Evaluation of the potential anti-inflammatory of *Ocimum americanum* L. essential oil in different experimental models of acute inflammation

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**Summary.** Anti-inflammatory effects of *Ocimum americanum* L. essential oil (OEO) were investigated using carrageenan-induced pleurisy in rats, carrageenan-induced rolling and adhesion of leucocytes by technique of intravital microscopy *in situ*, ear edema induced by croton oil and myeloperoxidase determination in mice. The OEO administered orally at doses of 100, 200 and 400 mg/kg significantly inhibited the exudate pleural volume (p<0.05), without interfere with the number of migrated cells in the carrageenan-induced pleurisy model. While evaluating ear edema induced by croton oil, OEO oral treatment (100, 200 and 400 mg/kg) and OEO topical application (1.0, 2.0 and 4.0 mg/ear) were able to reduce the edema formation (p<0.05). In myeloperoxidase determination, oral treatment with OEO at doses 100 and 200 mg/kg and topical application 1.0, 2.0 mg/ear showed significant inhibition (p<0.05). In the technique of intravital microscopy *in situ*, OEO orally treated at doses of 100, 200 and 400 mg/kg reduced the leukocyte rolling in 35, 37 and 51%, respectively, whereas, leukocyte adhesion was inhibited by 68% (100 mg/kg) and 43% (200 mg/kg). Thus, our results suggest that the screening with OEO realized in this new study can be useful in the future for the treatment of disease of origin inflammatory.

**Industrial relevance.** The leaves of *Ocimum americanum* are traditionally used in the treatment of various types of inflammatory diseases. However, there is no scientific data available given supporting. Hence, we conducted this study to evaluate the anti-inflammatory potential of the *Ocimum americanum* essential oil in different experimental models of acute inflammation. The screening of the biological activity of this essential oil responsible for the anti-inflammatory property may be useful to development of new drug anti-inflammatory, on a commercial scale.

**Keywords.** *Ocimum americanum*; leukocyte migration; essential oil; edema

**INTRODUCTION**

Inflammation is a dynamic process that occurs in response to tissue injury and involves a complex array of enzyme activation, mediator release, with consequent fluid extravasation, cell migration and the breakdown and repair of tissue. However, when sustained it can also has deleterious effects (Agbaje and Fageyinbo, 2012). Inflammatory processes are well established to be associated with the development of several human diseases, including atherosclerosis, rheumatoid arthritis, type 2 diabetes and cancer (Formagio et al., 2013). Moreover, important correlation among these diseases and increased morbidity and mortality have been reported (Gurven et al., 2008).

Although the therapeutic efficacy of some drugs to treatment of inflammatory disorders are established (McGettigan and Henry, 2013), the prolonged use of anti-inflammatory drugs currently used often leads to adverse reactions (Wani et al., 2012). Therefore, the development of new anti-inflammatory compounds with high efficacy and less side effects is necessary. The screening of the biological activities of plants used in folk medicine have been investigated for their possible therapeutic benefits (Abdelwahab et al., 2011), especially their essential oils that showed promising new sources of anti-inflammatory drugs (Abad et al., 2012).

![Figure 1. Leaves of *Ocimum americanum* collected from a rural property in the city of Maringá, Paraná, Brazil. These leaves were used to essential oil extraction and subsequently used in the study.](http://www.ijarnp.org)
Pharmacological studies with essential oils of the genus Ocimum spp (family Lamiaceae) include the specie Ocimum americanum, suggested anti-inflammatory, antinociceptive, antibacterial and insecticidal properties (Lino et al., 2005; Pinho et al., 2012, Dhale and Birari, 2010; Vieira et al., 2003).

Some pre-clinical and clinical studies showed therapeutic properties of O. americanum essential oil and their isolated constituents (Batista et al., 2010; Juergens et al., 2003). Recently, we demonstrated anti-inflammatory effect of this essential oil and some of the compounds isolated in zymosan-induced arthritis model (Yamada et al., 2013). However, in the present study we continue the investigations regarding of O. americanum essential oil in different experimental models of inflammation to sustain its anti-inflammatory activity.

MATERIALS AND METHODS

Plant material and essential oil. The leaves of Ocimum americanum L. were collected in March and April 2010 from a rural property in the city of Maringá, Paraná, Brazil. The samples were identified and authenticated by Dr. Roberto Fontes Vieira taxonomist Embrapa in Brasilia, Brazil. A voucher specimen was deposited in the herbarium of the botanical department of the State University of Maringá (no. 11, 160). The Ocimum americanum L. fresh leaf samples (200 g) were subjected to a hydrodistillation process in a Clevenger-type apparatus for 3 h at 70°C. The collected essential oil was stored at 4°C in dark vials for experimental use.

Animals. Male Balb/c mice (weighing 18 ± 6 g) and male Wistar rats (weighing 200-220 g) were provided by the Central Animal House of the State University of Maringá. The animals were housed at 22 ± 2°C under a 12 h/12 h light/dark cycle. Prior to the experiments, the animals were fasted overnight, with water provided ad libitum. All of the experimental protocols were approved by and followed the guidelines of the Ethics Committee for Animal Experimentation of the State University of Maringá (CAEA/UEM 066/2010).

Acute oral toxicity of OEO. Balb/c mice, weighing 22 ± 3 g, were divided into six groups with three mice in each group. The animals were fasted overnight, with water provided ad libitum, prior to the experiment. O. americanum essential oil was administered orally at doses of 1000, 1500, 2000 and 2500 mg/kg to determine the dose necessary to produce lethality in 50% of the animals (LD50). An equivalent dose of vehicle (1% Tween 80 solution) was administered to the control group. The mice were observed for 7 days following the treatments to determine lethality during the study period in each group. The LD50 was calculated according to the literature (Lorke, 1983).

Anti-inflammatory activity of OEO. Carrageenan-induced pleurisy in rats. The carrageenan-induced pleurisy test was performed according to the technique described by Vinegar et al. (1973). The rats (n = 6-8 per group) were orally pretreated with OEO (100, 200 and 400 mg/kg), vehicle (1% Tween 80 solution) as a negative control, or indomethacin (5 mg/kg) as a reference drug. One hour after treatment the animals received an intrapleural injection of carrageenan (200 µg/animal) or sterile saline. The animals were euthanized 4 h after carrageen injection and the pleural exudate was collected. The volume was determined and the pleural cavity was washed immediately afterward with 2.0 mL phosphate-buffered saline (PBS) that contained ethylenediaminetetraacetic acid to count the number of total and differentiated cells. To perform the total leukocyte count the pleural fluid (50 µL) was diluted in Turk’s solution (950 µL) and counting was performed in a Neubauer chamber. The results were expressed as the mean ± SEM number of leukocytes in the pleural fluid. Part of the pleural fluid was then centrifuged at 2500 rotations per minute for 10 min and the exudate was prepared and fixed on slides. The cells were then stained with May-Grunwald-Giemsa and counted under a light microscope (Zeiss, Wetzlar, Germany). The number of differentiated cells was calculated as a percentage of the total number of cells (100 cells in total). The results are expressed as mean ± SEM.

In situ intravital microscopy in Balb/c mice. The microscopic analysis was performed after the induction of leukocyte migration by an injection of carrageenan (500 µg/cavity, i.p.) in sterile saline solution. The Balb/c mice were treated orally with OEO (100, 200, and 400 mg/kg), vehicle (1% Tween 80 solution) as a negative control or indomethacin (5 mg/kg) as a reference drug 60 min before the carrageenan injection. Additional mice that were used as a negative control were orally treated with vehicle and injected with saline in the peritoneal cavity. Two hours after the carrageenan or saline injection the mice were anesthetized with an intramuscular injection of ketamine (0.1%) and xylazine (0.5%) and a volume of 20 µL per animal. A lateral incision was made in the abdominal and the mesenteric tissue was exposed for microscopic examination. The animals were kept on a special board thermostatically controlled (37°C) adapted to the chariot of an optical microscope with a video camera and monitor to project and record the images (Nunes et al., 2009). The preparation was kept moist and warm with Ringer-Locke’s solution (pH 7.2-7.4) that contained 1% gelatin. Vessels selected for study were third-order venules, defined according to their branch-order location within the microvascular network. These vessels corresponded to postcapillary venules, with a diameter of 10–18 µm. Rolling leukocytes were defined as white blood cells that moved within a given vessel over 10 min. The flux of rolling cells was determined as the number of rolling leukocytes that passed a given point in the venule per minute. Leukocyte adherence was determined when white blood cells remained static in the endothelium for more than 30 s. The results are expressed as the number of adherent leukocytes per 100 µm² of venule. The time points selected to determine rolling (2 h) and adhesion (2 h), after challenge with carrageenan. The number rolling and adhesion of leukocytes were determined from images that were recorded using four results were expressed as mean ± SEM.

Ear edema induced by croton oil. Ear edema was induced by the topical application of 10 µL of an acetone solution that contained the irritant agent 5% croton oil on the inner surface of the right ear of each mice (n = 6-8). The left ear was used as a negative control, which received an equal volume of vehicle (acetone). Sixty minutes before the application of the phlogistic agent, the animals were topically pretreated with vehicle (left ear), OEO (1.0, 2.0 and 4.0 mg/ear) or the antiinflammatory reference drug dexamethasone (1 mg/ear). Four hours after inflammatory stimulation, the mice were euthanized. Both ears were sectioned, and 7 mm plugs were removed. This same approach was used to evaluate the systemic anti-inflammatory effect of OEO. In this experiment, the mice were orally pretreated with OEO (100, 200 and 400 mg/kg) dissolved in vehicle (1% Tween 80 solution), dexamethasone as the reference drug (0.5 mg/kg), or vehicle 60 min before...
croton oil application. The antiedematogenic response was determined as the weight difference between the two plugs, and the data are expressed as the mean ± SEM weight of the ears.

**Myeloperoxidase activity.** The plugs obtained from the right and left ears were used to analyze myeloperoxidase (MPO) activity (Bradley et al., 1982). The ears were placed in 50 mM potassium phosphate buffer (pH 6.0) that contained 0.5% hexadecyl trimethyl ammonium bromide (Sigma, St. Louis, MO, USA; 1 mL/50 mg of tissue) in a Potter homogenizer. The homogenate was shaken in a vortex-mixer and centrifuged for 5 min. A 10 µL aliquot of the supernatant was added in triplicate to each well of a 96-well microplate. The supernatant solution was then mixed with 200 µL of the buffer solution that contained O-dianisidine dihydrochloride (16.7 mg; Sigma), double distilled water (90 mL), potassium phosphate buffer (10 mL), and 1% H₂O₂ (50 µL). The enzymatic reaction was stopped by the addition of sodium acetate. Enzyme activity was determined by measuring absorbance at 460 nm using a microplate spectrophotometer (Spectra Max Plus).

**Statistical analysis.** The data are expressed as the mean ± SEM for each group. The results were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s test. Differences were considered significant at *p < 0.05.*

### RESULTS AND DISCUSSION

The OEO chemical composition was investigated using chromatographic analysis and resulted in the identification of 26 components, representing 98% of the total OEO. The essential oil obtained from the leaves of *Ocimum americanum* (Fig. 1) is rich in monoterpenes and phenols. Its major components were linalool (19.63%), 1,8-cineole (17.27%), eugenol (14.67%), and camphor (14.06%), representing 65.63% of the essential oil. Minority phytoconstituents as α-pinene, Camphene, Sabinene, β-pinene, β-mircene, Limonene, linalool oxide, Fenchone, Borneol, 4-terpineol, cis-pipertol, Estragole, β-selinene, β-caryophyllene, α-trans-bergamotene, α-humulene, germacrene, bicyclogermaene, β-bisabolene, Y-murolene Y-cadinene also found in this plant. The percentage composition was provided in our previous work (Yamada et al., 2013).

**Acute toxicity test.** *O. americanum* essential oil was tested orally at doses of 1000-2500 mg/kg. None of the doses used in this study caused mortality during the observation period (7 days), suggesting that OEO is safe and nontoxic (data not shown). Similar results were found in previous studies of other species of this family (Chandrasekaran et al., 2013). Thus, the present study was performed to investigate the possible antiedematogenic activity of OEO and its effect on leukocyte migration using different models of inflammation (i.e., ear edema, pleurisy, and intravital technique).

**Effect of *O. americanum* essential oil on carrageenan-induced pleurisy in rats.** The intrapleural injection of carrageenan induced an acute inflammatory response, characterized by an increase in the volume of the pleural exudate and number of leukocytes that migrated to the cavity in 4 h compared with the saline injection. The oral treatment with OEO at doses of 100, 200 and 400 mg/kg significantly reduced the intensity of the pleural inflammatory response, reducing the exudate volume by 53, 50 and 71% at the doses tested, respectively. None of the OEO doses influenced the number of cells that migrated to the pleural cavity. The animals treated with indomethacin (5 mg/kg) as the standard drug exhibited a significantly reduction of inflammatory exudate, but no alterations in leukocyte migration to the pleural cavity were observed compared with the animals injected with saline (Table 1). The pleurisy model is considered a screening tool anti-inflammatory drugs. Exudate accumulation in the pleural cavity and leukocyte migration can be evaluated (Iwata et al., 2009; Melo et al., 2009). In the pleurisy model the OEO (orally) significantly reduced inflammatory exudates, similar the groups treated with indomethacin. OEO and indomethacin reduced inflammatory exudates, but not a reduction in leukocytes migration. Our data suggest that OEO inhibits inflammatory edema. However, OEO was not able to reduce cell migration to the injury site, corroborated with study that demonstrated the anti-inflammatory effects of different essential oils. (i.e., inhibition of inflammatory edema but did not chemotaxis) in this experimental model (Fachini-Queiroz et al., 2012).

![Table 1: Effect of *Ocimum americanum* L essential oil (OEO) treatment on exudate volume and leukocytes number 4h after carrageenan injection (200 µg/pleural cavity) in rats](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Exudate volume (mL)</th>
<th>Inhibition (%)</th>
<th>Total leukocytes (Cell/mm²) x 10²</th>
<th>MN</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.72±0.10</td>
<td></td>
<td>6106.7±7319</td>
<td>10914±1361</td>
<td>50125±6970</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.25±0.05*</td>
<td>65</td>
<td>53126±6334</td>
<td>10513±1168</td>
<td>42613±6359</td>
</tr>
<tr>
<td>(5 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OEO (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.34±0.05*</td>
<td>53</td>
<td>51775±8643</td>
<td>11732±4428</td>
<td>40043±6196</td>
</tr>
<tr>
<td>200</td>
<td>0.36±0.04*</td>
<td>50</td>
<td>48758±8643</td>
<td>13409±2710</td>
<td>3549±7079</td>
</tr>
<tr>
<td>400</td>
<td>0.21±0.07*</td>
<td>71</td>
<td>53292±3817</td>
<td>13260±2905</td>
<td>40032±3409</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of 6-8 animals/group Treatment orally with OEO. Animals control treated with vehicle and injected intraperitoneally with saline. Indomethacin orally used as standard drug. Except the control group, all other groups were injected with carrageenan. MN: mononuclears cells. PMN: polymorphonuclears cells. *P < 0.05 compared to control group (ANOVA, Tukey’s test).

**Effect of *O. americanum* essential oil on rolling leukocytes and leukocyte adhesion in mice.** The carrageenan injection significantly increased rolling leukocytes and leukocyte adhesion on the endothelium 2 h after stimulation compared with the mice pretreated only with an intraperitoneal injection of saline (Fig. 2). Oral pretreatment with OEO (100, 200 and 400 mg/kg) significantly decreased rolling leukocytes by 35, 37 and 51%, respectively (Fig. 2A) and decreased leukocyte adhesion by 68% and 43% at the doses of 100 and 200 mg/kg, respectively (Fig. 2B), compared with the mice treated orally.
with only vehicle (control group). Indomethacin inhibited rolling leukocytes by 65% and leukocyte adhesion by 63%. However, 400 mg/kg OEO did not reduce leukocyte adhesion compared with the control. Our data indicate that OEO is able to reduce leukocyte migration and adhesion in mesentery vessels after carrageenan injection. Additionally, OEO reduced the number of adherent leukocytes in mesentery vessels. *O. americanum at a dose of 400 mg/kg did not inhibit leukocyte adhesion. Different to that obtained from other plants with showed effect on migration and adhesion of leukocytes (Nogueira de Melo et al., 2011).

**Figure 2.** Effects of oral pretreatment with OEO or indomethacin in mesenteric microcirculation in mice. Carrageenan-induced leukocyte rolling (Figure 2A) and adhesion (Figure 2B). The data are expressed as mean ± SEM (n = 5-8 per group) and are representative of three independent experiments. *P < 0.05 control versus carrageenan; #P < 0.05 OEO versus carrageenan (ANOVA followed by Tukey’s test). Indo (Indomethacin), C (Control) Cg (carrageenan).

**Effects of O. americanum essential on ear edema in mice.** Topical pretreatment with OEO (1.0, 2.0, and 4.0 mg/ear) inhibited croton oil-induced ear edema by 79, 67 and 36%, respectively. Oral OEO treatment (100, 200 and 400 mg/kg) inhibited croton oil-induced ear edema by 73, 86 and 27% respectively. Topical (0.1 mg/ear) and oral (0.5 mg/kg) pretreatment with the reference drug dexamethasone inhibited ear edema by 80% and 83%, respectively (Fig. 3 A and 4A).

**O. americanum essential oil reduced myeloperoxidase activity.** The activity of MPO was decreased by 77% and 69% in the group treated topically with 1.0 and 2.0 mg OEO per ear, and MPO activity was decreased by 84% and 82% in the group treated orally with 100 and 200 mg/kg OEO compared with the control group. Dexamethasone reduced MPO activity by 89% (topical) and 85% (oral). The OEO-induced inhibition of MPO activity at doses of 4.0 mg/ear (topical) and 400 mg/kg (orally) significantly reduced ear edema, but did not inhibit neutrophil migration (Fig. 3B and 4B).

The topical and systemic effect of OEO on ear edema induced by croton oil in mice was demonstrated in the present study. Ear edema induced by croton oil is a model that screens compounds with topical anti-inflammatory activity, in which the conditions induced by croton oil resemble some types of dermatitis observed in humans. Such a model can be potentially useful to test natural products with purported anti-inflammatory activity (Veras et al., 2013; Vinegar, 1979). The local irritation provoked by croton oil induces an inflammatory response, with increased vascular permeability, leukocyte migration, and consequently edema formation.

Topical or oral pretreatment with OEO reduced edema formation 4 h after croton oil application. Its effects may be related to its ability to inhibit the formation of inflammatory mediators stimulated by the PKC pathway or adjacent pathways involved the inflammation response. Topical and oral OEO treatment inhibited cell influx, indirectly reflected by a reduction of MPO activity. Since this enzyme is considered a marker of neutrophil influx into inflamed tissues; therefore, its inhibition suggests an anti-inflammatory effect of the tested agent (Noufou et al., 2012; Saraiva et al., 2011).

The present results showed that OEO at the highest concentration tested (4.0 mg/ear, topical; 400 mg/kg, oral) increased neutrophil influx into the inflamed ear. As observed in the MPO test, the irritative response was similar between OEO and croton oil. This response could be attributable to the release of histamine and other mediators that act in response to irritative agents, which has been observed for other essential oils (Fachini-Queiroz et al., 2012). The anti-inflammatory effect of OEO may be attributable to a single or synergistic effect of its main components or other minor constituents present in the oil. Many anti-inflammatory activity of isolated compounds obtained from essential oil were studied, where linalool has been shown to inhibit paw edema induced by Complete Freund's Adjuvant (Batista et al., 2010). 1,8-Cineole has also been shown to have anti-inflammatory properties (Juergens et al., 2003; Martinez-Velazquez et al., 2011). The effect of linalool and 1,8-cineole on paw edema and leukocyte migration induced by zymosan was previously studied (Yamada et al., 2013).
Ocimum americanum essential oil prevents experimental inflammation

Figure 3. Effect of topical pretreatment with OEO (1.0, 2.0, and 4.0 mg/ear) or dexamethasone (0.1 mg/ear) (fig. 3A) myeloperoxidase (MPO) activity (fig. 3B) ear edema induced by croton oil in mice. The data are expressed as mean ± SEM (n = 6-8 per group). #p < 0.05, compared with control group (croton oil; ANOVA followed by Tukey’s test). Dex (dexamethasone)

Figure 4. Effect of oral pretreatment with OEO or dexamethasone (0.5 mg/ear) on ear edema (fig. 4A) and myeloperoxidase (MPO) activity (fig. 4B) induced by croton oil in mice. The data are expressed as mean ± SEM (n = 6-8 per group) compared with control group (croton oil) (ANOVA followed by Tukey’s test) and representative of three independent experiments. Dex (dexamethasone)

CONCLUSION

These data show that OEO has significant anti-inflammatory effects in acute inflammation models. Treatment with OEO inhibited ear edema formation, inflammatory exudates, and leukocyte migration and adhesion. Our results support the possible use of OEO in the development of new anti-inflammatory drugs. However, further studies are needed to confirm this possibility.

ACKNOWLEDGMENTS

This study was supported by grants from the CAPES (Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior), Fundação Araucária and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil. We thank Mr. Jailson Araujo Dantas and Mrs. Celia Regina Miranda for technical assistance.
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