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Full Length Research Paper

Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats

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Zerumbone a monocyclic sisquiterpene, is the major constituent of *Zingiber zerumbet* Smith. In Southeast Asia, *Z. zerumbet* is being used for its anti-inflammatory and analgesic properties. The objective of this current investigation was to evaluate the *in vivo* anti-inflammatory and analgesic activities of zerumbone isolated from *Z. zerumbet* in rats. The results revealed zerumbone potently inhibit inflammation induced by both λ-carrageenan and prostaglandin E_2, which was statistically similar to the nonsteroidal anti-inflammatory drug (NSAID) piroxicam. Zerumbone also potently inhibited pain in the sabdorminal writhing test similar to the NSAID. Collectively, our results validate the traditional use of this plant and zerumbone has been demonstrated to be a potent anti-inflammatory and analgesic agent.

Key words: Zerumbone, *Zingiber zerumbet;* anti-inflammation, analgesic.

INTRODUCTION

Zingiber zerumbet, locally known as lempoyang, belongs to the Zingiberaceae family. It is cultivated in the village gardens in the tropics for its medicinal properties (Somchit and Shukriah, 2003). The rhizomes of Z. *zerumbet* are used for stomachache relieve and macerated in alcohol as tonic or stimulant in China (Perry, 1980). The volatile oils of the rhizomes have been shown to contain natural compounds such as zerumbone, humulene and camprene (Hasnah, 1991).

Zerumbone, the main component of the volatile oils, is a monocyclic sesquiterpene containing a cross-conjugated dienone system (Kitayama et al., 2002) (Figure 1). Recently, Chien et al. (2008) reported anti-inflammatory effects of constituents from *Z. zerumbet*. In addition, zerumbone was reported to inhibit inducible nitric oxide synthase (iNOS) and COX-2 expression in macrophages treated with lipopolysaccharide (LPS) and interferon (IFN)-γ (Tanaka et al., 2001). Therefore, the objectives of this current study were to investigate the *in vivo* anti-inflammatory and analgesic properties of zerumbone isolated from *Z. zerumbet*.

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MATERIALS AND METHODS

Natural plant-derived compound, zerumbone

Zerumbone was extracted and isolated from the rhizomes of the plant, *Z. zerumbet* as described in our previous report (Ahmad Bustamam et al., 2008). Briefly, 5 kg of fresh rhizomes were cut into slices and dried in oven at 37ºC. The dried samples were grinded and soaked in methanol for 3 days before rotavaped to fore dried crude extract. This extract was later column fractionated in silica gel and eluted using organic solution mixture of hexane: ethyl acetate (8:2; $^{v}/_{v}$). Upon evaporation, 13.5 g (0.27% of fresh weight) was obtained. Zerumbone (Figure 1) was obtained from crystallization. The identity and purity (more than 99%) were confirmed by

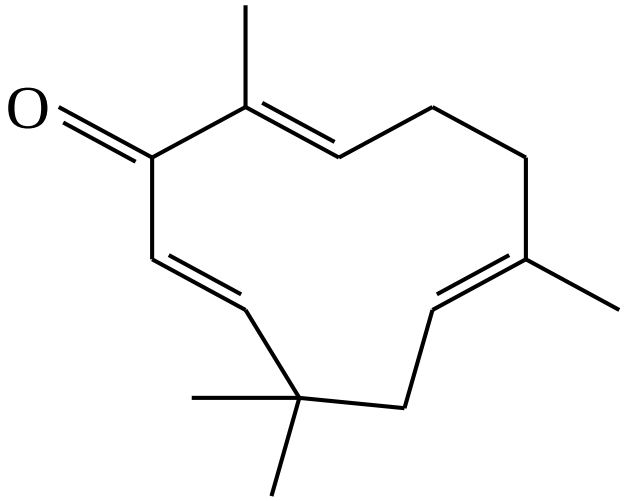

Figure 1. Chemical structure of zerumbone.

structure elucidation and comparison with previous results (Matthes et al., 1980) and using high performance liquid chromatography (HPLC).

Laboratory animals

Female Sprague–Dawley rats (200 to 250 g in body weight, n = 6/group) were housed in plastic cages with wood shavings as bedding. The rats were fed on standard rat pellets and tap water *ad libitum*. The care and experimental procedures were carried out in strict compliance with the Animal Ethics Committee rules and regulation followed in this institute. This experiments also in accordance with the current guidelines for pain experimentation in conscious animals (Zimmerman, 1983).

Anti-inflammatory assessment using paw edema model

Thirty minutes after zerumbone (10 and 20 mg/kg) intraperitoneal administration (i.p.), the rats were treated with 0.05 ml of λ-carrageenan (1%) or Prostaglandin E_2 (100 IU) by intraplantar injection into the right hind paw. The control group received vehicle (0.9% NaCl solution), and the positive group received 20 mg/kg piroxicam. The paw volumes were measured before (initial volume) and after the λ-carrageenan or PGE_2 injection at 1, 2, 3, 4 and 5 h using Ugo Basile 7140 Plethysmometer. Results are expressed in the differences of paw volume when compared to initial volume.

Acetic acid-induced abdominal writhing test

The acetic acid-induced abdominal writhing test was performed as described previously (Zakaria et al., 2005) with slight modification. Rats were administered with zerumbone (10 and 20 mg/kg; i.p.) and, 30 min later, were given 0.6% acetic acid (10 ml/kg; subcutaneous). The total number of writhings following the acetic acid administration was recorded for 30 min, starting 5 min after the injection. Negative control animals received a similar volume of 0.9% NaCl solution whereas positive control animals received piroxicam (20 mg/kg, i.p.). Antinociceptive activity was expressed as the percentage reduction or inhibition of the number of abdominal writhes.

Statistical analysis

Data was expressed as mean ± SD and analysed using analysis of variance (ANOVA). When interactions were significant, Duncan multiple post-test was performed. Values of $p \leq 0.05$ was considered as the limit of significant.

RESULTS

Results obtained from the carrageenan-induced paw edema are shown in Table 1. 10 and 20 mg/kg zerumbone exhibited significant ($P<0.05$) anti-inflammatory activity with maximum inhibition of 45.67 and 70.37%, respectively. The anti-inflammatory activity of zerumbone was statistically similar to piroxicam. Similar trends were also observed with prostaglandin E_2-induced paw edema test (Table 2). Zerumbone further inhibited the rat paw edema by 87.80%. However, piroxicam was the most potent where it inhibited the inflammation by 92.68%.

As shown in Table 3, zerumbone exhibited dose-dependent antinociceptive activity by inhibiting abdominal writhing at 47.89 and 71.05% for 10 and 20 mg/kg zerumbone, respectively. The high dose of zerumbone showed similar potency as the NSAID piroxicam.

DISCUSSION

The present study confirms that zerumbone has both anti-inflammatory and analgesic properties. Its anti-inflammatory activity was deduced from its inhibitory effects on chemicals (prostaglandin E_2 and λ-Carrageenan) induced inflammation. At 20 mg/kg, the anti-inflammatory action of zerumbone on λ-carrageenan inflammation was found to be comparable to 20 mg/kg of

Table 1. Anti-inflammatory effects of zerumbone tested by Carrageenan-induced paw edema.

	Paw volume (ml)					
	0	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control (0.9% NaCl)	0	0.42 ± 0.03^{ax} (-)	0.68 ± 0.09^{ay} (-)	0.82 ± 0.11^{az} (-)	0.90 ± 0.07^{az} (-)	0.81 ± 0.05^{az} (-)
10 mg/kg Zerumbone	0	0.38 ± 0.04^{ax} (9.52)	0.49 ± 0.05^{by} (27.94)	0.47 ± 0.03^{bxy} (42.68)	0.49 ± 0.04by (45.56)	0.44 ± 0.09^{bxy} (45.67)
20 mg/kg Zerumbone	0	0.29 ± 0.04^{bx} (30.92)	0.32 ± 0.02^{cx} (52.94)	0.32 ± 0.03^{cx} (60.98)	0.30 ± 0.02^{cx} (66.67)	0.24 ± 0.06^{cx} (70.37)
20 mg/kg Piroxicam	0	0.22 ± 0.05^{bx} (47.61)	0.29 ± 0.02^{cy} (57.35)	0.30 ± 0.06^{cxy} (63.41)	0.25 ± 0.03^{dxy} (72.22)	0.20 ± 0.01^{cx} (75.31)

Values are mean ± sd of 6 animals. $^{a-d}$Means with different superscript differ significantly ($p<0.05$) in the same column. $^{x-z}$Means with different superscript differ significantly ($p<0.05$) in the same row. Numbers in parentheses are % inhibition when compared to controls.

Table 2. Anti-inflammatory effects of zerumbone tested by Prostaglandin E_2-induced paw edema.

	Paw volume (ml)					
	0	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control (0.9% NaCl)	0	0.27 ± 0.02^{ax} (-)	0.43 ± 0.04^{ay} (-)	0.52 ± 0.01^{az} (-)	0.50 ± 0.03^{az} (-)	0.41 ± 0.01^{ay} (-)
10 mg/kg Zerumbone	0	0.18 ± 0.03^{bx} (33.33)	0.22 ± 0.04^{bx} (48.84)	0.37 ± 0.08^{by} (28.85)	0.29 ± 0.03^{bxy} (42.00)	0.24 ± 0.05^{bxy} (41.46)
20 mg/kg Zerumbone	0	0.12 ± 0.01^{cx} (55.56)	0.12 ± 0.02^{cx} (72.09)	0.10 ± 0.01^{cx} (80.77)	0.09 ± 0.02^{cx} (82.00)	0.05 ± 0.01^{cx} (87.80)
20 mg/kg Piroxicam	0	0.09 ± 0.02^{cx} (66.67)	0.10 ± 0.03^{cx} (76.75)	0.07 ± 0.02^{dx} (86.54)	$0.05 \pm 0.03c^{xy}$ (90.00)	0.03 ± 0.01^{cy} (92.68)

Values are mean ± sd of 6 animals. $^{a-d}$Means with different superscript differ significantly ($p<0.05$) in the same column. $^{x-z}$Means with different superscript differ significantly ($p<0.05$) in the same row. Numbers in parentheses are % inhibition when compared to controls.

Table 3. Antinociceptive effects of Zerumbone tested by acetic acid induced abdominal writhing.

	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**	**Total**	**Inhibition (%)**
Control (0.9% NaCl)	4.80 ± 0.35	7.50 ± 0.53	6.75 ± 0.41	4.14 ± 0.26	4.15 ± 0.37	2.33 ± 0.21	29.67	-
10 mg/kg Zerumbone	3.21 ± 0.15	5.23 ± 0.91	3.75 ± 0.62	1.50 ± 0.36	1.25 ± 0.27	0.52 ± 0.15	15.46	47.89
20 mg/kg Zerumbone	2.52 ± 0.31	3.48 ± 0.28	2.42 ± 0.15	0.17 ± 0.17	0.00	0.00	8.59	71.05
20 mg/kg Piroxicam	2.15 ± 0.48	4.00 ± 0.31	1.98 ± 0.22	0.33 ± 0.24	0.00	0.00	8.46	71.49

Values are mean ± sd of 6 animals. $^{a-d}$Means with different superscript differ significantly ($p<0.05$) in the same column. $^{x-y}$Means with different superscript differ significantly ($p<0.05$) in the same row. % inhibition when compared to total of controls.

piroxicam but less so with inflammation induced by prostaglandin E_2 (Tables 1 and 2). Furthermore, injection of λ-carrageenan rapidly induce local oedema by increasing vasular permeability (Guo et al., 2008), and promote the release of prostaglandin (Seibert et al., 2004). Our results indicate zerumbone supressed oedema (that is, swelling) and may atteuate the vascular permeability. It has been reported that nitric oxide (NO) plays an important role in λ-carrageenan induced paw oedema by inhibiting iNOS activity, which will produce an anti-inflammatory effect on paw oedema (Rioja et al., 2000).

In the analgesic experiment, which used the acetic acid-induced abdominal writhing assay, the activity of 20 mg/kg zerumbone was found to be similar to that of 20 mg/kg piroxicam until the end of the experiment. The anti-inflammatory effects of zerumbone on acute inflammatory process such as carrageenan-induced oedema was dose-dependent. At 10 mg/kg, zerumbone showed approximately 45% inhibitory activity throughout the measurement intervals and the efficacy of piroxicam (20 mg/kg) was comparable to 20 mg/kg of zerumbone.

Somchit and Shukiryah (2003) had demonstrated the anti-inflammatory activity of crude extract of *Z. zerumbet*, which was expectedly lower than the activity of the pure active compound, zerumbone. Furthermore, Somchit et al. (2005) had reported that the crude extract of *Z. zerumbet* also possesses potent anti-pyretic activity. The acetic acid-induced abdominal constriction is believed to show the involvement of peripheral mechanisms, whereas the hot plate test is believed to show that of central mechanisms (Abbott and Melzack, 1982). It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs (Derardt et al., 1980). Therefore, the results of the acetic acid-induced writhing strongly suggest that the mechanism of this extract may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, thereby reducing PGE_2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor.

These data validated the traditional uses of this plant to assuage pain resulting from headache and dysmenorrhoea as well as inflammatory diseases like gout and rheumatism. Recently, Ganabadi et al. (2009) reported treatment of osteoarthritis using zerumbone as a condroprotective agent. It is also widely used during post-partum to reduce pain, inflammation and for general mothers' health in Malay folk medicine. However, further studies should be performed to elucidate the exact mechanism of analgesic and anti-inflammation. In conclusion, results from our present and previous studies suggested that zerumbone is a potent anti-inflammatory and analgesic agent similar to any commercial NSAIDs.

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