Analytic 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, flu, 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, flu, and sinus congestion. Essential oils from Eucalyptus species are also widely used in modern cosmetics, food, and pharmaceutical industries (Gomes-Carneiro et al., 1998a). 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 shown to inhibit the production/synthesis of tumor necrosis factor-α, interleukin-1β, leukotriene B4, and thromboxane B2 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-Mayer Squibb, Brazil), carrageenan (BDH, UK), dextran 70 (Pharmacia, USA), histamine, prostaglandin I2, mepipramine (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% extractor which provided a yield of 1%. The essential oils were then 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 Laboratório 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 to the start of the experiments, 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). 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 I2 (PGL2, 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
prostaglandin I₂ in a total volume of 0.2 ml after 30 min
(400 µg). The permeability test was initiated by injection of carrageenan
2.5.3. Cutaneous vascular permeability in rats
in PBS). Control animals for basal value determi-
nation, received equivalent volume of vehicle (4% Tween-20
subcutaneously), was used as a positive control for inhibiting neu-

nephritis migration. Control animals for the basal value determi-
nation, received equivalent volume of vehicle (4% Tween-20
and mepiramine were used as positive controls for the in-
hibition 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
excised tissue 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 evalu-
ations were made using ANOVA or Student’s t-test, and val-
ues 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 de-
creased the number of acetic acid-induced writhes in mice
compared to the animals that received vehicle only (Table 1).
The writh 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) analge-
sia in this nociception model. While the analgesic effect in-
duced 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 intraperi-
tonal 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 cor-
responding control groups (Table 2). These effects of es-

cential 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 stimula-
tion, 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,
esential 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 exper-
iments, the oil extracts from ET were more effective against
dextran-induced paw edema compared to the other two ex-
tracts 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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>0.1 mg/kg</th>
<th>10 mg/kg</th>
<th>100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.M.</td>
<td>Percent inhibition</td>
<td>Mean ± S.E.M.</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>38 ± 3 (14)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>3.4 ± 0.4* (10)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>EC</td>
<td>16 ± 2* (21)</td>
<td>57</td>
<td>19 ± 1* (20)</td>
</tr>
<tr>
<td>ET</td>
<td>19 ± 2* (13)</td>
<td>50</td>
<td>17 ± 2* (13)</td>
</tr>
<tr>
<td>EG</td>
<td>14 ± 3* (9)</td>
<td>53</td>
<td>21 ± 2* (16)</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Time needed to respond after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
</tr>
<tr>
<td>Control (vehicle) (10)</td>
<td>–</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Morphine 10 (7)</td>
<td>6.0 ± 0.6</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Morphine 100 (10)</td>
<td>5.0 ± 0.6</td>
<td>14.5 ± 1.7*</td>
</tr>
<tr>
<td>EC 10 (10)</td>
<td>7.6 ± 0.6</td>
<td>12.5 ± 1.3*</td>
</tr>
<tr>
<td>ET 10 (8)</td>
<td>13.3 ± 1.8*</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>ET 100 (19)</td>
<td>63.0 ± 0.6</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>EG 10 (8)</td>
<td>6.7 ± 0.7</td>
<td>10.8 ± 1.7*</td>
</tr>
<tr>
<td>EG 100 (19)</td>
<td>5.0 ± 0.6</td>
<td>12.6 ± 1.6*</td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. of the number of animals in parentheses.

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 PGEi2 (200 µg/paw)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Edema volume (ml) during several hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control (vehicle) (10)</td>
<td>–</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>EC 10 (6)</td>
<td>0.70 ± 0.05</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>ET 10 (6)</td>
<td>0.60 ± 0.05</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>ET 100 (6)</td>
<td>0.30 ± 0.10*</td>
<td>0.20 ± 0.10*</td>
</tr>
<tr>
<td>EG 10 (6)</td>
<td>0.60 ± 0.03</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>EG 100 (6)</td>
<td>0.30 ± 0.04*</td>
<td>0.30 ± 0.05*</td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. of the number of animals in parentheses.

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 neutrophil 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 (100 µg/paw) plus PGEi2 (200 µg/paw)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Edema volume (ml) during several hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control (vehicle) (10)</td>
<td>–</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>EC 10 (6)</td>
<td>0.60 ± 0.06</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>EC 100 (6)</td>
<td>0.40 ± 0.08*</td>
<td>0.50 ± 0.08*</td>
</tr>
<tr>
<td>ET 10 (6)</td>
<td>0.50 ± 0.03</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>ET 100 (6)</td>
<td>0.40 ± 0.03*</td>
<td>0.02 ± 0.01*</td>
</tr>
<tr>
<td>EG 10 (6)</td>
<td>0.70 ± 0.05</td>
<td>0.70 ± 0.06</td>
</tr>
<tr>
<td>EG 100 (6)</td>
<td>0.40 ± 0.03*</td>
<td>0.40 ± 0.06*</td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. of the number of animals in parentheses.

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

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).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Evans blue dye (μg/g of tissue)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle) (6)</td>
<td>~</td>
<td>17.2 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>2 (8)</td>
<td>5.0 ± 0.6</td>
<td>70</td>
</tr>
<tr>
<td>EC 10 (10)</td>
<td>18.0 ± 1.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>100 (9)</td>
<td>15.4 ± 1.3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>ET 10 (10)</td>
<td>13.2 ± 0.8</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>100 (10)</td>
<td>11.1 ± 1.0</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>EG 10 (9)</td>
<td>10.0 ± 0.4</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>100 (10)</td>
<td>10.4 ± 1.0</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. of the number of animals in parentheses.

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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg)</th>
<th>Evans blue dye (μg/g of tissue)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle) (31)</td>
<td>~</td>
<td>34.5 ± 2.4</td>
<td>0</td>
</tr>
<tr>
<td>Meperidine 0.01 (8)</td>
<td>13.4 ± 1.7</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>EC 10 (8)</td>
<td>13.0 ± 0.7</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>100 (8)</td>
<td>23.0 ± 1.0</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>ET 10 (8)</td>
<td>33.0 ± 3.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>100 (8)</td>
<td>27.0 ± 1.8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>EG 10 (8)</td>
<td>38.0 ± 1.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>100 (8)</td>
<td>39.0 ± 2.1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The data represent the mean ± S.E.M. of the number of animals in parentheses.

4. Discussion and conclusion

Previously, it was determined that the lethal dose 50 (LD₅₀) 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 LD₅₀ values. Consequently, no apparent behavioral side effects were observed in the animals during our experimentation. The high LD₅₀ 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 (writhes 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 B2, prostaglandin E2, 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 LD50 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 LD50 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 of new class of analgesic and anti-inflammatory drugs.

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