Preliminary Analysis of the Anti-Inflammatory Activity of Essential Oils of Zingiber zerumbet

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Abstract
Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for the treatment of inflammation. However, despite their effectiveneseness, most NSAIDs cause various side effects that negatively affect the management of inflammation and, in part, pain. Thus, there is a need to search for new anti-inflammatory agents with few, or no, side effects. Natural products of plant, animal, or microorganism origin have been good sources of new bioactive compounds. The present study was carried out to evaluate the acute and chronic anti-inflammatory activities of the essential oil of the rhizomes of Zingiber zerumbet (Zingiberaceae) using the carrageenan-induced paw edema and cotton pellet-induced granuloma tests, respectively. The effect of the essential oil on inflammatory- and noninflammatory-mediated pain was also assessed using the formalin test. Essential oil of Z. zerumbet, at doses of 30, 100, and 300 mg/kg, was administered intraperitoneally to rats. The substance exhibited significant anti-inflammatory activity both in acute and chronic animal models. The essential oil also inhibited inflammatory- and noninflammatory-mediated pain when assessed using the formalin test. In conclusion, the essential oil of Z. zerumbet possessed anti-inflammatory activity, in addition to its antinociceptive activity, which may explain its traditional uses to treat inflammatory-related ailments.

Keywords
Zingiber zerumbet, Zingiberaceae, essential oil, anti-inflammatory, antinociceptive

Inflammation, defined as a localized protective response elicited by injury or destruction of tissue that serves to destroy, dilute, or wall off both the injurious agent and the injured tissue, is characterized in the acute form by the classical symptoms of pain, heat, redness, swelling, and loss of function. Histologically, inflammation involves a complex series of events, including dilatation of arterioles, capillaries, and venules with increased permeability and blood flow, exudation of fluids including plasma proteins, and leukocytic migration into the inflammatory site (Mariotti, 2004).

Nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, acetaminophen, ibuprofen, and ketoprofen) have been widely used to treat inflammation due to their ability to attenuate the inflammatory process via inhibition of the cyclooxygenase 1 (COX1) action. COX1 is responsible for converting arachidonic acid (AA) into several inflammatory mediators of the prostaglandin (PG) types (e.g., PGD2, PGE2, and PGF2α; Mariotti, 2004). Despite their effectiveness in reducing inflammation, most of the NSAIDs tend to cause various side effects. For example, aspirin causes stomach irritation and bleeding and increased risk of Reye’s syndrome in children and, if taken repeatedly, may lead to tinnitus, impaired hearing, rapid breathing, and confusion (Stephens, Laskin, Pashos, Pena, & Wong, 2003).

The unwanted side effects of NSAIDs have contributed negatively to the management of inflammation as well as pain (Du Pen, Shen, & Ersek, 2007). Alternative treatments are, therefore, desirable. One of the alternative approaches used in the treatment of pain and inflammation is herbal therapy (Astin, 1998; Wirth, Hudgins, & Paice, 2005). It is important for clinical, research, and advanced practice nurses to gain more knowledge regarding herbal therapies and their uses in the treatment of various ailments, particularly inflammation and pain. Furthermore, it would be beneficial for them to understand assays that are used to evaluate the anti-inflammatory or pain-relieving activities of certain natural products.

In the present study, three types of inflammatory assays were used: the carrageenan-induced paw edema test, the cotton

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pellet-induced granulomatous test, and the formalin-induced paw-licking test. The carrageenan-induced paw edema test is considered an acute inflammatory model (Di Rosa, Giroud, & Willoughby, 1971) and is used to measure the ability of drugs/extracts to attenuate carrageenan-induced locally produced edema (Chan, Tsi, & Wu, 1995; Winter, Risley, & Nuss, 1962). The inflammatory response induced by carrageenan is a COX-dependent response and involves the release of edema-producing proinflammatory mediators (e.g., PG), kinins, and polymorphonuclear leucocytes (Damas, Remacle-Volol, & Deflandle, 1986). The cotton pellet-induced granulomatous test, on the other hand, is considered a chronic inflammatory model (Sulaiman et al., 2009) and can be used to evaluate the transudative and proliferative components of chronic inflammation (Bhattacharya, Pal, & Nag-Chaudhuri, 1992). The tissue repair processes that occur during inflammation are associated with the proliferation of macrophages, neutrophils, and fibroblasts and the multiplication of small blood vessels to form a highly vascularized reddish mass called granulation tissue. The formalin-induced paw-licking test, in addition to being considered a reliable model for persistent nociception (Vasudevan, Gunnam, & Parle, 2007), can also be used to determine the inhibitory effect of compounds/extracts on inflammatory-mediated nociception. There are two distinct phases, known as the early and late phases, that characterize the formalin-induced paw-licking test. The early phase corresponds to neurogenic pain, while the late phase corresponds to inflammatory-mediated pain that results from the release of inflammatory mediators (Leal, Ferreira, Bezerra, Matos, & Viana, 2000). Interestingly, the formalin-induced paw-licking test can also be used to determine whether a compound/extract produces antinociceptive activity at the peripheral or central levels (Adzu, Amos, Kapu, & Gamaniel, 2003). Centrally acting drugs (e.g., opioids) inhibit both phases equally, whereas peripherally acting drugs (e.g., NSAIDs) inhibit only the late phase. These tests are being used widely as in vivo tools for confirming the anti-inflammatory property of certain medicinal plant extracts.

Natural products in general are an important source of new chemical substances, and medicinal plants in particular have proven to be a potential source of agents with high therapeutic efficacy. The use of readily available natural resources, such as herbs or medicinal plants, as anti-inflammatory and pain agents commenced around 2000 years ago (Almeida, Navarro, & Barbosa-Filho, 2001). For example, the ancient Greeks used an extract of willow bark to relieve pain during labor. The extract is a rich source of salicylic acid, which is one of the most widely used agents for the treatment of inflammation and pain (Vane & Botting, 1995). Keeping this example in mind, the use of plant species in the search for new anti-inflammatory and pain-relieving drugs based on their traditional uses should still be seen as a fruitful research strategy (Shanmugasundaram & Venkataraman, 2005).

In our laboratory, we are studying Zingiber zerumbet Smith. We have successfully isolated its major constituent, zerumbone, and are now exploring its pharmacological properties using various in vivo models. Z. zerumbet Smith, or lempoyang, as it is known to the Malays, belongs to the tropical and subtropical family Zingiberaceae, from which approximately 160 species from 18 genera are found in Peninsular Malaysia alone (Larsen, Ibrahim, Khaw, & Saw, 1999; Park & Pizzuto, 2002). The plant originated from Southeast Asia and has been cultivated for thousands of years as a marketable spice and also for its medicinal properties (Bhuiyan, Chowdhury, & Begum, 2009; Somchit & Nur Shukriyah, 2003). The underground stem, or rhizome, of this plant has been used as a medicine in Asian, Indian, and Arabic herbal traditions since ancient times (Altman & Marcussen, 2001). The rhizome of Z. zerumbet is commonly applied in Malay traditional medicine to treat ailments such as swelling, sores, cuts, toothaches, stomach aches, and muscle sprains (Burkill, 1966; Habbsah et al., 2000). The Chinese have extensively used those parts for more than 2500 years to treat headaches, colds, and nausea (Grant & Lutz, 2000). In Mediterranean and Western areas, they were used to treat rheumatological conditions, arthritis, and muscular discomforts (Bordia, Verma, & Srivastava, 1997; Langner, Greifenberg, & Gruenwald, 1998; Sharma & Clark, 1998). According to Liang (1992), Z. zerumbet has also been used for the treatment of migraines, ulcers, impotence, atherosclerosis, and depression. Researchers have isolated zerumbone, a flavone, and two flavonoid glycosides from the rhizome of Z. zerumbet (Chien, Chen, Lee, Lee, & Wang, 2008). Previous studies revealed that the volatile oil of the rhizomes of Z. zerumbet contained zerumbone as one of its major components (Bhuiyan et al., 2009; Chhabra, Dhillon, Wadia, & Kalsi, 1975; Damodaran & Dev, 1968; Dung, Chinh, Rang, & Leclercq, 1993; Duve, 1980; Hasnah, 1991; Srivastava, Srivastava, & Shah, 2000).

We have recently reported on the peripheral and central antinociceptive activity of zerumbone when assessed using the abdominal constriction and hot plate tests. Furthermore, our findings revealed that the antinociceptive activity of zerumbone involves modulation of the opioid receptors (Sulaiman, Perimal, et al., 2009). We have also reported on the antinociceptive activity of the essential oil from the rhizome of Z. zerumbet (EOZZ) when assessed using the abdominal writhing test, the hot plate test, and the formalin-induced paw-licking test. This activity was reversed by naloxone, indicating again that the opioid system is partly involved in the analgesic mechanism of zerumbone’s action (Sulaiman, Tengku Mohamed, et al., 2010). To our knowledge, no researchers have attempted to determine the antinflammatory activity of the EOZZ. Thus, we conducted the present study to investigate the anti-inflammatory activity of the EEOZ using the various in vivo models.

Method

Study Design

We measured the anti-inflammatory activity of EOZZ using three animal models to assess acute, chronic, and
We purchased acetylsalicylic acid (ASA) from Sigma and obtained 6.2 g of essential oil from the rhizomes of Z. zerumbet. We kept it for analysis. Upon evaporation of the hexane layer and condensate-receiving tube to allow recycling of the condensed solvent back to the distillation flask. During distillation, we introduced 2.0 ml distilled hexane to the receiving tube filled to the maximum level, which acted as an organic extractant for investigations of experimental pain in conscious animals (Zimmermann, 1983).

**Plant Material**

We purchased Z. zerumbet rhizomes from a local wet market on Chow Kit Road, Kuala Lumpur, Malaysia, May–July, 2007, and had them identified by Dr. Shamsul Khamis, a botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. We deposited a voucher specimen (SK 622/07) at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia.

**Extraction of EOZZ**

We sliced the fresh material into 0.5–1 mm pieces using a commercial food processor and then used hydrodistillation to obtain the essential oil from this plant sample. We used the cotton pellet-induced granulomatous test in which EOZZ’s effect on the weight of wet and dried cotton pellets subcutaneously implanted into the animals and then dissected out again after the animal was sacrificed on the eighth day after administration of the tests suggested its ability to attenuate the transudative and proliferative stages seen in chronic inflammation. Based on the recent reports of the antinociceptive effect of EOZZ, we also assessed the ability of EOZZ to attenuate inflammatory-induced pain using the formalin-induced paw-licking test, in particular, the second phase of the test, which occurred 15–30 min after formalin injection.

The dosages of EOZZ used (30, 100, and 300 mg/kg) in these tests were based on a recent publication (Sulaiman et al., 2008) in which the selected doses were reported to produce no toxic effects. We administered the test solutions via intraperitoneal (i.p.) injection 30 min prior to induction of inflammation due to the nature of the tests, particularly the formalin test.

**Anti-Inflammatory Assays**

**Carrageenan-induced paw edema test.** We used the carrageenan-induced paw edema test to determine the anti-inflammatory activity of EOZZ against acute inflammation (Sulaiman et al., 2008). For this test, we divided the rats into six groups (n = 6 in each group), each of which received one of the following treatments i.p.: 0.9% NaCl, DMSO, ASA (100 mg/kg), or 30, 100, or 300 mg/kg EOZZ. Intraplantar administration (i.p.l.) of 50 μl of 1% carrageenan suspension into each rat’s right hind paw followed 30 min later. We measured paw volume before (V₀) and at 1, 2, 3, 4 and 5 hr (V₁) following the carrageenan injection using a plethysmometer (Model 7140, Ugo Basile, Italy). We quantified the degree of inflammation by measuring the volume displaced by the paw between the final volume (V₁) and the initial volume (V₀). The percentage of anti-inflammation activity was calculated using the following formula

\[
\text{Percentage of anti-inflammation} = \left( \frac{V₁ - V₀}{V₁ - V₀}\right)_\text{Control} \times 100
\]

where f = the time interval.

**Cotton pellet-induced granuloma test.** We used the cotton pellet-induced granuloma test to determine the anti-inflammatory activity of EOZZ against chronic inflammation (Sulaiman et al., 2009). For this test, we divided the animals into five groups (n = 10 in each). On Day 1, we pretreated the rats subcutaneously with DMSO, 100 mg/kg ASA, or 30, 100, or 300 mg/kg EOZZ. We subcutaneously introduced a sterilized cotton pellet (30 ± 1 mg) in the dorsum of rats anesthetized with averitin (1 ml/kg, i.p.) 30 min after pretreatment. We then treated the animals with a single injection of 0.9% NaCl, 100 mg/kg ASA, or 30, 100, or 300 mg/kg EOZZ daily for 7 consecutive days. On Day 8, we sacrificed the animals, dissected the pellets out, and weighed them to obtain the wet weight.
We dried the wet pellets at 60°C overnight to determine the final dry weight. The difference between the initial (30 mg) and wet weight was considered the weight of transudates produced, whereas the difference between the initial and final dry weight was considered the weight of the granulomatous tissues produced. We calculated the level of inhibition of granuloma tissue development (Okoli et al., 2007) using the following formula

\[
\% \text{Inhibition} = \left( \frac{T_c - T_i}{T_c} \right) \times 100
\]

where \(T_c\) = weight of granuloma tissue of control group and \(T_i\) = weight of granuloma tissue of treated group.

**Formalin test.** We used the formalin test (Adzu et al., 2003) to study the effect of EOZZ on inflammatory-mediated pain seen in the second, or late, phase of the test and to further confirm our recently published findings on the ability of EOZZ to inhibit pain in this second phase (Sulaiman, Tengku Mohamed, et al., 2010). Briefly, we divided the animals into six groups (n = 10 in each group) and administered 50 μl of 2.5% formalin (v/v) i.pl. on the left hind paw 30 min after the i.p. administration of DMSO, 5 mg/kg morphine, 100 mg/kg ASA, or 30, 100, or 300 mg/kg EOZZ. We measured the time spent biting, licking, and scratching the injected paw 15–30 min after test solution administration, which is the period of the test associated with inflammatory-mediated pain.

**Statistical Analysis**

We analyzed the data obtained in the cotton pellet-induced granulomatus edema test and formalin-induced paw edema test using one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison tests. We analyzed data from the carrageenan-induced paw edema test using two-way ANOVA. We expressed the results as mean ± SEM and consider differences to be significant when \(p \leq .05\).

**Results**

**Carrageenan-Induced Paw Edema Test**

Systemic (i.p.) administration of EOZZ at doses of 30, 100, and 300 mg/kg produced significant (\(p < .05\)) anti-inflammatory activity in a dose-dependent manner when assessed by the carrageenan-induced paw edema test (Table 1), an acute model of inflammation. The anti-inflammatory activity, which continued until the end of the experiment, started 3 hr after EOZZ administration for all doses used. In comparison, 100 mg/kg ASA began to exert anti-inflammatory activity 2 hr after administration; only 100 and 300 mg/kg EOZZ produced greater activity in the last 2 hr of the experiment. DMSO alone did not exhibit significant anti-inflammatory activity until the end of the experiment. ASA reduced edema by 34.4% at 1 hr, which increased to a peak of 76.1% at 3 hr before subsided to 66.7% at the end of the experiment (fifth hour). Anti-inflammatory activity of EOZ increased significantly between 1 and 5 hr, going from 16.9% to 55.0%, 16.2% to 83.9%, and 22.7% to 83.9% reduction for 30, 100, and 300 mg/kg, respectively.

**Cotton Pellet-Induced Granuloma Test**

EOZZ, in doses of 30, 100, and 300 mg/kg, also exhibited dose-dependent antitransudative and antiproliferative activity against granulomatus edema when assessed using a chronic model of inflammation, the cotton pellet-induced granuloma test (Table 2). Interestingly, 30 mg/kg EOZZ produced antitransudative activity that was equal in strength to that of 100 mg/kg ASA (57.1% and 59.5% inhibition of granuloma formation, respectively), while the 100 and 300 mg/kg doses of EOZZ produced antiproliferative activity equal to 100 mg/kg ASA (56.6%, 65.8%, and 60.3%, respectively).

**Formalin Test**

EOZZ also exhibited significant (\(p < .05\)) antinociceptive activity at all doses against inflammatory-mediated pain, as indicated by a reduction in the amount of time during which

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**Table 1. Anti-Inflammatory Effect of the Essential Oil from the Rhizome of Zingiber zerumbet (EOZZ) on Acute Inflammation Represented by Hind Paw Edema in the Carrageenan-Induced Paw Edema Test in Rats**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>0.158 ± 0.015</td>
<td>0.311 ± 0.021</td>
<td>0.392 ± 0.013</td>
<td>0.459 ± 0.025</td>
<td>0.453 ± 0.011</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.154 ± 0.017</td>
<td>0.290 ± 0.018</td>
<td>0.381 ± 0.019</td>
<td>0.443 ± 0.019</td>
<td>0.429 ± 0.019</td>
</tr>
<tr>
<td>100 mg/kg ASA</td>
<td>0.101 ± 0.014</td>
<td>0.172 ± 0.036</td>
<td>0.091 ± 0.021</td>
<td>0.218 ± 0.028</td>
<td>0.143 ± 0.024</td>
</tr>
<tr>
<td>30 mg/kg EOZZ</td>
<td>0.128 ± 0.095</td>
<td>0.227 ± 0.024</td>
<td>0.207 ± 0.023</td>
<td>0.242 ± 0.024</td>
<td>0.193 ± 0.027</td>
</tr>
<tr>
<td>100 mg/kg EOZZ</td>
<td>0.129 ± 0.024</td>
<td>0.233 ± 0.029</td>
<td>0.168 ± 0.040</td>
<td>0.119 ± 0.031</td>
<td>0.069 ± 0.032</td>
</tr>
<tr>
<td>300 mg/kg EOZZ</td>
<td>0.119 ± 0.018</td>
<td>0.221 ± 0.013</td>
<td>0.162 ± 0.031</td>
<td>0.106 ± 0.027</td>
<td>0.069 ± 0.032</td>
</tr>
</tbody>
</table>

Note. ASA = acetylsalicylic acid; DMSO = dimethyl sulfoxide (vehicle); NaCl = saline solution; SEM = standard error of mean. Hind paw edema is expressed as mean ± SEM; n = 6 in each group. % reduction represents the percentage of edema reduced in each group as compared to the NaCl (control) group at each timepoint.

* \(p \leq .05\) compared with DMSO-treated rats in the same period.
animals exhibited behaviors indicative of discomfort in the second phase of the formalin test (Table 3). The 100 mg/kg dose of EOZZ produced an activity that was equal in strength to that of 100 mg/kg ASA, with percentages of inhibition of 56.2% and 58.2%, respectively.

Table 2. Anti-Inflammatory Effect of the Essential Oil of the Rhizome of Zingiber zerumbet (EOZZ) on Chronic Inflammation, as Assessed by Cotton Pellet-Induced Granuloma Test in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Antitransudative</th>
<th>Antiproliferative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>DMSO</td>
<td>663.11 ± 20.96</td>
<td>–</td>
</tr>
<tr>
<td>ASA (100 mg/kg)</td>
<td>268.76 ± 7.56*</td>
<td>59.47</td>
</tr>
<tr>
<td>EOZZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>284.47 ± 22.17*</td>
<td>57.10</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>211.25 ± 19.52*</td>
<td>68.14</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>168.56 ± 19.39*</td>
<td>74.58</td>
</tr>
</tbody>
</table>

Note. ASA = acetylsalicylic acid; DMSO = dimethyl sulfoxide (vehicle); SEM = standard error of mean. n = 10 in each treatment group. * Differs significantly (p < .05) when compared against the DMSO-treated group.

Table 3. Antinociceptive Effect of the Essential Oil of the Rhizome of Zingiber zerumbet (EOZZ) on Inflammatory-Mediated Nociception Assessed in the Second Phase of the Formalin Test in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paw-Licking Time (s), Mean ± SEM</th>
<th>% Anti-Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>53.20 ± 2.00</td>
<td>–</td>
</tr>
<tr>
<td>ASA (100 mg/kg)</td>
<td>22.22 ± 3.65*</td>
<td>58.23</td>
</tr>
<tr>
<td>Morphine (5 mg/kg)</td>
<td>31.00 ± 1.90*</td>
<td>41.73</td>
</tr>
<tr>
<td>EOZZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>35.90 ± 1.98*</td>
<td>32.52</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>23.30 ± 3.88*</td>
<td>56.20</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>16.50 ± 2.13*</td>
<td>68.98</td>
</tr>
</tbody>
</table>

Note. ASA = acetylsalicylic acid; DMSO = dimethyl sulfoxide (vehicle); SEM = standard error of mean. n = 10 in each treatment group. * Differs significantly (p < .05) when compared against the DMSO-treated group.

Discussion

EOZZ exhibited anti-inflammatory activity in both acute and chronic models of inflammation in the current study. It also attenuated inflammatory-mediated pain. The inflammatory response in the carrageenan-induced rat paw edema test, our model of acute inflammation (Di Rosa et al., 1971), is a COX-dependent response (Gamache, Povlishock, & Ellis, 1986). The assay measures biphasic events whereby the early phase (1–3 hr) of inflammation is due to the release of inflammatory mediators (e.g., histamine, bradykinin, and serotonin), while the late phase (4–6 hr) is linked with the activation of kinin-like mediators (e.g., PGs, proteases, and lysozyme; Olajide, Makinde, & Awe, 1999). These mediators, which are the metabolites of AA produced via COX and lipooxygenase (LOX) pathways, act alone or in combination to produce the characteristic signs of inflammation (e.g., vasodilatation, hyperemia, pain, edema, and cellular filtration; Issekutz & Movat, 1982; Lewis & Austen, 1981). The COX products increase blood flow through vasodilatation, but the LOX products are necessary for vascular leakage, edema, and subsequent cellular infiltration (Wedmore & Williams, 1981). Researchers have found that the edema induced in the carrageenan-induced paw edema test is effectively controlled with arachidonate COX, rather than arachidonate LOX, inhibitors (Gamache et al., 1986). In the present study, 30, 100, and 300 mg/kg of EOZZ reduced edema by 16%–55%, 16%–84%, and 22%–84%, respectively, with anti-inflammatory activity starting 3 hr after administration of the phlogistic agent and lasting until the end of the experiment. The late onset of the anti-inflammatory action of EOZZ (starting in the third measurement interval) as compared to that of ASA (starting in the second interval) indicates that EOZZ was effective toward the late phase of the test, which involves arachidonate COX products, particularly PG. Another possible explanation for the late onset of EOZZ action is delayed absorption of EOZZ (Ahmad, Khan, & Rasheed, 1992). Interestingly, the anti-inflammatory activity of EOZZ increased over time, with the highest level of activity seen at the end of experiment, while the activity of ASA peaked at the third measurement interval. These findings suggest that the duration of action of EOZZ is longer than that of ASA and that EOZZ is an effective arachidonate COX inhibitor.

The cotton pellet-induced granuloma test is a model for studying the transudative and proliferative components of chronic inflammatory processes (Bhattacharya et al., 1992). Chronic inflammation, which results from an acute response insufficient for eliminating proinflammatory mediators (Arrigoni-Maratellie, 1988, pp. 119–120), involves fibroblast proliferation and neutrophil infiltration and exudation (Dunne, 1990). The proliferative cells that develop during chronic inflammation can either spread or be a granuloma. In the present study, EOZZ reduced the weight of granulomatous pellets at both transudative and proliferative levels. These findings suggest that EOZZ is effective in reducing chronic inflammation (Recio, Giner, Manez, & Ros, 1995). Like EOZZ, NSAIDs (e.g., indomethacin) have been reported to inhibit both levels of the chronic inflammatory response (Choi et al., 2006), which reflect their efficacy in inhibiting the increase in the number of fibroblasts and the synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Recio et al., 1995).

We examined the effect of EOZZ in the late phase of the formalin-induced paw-licking test, a model of inflammatory pain (Lee et al., 2000), in order to strengthen the evidence of the anti-inflammatory activity of EOZZ seen in the prior tests. It is well known that inflammatory mediators like histamine, serotonin, bradykinin, and PGs are involved in the late phase of this test (Shibata, Ohkubo, Takahashi, & Inoki, 1989). NSAIDs, such as ASA and indomethacin, reduce nociceptive behavior during the late phase, while the early phase seems unaffected by these medications (Rosland, Tjolsen, Maehle, & Hole, 1990).
Based on the data we obtained in the present study, EOZZ successfully attenuated the inflammatory-mediated pain associated with the late phase, which reflects its effect on the synthesis and/or release of PGs and other inflammatory mediators (Choi et al., 2006), further supporting the anti-inflammatory activity seen earlier.

The involvement of COX in inflammation is widely accepted (Katzung, 2003). The ability of EEOZ to exert anti-inflammatory activity could be due partly to its ability to inhibit the peripheral and central COX action (Ballou, Botting, Goorha, Zhang, & Vane, 2000; Pini, Vitale, Ottani, & Sandrini, 1997). The ability of EOZZ to inhibit edema in the carrageenan-induced paw edema test suggests peripheral COX involvement (Gamache et al., 1986), as seen in the writhing response reported earlier (Sulaiman, Zakaria, et al., 2010). In addition, the writhing test has been associated with an increase in the release of prostanoids (PGE2 and PGF2α) as well as other LOX products in peritoneal fluids (Choi et al., 2006; Vasudevan et al., 2007). Carrageenan-induced inflammation is more effectively controlled with arachidonate COX inhibitors (Gamache et al., 1986). The involvement of central COX in the pain response has been reported previously (Pini et al., 1997).

Interestingly, phytochemical study of EOZZ revealed zerumbone as its major constituent (Sulaiman, Zakaria, et al., 2010). Zerumbone has been shown to attenuate expression of COX-2 and inducible nitric oxide synthase (iNOS) in vitro. COX-2 and iNOS are known to participate in the mechanism of inflammation (Murakami & Ohigashi, 2007; Murakami, Shigemori, & Ohigashi, 2005). We have previously reported on the antinociceptive activity of zerumbone assessed by the writhing and hot plate tests (Sulaiman, Zakaria, et al., 2010). This activity was mediated at peripheral and central levels via the opioid receptor system. The ability of anti-inflammatory compounds to exert antinociceptive activity is widely acknowledged (Attaway & Zaborsky, 1993, pp. 1–23).

In conclusion, the present study provides convincing evidence that EOZZ, isolated from Z. zerumbet rhizomes, possesses significant anti-inflammatory activity in both acute and chronic models of inflammation, which can be at least partly attributed to the presence of the antinociceptive substance zerumbone. Our study, thus, supports the traditional uses of Z. zerumbet rhizomes in the treatment of inflammatory-mediated ailments.

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