against bacterial and fungal infections (Pattnaik et al., 1996), eucalyptol has been reported to inhibit the production/synthesis of tumor necrosis factor-α, interleukin-1β, leukotriene B_4, and thromboxane B_2 in inflammatory cells (Juergens et al., 1998a,b). These findings provide support at least for some of the traditionally accepted medicinal uses of eucalyptus in the Brazilian society, although the relationship may not be that direct. The aim of the present study was to evaluate the analgesic and anti-inflammatory effects of the essential oils of the *Eucalyptus* species, EC, ET, and EG, using various standard experimental test models. To our knowledge, this is the first attempt addressing such ethnopharmacological properties of eucalyptus in a comprehensive manner.

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2. Materials and methods

2.1. Animals

Male Wistar rats (150–250 g), and either male or female Swiss albino mice (20–25 g) were used. These animals were obtained from colonies maintained at the Departamento de Biologia, Centro de Ciências, Universidade Federal do Ceará (Fortaleza, Brazil). The animals were housed in groups of 6–10 under environmentally controlled conditions with free access to water and standard food. Food was withheld overnight prior to experiments while water was still provided ad libitum. The handling and use of animals were in accordance to the institutional guidelines.

2.2. Drugs and chemicals

The following drugs and chemicals were used: morphine (Laboratório Enila Ltda., RJ, Brazil), acetylsalicylic acid (Bristol-Mayers Squibb, Brazil), carrageenan (BDH, UK), dextran 70 (Pharmacia, USA), histamine, prostaglandin I_2, mepiramine (Sigma Chemical Co., MO, USA), dexamethasone (MSD, Brazil), and Evans blue dye (Aldrich Chemical Co., USA). All other chemicals were of analytical grade.

2.3. Plant materials and preparation of essential oil extracts

Essential oils were extracted from samples of the aerial parts of EC and ET, cultivated in the Medicinal Garden of the Federal University of Ceará (Fortaleza, Brazil). The taxonomic identity of the plants was confirmed by Professor G. Fernandes, Department of Biology, Federal University of Ceará and voucher samples were deposited in the Prisco Bezerra Herbarium No. 21 444 (EC) and No. 24 743 (ET), Department of Biology, Federal University of Ceará, Fortaleza, Ceará, Brazil. In the extraction process, leaves were air-dried and ground into a fine powder. The oils were then extracted according to Craveiro et al. (1981), using a vapor extractor which provided a yield of 1%. The essential oils of EG, which were also extracted as above providing a 1% yield, were generously provided by Dr. F.A. Matos of Laboratorio de Produtos Naturais-UFC, Brazil.

2.4. Analgesic activity

2.4.1. Acetic acid-induced writhing in mice

The writhing acetic acid test was performed in mice as originally described by Siegmund et al. (1957). This method was used to preferentially evaluate possible peripheral effects of the essential oils as analgesic substances. Groups of 10 mice were fasted overnight prior the start of the experiment, while given free access to water. The essential oils of EC, ET, and EG (0.1, 10, and 100 mg/kg), acetylsalicylic acid (250 mg/kg), or equivalent volumes of vehicle (0.9% saline plus 4% Tween-20) were injected subcutaneously 30 min prior to the injection of acetic acid (0.6%, 10 ml/kg). Acetylsalicylic acid is a well known peripheral analgesic drug and it was used as a positive control in the present investigation. The mice were then placed in an observation box, and the number of writhes was counted for 20 min after acetic acid injection (Siegmund et al., 1957).

2.4.2. Hot plate test in rats

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of the essential oils (MacDonald et al., 1946). The central analgesic drug, morphine, was used as a positive control substance. For three consecutive days preceding the experiments, rats (200–250 g) were placed on a plate maintained at room temperature for 15 min each day. Essential oil extracts from EC, EG, or ET were given by intraperitoneal injection at a dose of 10 or 100 mg/kg. Morphine (10 mg/kg), and vehicle were also administered in some animals by the same route. Each animal was then placed gently on to a 55 °C hot plate. Latency to exhibit nociceptive responses, such as licking paws or jumping off the hot plate, was determined 15, 30, 45, 60, and 90 min after administration of the test substances or vehicle (MacDonald et al., 1946).

2.5. Anti-inflammatory activity

2.5.1. Inflammatory paw edema in rats

This assay was determined as described by Winter et al. (1962). Paw edema was induced by a single 0.1 ml subplantar injection of carrageenan (200 μg/paw) or dextran (300 μg/paw), containing prostaglandin I_2 (PGI_2, 200 ng/paw), into the right hind paw of conscious rats (150–170 g). While carrageenan is known to result in at least neutrophil-linked edematous inflammation, the effect of dextrane is associated with mast cell degranulation and is less related to the action of neutrophils (Lo et al., 1982). Rat paw volume was measured immediately before the injection of the "irritant" substance and at regular selected time intervals (1, 2, 3, and 4 h) after injection of each of the *Eucalyptus* essential oils (10 or 100 mg/kg) or equivalent volume of vehicle, using a plethysmograph (model 7150, UGO Basile, Milan, Italy). Results were expressed as the increase in paw volume (in ml) calculated after subtraction of basal paw volume prior to irritant injection (Winter et al., 1962).

2.5.2. Neutrophil migration into peritoneal cavity of rats

Carrageenan diluted in sterile physiological buffer solution (PBS) (400 μg/ml) was injected intraperitoneally into rats (150–170 g) 1 h after subcutaneous injection of EC, ET, or EG essential oils at a dose of 100 mg/kg each (Flores et al., 1993). Three hours later, the animals received an injection of Evans blue dye (25 mg/kg in 2.5% PBS) and were immediately sacrificed. The peritoneal cavity was washed with 10 ml of PBS containing 5 U/ml heparin. The lavage fluid was removed, and total and differential cell counts

were performed (Flores et al., 1993; Brito et al., 1997). The results are expressed as the number of cells per milliliter of peritoneal wash fluid (Flores et al., 1993). The steroidal anti-inflammatory drug, dexamethasone (1 mg/kg, subcutaneously), was used as a positive control for inhibiting neutrophil migration. Control animals for basal value determination received equivalent volume of vehicle (4% Tween-20 in PBS).

2.5.3. Cutaneous vascular permeability in rats

The dorsal region of a rat was shaved and skin vascular permeability test was initiated by injection of carrageenan (400 μg) or histamine (10 μg), together with 200 ng prostaglandin I_2 in a total volume of 0.2 ml after 30 min pretreatment with either essential oils (10 or 100 mg/kg), dexamethasone (2 mg/kg), mepiramine (0.01 mg/kg) or vehicle (4% Tween-20 in PBS) alone administered per via intraperitoneal (Brito et al., 1997). In this regard, histamine is different from carrageenan in that it has a more direct effect of causing vascular permeability. Dexamethasone and mepiramine were used as positive controls for the inhibition of the carrageenan and histamine-induced vascular permeability, respectively. One hour after the injection of inflammatory stimulus (carrageenan or histamine), each rat received an intravenous injection of Evans blue dye (25 mg/kg in 2.5% PBS). The animals were sacrificed 1 h after the dye injection, and the skin was carefully dissected to reveal the blue spots of the invaded dye. Each spot was excised, and the Evans blue dye was extracted from the tissue with formamide (2 ml per spot) overnight at room temperature (Brito et al., 1997). The concentration of Evans blue dye was assessed by spectrophotometry at 600 nm. The results were expressed as Evans blue dye extracted per gram of tissue/spot in each animal.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. Statistical evaluations were made using ANOVA or Student's *t*-test, and values were considered significantly different when $P < 0.05$.

3. Results

3.1. Analgesic effects of essential oils of Eucalyptus

Intraperitoneal administration of essential oils of EC, ET, and EG (0.1, 10, and 100 mg/kg each) significantly decreased the number of acetic acid-induced writhes in mice compared to the animals that received vehicle only (Table 1). The writhe inhibitory effects of the oil extracts ranged from 43 to 73%. By comparison, 250 mg/kg acetylsalicylic acid produced nearly complete (i.e. 91% effectiveness) analgesia in this nociception model. While the analgesic effect induced by the essential oil of ET was dose-related, the effects produced by the oils of EC and EG were not. At the highest dose administered, EC was the most effective followed by ET and then EG (Table 1).

Using the hot plate test, it was also shown that intraperitonial administration of EC, ET, or EG (10 and 100 mg/kg each) significantly prolonged the reaction time at several time points after 30 min treatment, as compared to the corresponding control groups (Table 2). These effects of essential oils were dose-independent. Although the oil of ET appeared to induce higher analgesic activity in most cases, there was no consistent pattern of activity among the three essential oils (Table 2). As expected, in this analgesic testing model, morphine significantly prolonged the reaction time of the animals with relatively extended duration of stimulation, confirming centrally mediated activity.

3.2. Anti-inflammatory effects of essential oils of Eucalyptus

As shown in Tables 3 and 4, 100 mg/kg, but not 10 mg/kg, essential oils of EC, ET, and EG significantly reduced edema of the rat hind paws induced by carrageenan and dextran 1–4 h after administration, relative to control values. While, there was no clear difference in "edema-reducing" activity among the three types of essential oils in most of the experiments, the oil extracts from ET were more effective against dextran-induced paw edema compared to the other two extracts at the dose of 100 mg/kg (Table 4).

Table 1
Analgesic effects of essential oils of EC, ET, and EG (0.1, 10, and 100 mg/kg each), and acetylsalicylic acid (250 mg/kg) on acetic acid-induced writhes in mice

Treatment group	0.1 mg/kg		10 mg/kg		100 mg/kg	
	Mean ± S.E.M.	Percent inhibition	Mean ± S.E.M.	Percent inhibition	Mean ± S.E.M.	Percent inhibition
Control (vehicle)	38 ± 3 (14)	–	–	–	–	–
Acetylsalicylic acid	3.4 ± 0.4* (10)	91	–	–	–	–
EC	16 ± 2* (21)	57	19 ± 1* (20)	50	10 ± 1* (21)	73
ET	19 ± 2* (13)	50	17 ± 2* (13)	56	14 ± 1* (20)	63
EG	18 ± 3* (9)	53	21 ± 2* (16)	43	20 ± 1* (20)	48

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to control.

Table 2
Analgesic effects of essential oils of EC, ET, and EG (10 and 100 mg/kg each), and morphine (10 mg/kg) on heat stimulation response in the hot plate test in rat

Treatment group	Dose (mg/kg)	Time needed to respond after				
		15 min	30 min	45 min	60 min	90 min
Control (vehicle) (10)	–	7.0 ± 0.6	7.5 ± 0.7	6.0 ± 0.3	8.0 ± 1.0	5.5 ± 0.4
Morphine	10 (7)	6.0 ± 0.6	7.6 ± 0.9	8.6 ± 1.0	13.7 ± 2.5*	20.6 ± 4.7*
EC	10 (10)	11.7 ± 1.1*	5.0 ± 0.6	14.5 ± 1.7*	6.5 ± 0.7	10.0 ± 1.0*
	100 (19)	7.6 ± 0.6	8.0 ± 0.7	12.5 ± 1.3*	6.3 ± 0.6	5.5 ± 0.6
ET	10 (8)	13.3 ± 1.8*	6.5 ± 0.5	11.0 ± 1.8*	15.0 ± 2.0*	26.0 ± 4.0*
	100 (8)	6.3 ± 0.6	7.0 ± 0.8	25.4 ± 3.4*	8.1 ± 1.3	9.3 ± 1.3
EG	10 (8)	6.7 ± 0.7	10.8 ± 1.7*	7.1 ± 0.9	11.3 ± 1.9*	11.0 ± 2.0*
	100 (9)	5.0 ± 0.4	12.0 ± 1.9*	6.0 ± 0.5	11.0 ± 2.0*	6.5 ± 0.9

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to corresponding control.

Table 3
Effect of essential oils of EC, ET, and EG (10 and 100 mg/kg each) on edema of rat hind paws induced by carrageenan (200 μg/paw) plus PGI_2 (200 ng/paw)

Treatment group	Dose (mg/kg)	Edema volume (ml) during several hours			
		1 h	2 h	3 h	4 h
Control (vehicle) (18)	–	0.60 ± 0.03	0.50 ± 0.03	0.30 ± 0.02	0.30 ± 0.03
EC	10 (6)	0.70 ± 0.05	0.60 ± 0.04	0.50 ± 0.04	0.30 ± 0.03
	100 (6)	0.10 ± 0.01*	0.09 ± 0.03*	0.10 ± 0.01*	0.03 ± 0.01*
ET	10 (6)	0.60 ± 0.05	0.60 ± 0.04	0.30 ± 0.04	0.20 ± 0.03
	100 (6)	0.30 ± 0.10*	0.20 ± 0.09*	0.09 ± 0.04*	0.04 ± 0.03*
EG	10 (6)	0.60 ± 0.03	0.60 ± 0.03	0.40 ± 0.02	0.40 ± 0.01
	100 (6)	0.30 ± 0.04*	0.30 ± 0.05*	0.07 ± 0.01*	0.08 ± 0.02*

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to corresponding control.

Subcutaneous administration of essential oils of EC, ET, or EG (100 mg/kg each) also significantly reduced carrageenan-induced neutrophil migration into peritoneal cavity of rats 3 h after injection (Table 5). Compared to the effect of the positive control anti-inflammatory drug, dexamethasone (1 mg/kg), which produced almost total inhibition of neutophil migration, the essential oils caused inhibition by 70, 80, and 76%, respectively, relative to control.

Pretreatment of rats with essential oils of ET and EG significantly diminished vascular permeability induced by carrageenan (Table 6). The effect of EG was, however, more pronounced than that of ET. Only the extract of ET produced dose-dependent inhibition of carrageenan-induced vascular permeability (Table 6). Compared to the effect of dexamethasone (2 mg/kg), which produced an inhibition of 70%, the vascular permeability inhibitions caused by ET

Table 4
Effect of essential oils of EC, ET, and EG (10 and 100 mg/kg each) on the edema of rat hind paws induced by dextran (300 μg/paw) plus PGI_2 (200 ng/paw)

Treatment group	Dose (mg/kg)	Edema volume (ml) during several hours			
		1 h	2 h	3 h	4 h
Control (vehicle) (18)	–	0.70 ± 0.03	0.50 ± 0.02	0.50 ± 0.02	0.50 ± 0.02
EC	10 (6)	0.6 0 ± 0.06	0.50 ± 0.06	0.50 ± 0.02	0.40 ± 0.03
	100 (6)	0.40 ± 0.08*	0.30 ± 0.05*	0.20 ± 0.05*	0.30 ± 0.06*
ET	10 (6)	0.50 ± 0.03	0.50 ± 0.03	0.50 ± 0.03	0.40 ± 0.04
	100 (6)	0.10 ± 0.03*	0.02 ± 0.01*	0.07 ± 0.03*	0.04 ± 0.01*
EG	10 (6)	0.70 ± 0.05	0.70 ± 0.06	0.60 ± 0.06	0.50 ± 0.04
	100 (6)	0.40 ± 0.03*	0.40 ± 0.06*	0.40 ± 0.03	0.30 ± 0.03*

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to corresponding control.

Table 5
Effect of essential oils of EC, ET, and EG (100 mg/kg each), and dexamethasone (1 mg/kg) on carrageenan-induced neutrophil migration into rat peritoneal cavities

Treatment group	Dose (mg/kg)	Neutrophils × 10^3/ml	Percent inhibition
Control (vehicle) (6)	–	12.8 ± 0.9	0
Dexamethasone	1 (6)	0.40 ± 0.03*	97
EC	100 (6)	4.0 ± 0.1*	70
ET	100 (6)	2.5 ± 0.4*	80
EG	100 (6)	3.0 ± 0.3*	76

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to control.

Table 6
Effect of essential oils of EC, ET, and EG (10 and 100 mg/kg each), and dexamethasone (2 mg/kg) on Evans blue dye extravasation induced by subcutaneous injection of carrageenan (400 μg per site)

Treatment group	Dose (mg/kg)	Evans blue (μg/g of tissue)	Percent inhibition
Control (vehicle) (13)	–	17.2 ± 1.1	0
Dexamethasone	2 (8)	5.0 ± 0.6*	70
EC	10 (10)	18.0 ± 1.5	0
	100 (9)	15.4 ± 1.3	11
ET	10 (10)	13.2 ± 0.8*	24
	100 (10)	11.1 ± 1.0*	35
EG	10 (9)	10.0 ± 0.4*	43
	100 (10)	10.4 ± 1.0*	40

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to control.

and EG ranged from 24 to 43% (Table 6). By contrast, the essential oil of EC was without significant effect on carrageenan-induced vascular permeability, although at a relatively high dose it tended to produce some inhibition.

Table 7 shows that essential oils of EC and ET significantly inhibited vascular permeability induced by histamine.

Table 7
Effect of essential oils of EC, ET, and EG (10 and 100 mg/kg each), and mepiramine (0.01 mg/kg) on Evans blue dye extravagation induced by subcutaneous injection of histamine (10 μg per site)

Treatment group	Dose (mg/kg)	Evans blue dye (μg/g of tissue)	Percent inhibition
Control (vehicle) (31)	–	34.5 ± 2.4	0
Mepiramine	0.01 (8)	13.4 ± 1.7*	61
EC	10 (8)	13.0 ± 0.7*	63
	100 (8)	23.0 ± 1.0*	34
ET	10 (8)	33.0 ± 3.0	5
	100 (8)	27.0 ± 1.8*	32
EG	10 (8)	38.0 ± 1.6	0
	100 (8)	39.0 ± 2.1	0

The data represent the mean ± S.E.M. of the number of animals in parentheses.
* $P < 0.05$ compared to control.

However, the effect of the oil extract of EC was greater than that of ET. ET, but not EC, produced a dose-dependent effect. On the other hand, essential oils of EG had no effect on histamine-induced vascular permeability (Table 7). The histamine receptor antagonist drug, mepiramine (0.01 mg/kg), which was used as a positive control, elicited marked reduction (i.e. by 61%) of the vascular permeability caused by histamine, and by comparison only 10 mg/kg EC generated a similar (i.e. 63%) inhibitory effect (Table 7).

4. Discussion and conclusion

Previously, it was determined that the lethal dose 50 (LD_{50}) values of essential oil extracts from EC, ET, and EG were 190 ± 22 mg/kg, 240 ± 34 mg/kg, and 353 ± 64 mg/kg ($n = 10$), respectively, in mice. Therefore, the dosages of the eucalyptus oils used in the present study (i.e. 0.1, 10, and 100 mg/kg) were far below the LD_{50} values. Consequently, no apparent behavioral side effects were observed in the animals during our experimentation. The high LD_{50} values also suggest that the oil extracts were relatively safe or non-toxic to the animals.

Two different analgesic testing methods were employed in the current investigation with the objective of identifying possible peripheral and central effects of the test substances. Using both acetic acid-induced writhes and hot plate thermal stimulation, it was observed that the essential oils of EC, ET, and EG possessed analgesic effects against both models. This observation indicates that these eucalyptus oils have both peripheral (writhe reduction) and central (thermal reaction time prolongation) effects. Among the oils tested, extracts from EC demonstrated the highest peripheral anti-nociceptive activity, while extracts from ET were the most potent central anti-nociceptive substance. On the other hand, while both of these oil extracts induced moderate effects in reverse manners to the above, the essential oils of EG appeared to be the least effective in both animal models of nociception. Taken together, the data presented demonstrate that the essential oils of eucalyptus induce variable degrees of peripheral and central analgesic effects depending upon the *Eucalyptus* species where they are obtained from. These observations can provide useful information if a choice is desired to be made regarding the species of eucalyptus as a source of analgesic drugs.

From the present work alone, the mechanism(s) of the analgesic effects of the eucalyptus oils tested is not readily apparent. It can, however, be speculated that it may be linked to processes involved in the prevention of sensitization of the nociceptor, down-regulation of the sensitized nociceptor and/or blockade of the nociceptor at peripheral and/or central levels (Ferreira, 1990). One of the well characterized signaling systems believed to participate in this mechanism(s) is the arachidonic acid metabolic pathway. In this regard, it has been previously shown that at least eucalyptol, one of the major components of eucalyptus oils, inhibits

the production of prostaglandins and thromboxanes similar to acetylsalicylic acid (Juergens et al., 1998a).

The anti-inflammatory properties of the eucalyptus oils were studied using several standard pharmacological methods. The results generally reveal that the essential oils of EC, ET, and EG elicited anti-inflammatory activities of different intensities, as reflected by inhibition of rat paw edema caused by either carrageenan or dextran, carrageenan-induced neutrophil migration into rat peritoneal cavity, and vascular permeability induced by carrageenan or histamine. Of the three eucalyptus oils, ET clearly demonstrated the highest anti-edematogenic activity against edema induced by dextran. These data suggest that the anti-edematogenic effect of the oils of ET has a strong non-neutrophil-mediated component as compared to the effects of the other two extracts of eucalyptus oils. On the other hand, the fact that all the essential oil extracts effectively inhibited neutrophil migration as well as carrageenan-induced edema with similar efficacies provides evidence for the important roles that neutrophil-linked mechanisms play in the anti-inflammatory action of the essential oils from the three *Eucalyptus* species. In addition, while the observed effects of the eucalyptus oils on carrageenan and histamine-induced vascular permeability can provide additional support for their anti-inflammatory activities, the lack of effect of extracts of either EC (in carrageenan-induced permeability) or EG (in histamine-induced permeability) on these inflammatory reaction models is hard to explain. It is, however, possible that this could be a reflection of differences in the mechanisms of vascular inflammatory processes caused by carrageenan and histamine, which can be differentially affected by the essential oils. Alternatively, this may indicate possible differences in the mechanisms of actions of the essential oils and/or other components present in the two types of extracts.

As with the analgesic effects, the exact mechanism(s) of the anti-inflammatory properties of the essential oils used in the present study is unclear. However, other investigators have previously reported that the monoterpene components of the oils of eucalyptus, such as eucalyptol, are potent inhibitors of the inflammatory mediators, cytokines (Juergens et al., 1998a). Furthermore, the production of leukotriene B_2, prostaglandin E_2, and other arachidonic acid metabolites in human monocytes was also shown to be inhibited by eucalyptol (Juergens et al., 1998b). There are also documents indicating that monoterpenes have secretolytic properties against several inflammatory mediators (Juergens et al., 1998b). These reports imply that the essential oil extracts from the *Eucalyptus* species we used can be associated with anti-inflammatory properties at least due to the presence of the above components.

Although attempts have been made to speculate the possible mechanisms for the analgesic and anti-inflammatory effects of the eucalyptus oil extracts based on the literature, our observation of inconsistent or multiphasic activities in some situations is difficult to be provided with any satisfactory explanations. However, as suggested by Jager et al. (1996), this problem may be related, at least in part, to the presence of different chemical components in the extracts. It is possible that these components can vary in kind as well as concentrations. This in turn may result in the manifestation of divergent intrinsic activities, depending upon the type of active ingredients present, and/or different magnitudes of responses, depending upon the concentrations of the active ingredients. The presence of multiple chemicals in a given extract can also interfere with the pharmacokinetic and pharmacodynamic properties of the individual active ingredients (Juergens et al., 1998a,b). A useful approach of shading light into this problem of crude extract application is the determination of the composition of the extracts. Such an effort can facilitate a better assessment whether or not the effects observed with the oil extracts are related to the presence of different components or different concentrations of the same active ingredients. In this regard, while the present investigation is meant to serve as a preliminary observation, future studies should consider the determination of the composition of the individual oil extracts and evaluation of the pharmacology of each active ingredient. It should also be recognized that the use of different experimental models can also play an important role in influencing the effects of the test substances.

In the present study, doses of oil extracts ranging from 0.1 to 100 mg/kg were used. These doses were chosen on the basis of effectiveness and the LD_{50} values determined previously. Doses lower than the minimum indicated for a particular type of experiment did not usually produce measurable responses and doses higher than 100 mg/kg were not desired to be used due to questionable practical significance and closeness to the LD_{50} values. Compared to the assumed doses of essential oil extracts used by patients in traditional therapy (i.e. in the form of hot water infusion), the doses of the extracts we used in the present experiments appear to be higher, although one cannot be sure about it. However, such variations in doses of drugs used in animal experiments and in humans are not unexpected, in which case the doses are usually higher in animal studies. In this respect, animal studies are considered useful in providing clues about what may happen in humans even at different doses. Based on the outcomes from such studies, further investigation can then be pursued. It is believed that the results presented in this communication have the potential to lead to this development.

In conclusion, the results reported in this communication demonstrate that the essential oils of EC, ET, and EG inhibit peripherally and centrally mediated nociception as well as neutrophil-dependent and independent inflammatory reactions. The variations in biological activities observed could be related, at least in part, to differences in the types and amounts of the components of the oils and/or experimental models used, as documented in the literature. Overall, our data provide support for the popular use of the eucalyptus plant in Brazilian folk medicine for pain, and for respiratory and other inflammatory conditions. Thus, the present

study warrants further investigations involving components of essential oil extracts from various species of eucalyptus for possible development new class of analgesic and anti-inflammatory drugs.

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