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Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice

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ABSTRACT

The anti-inflammatory activity of zerumbone (**1**), a natural cyclic sesquiterpene isolated from *Zingiber zerumbet* Smith was investigated using carrageenan-induced paw edema and cotton pellet-induced granuloma tissue formation test in mice. It was demonstrated that intraperitoneal administration of **1** at a dose of 5, 10, 50 and 100 mg/kg produced significant dose-dependent inhibition of paw edema induced by carrageenan. It was also demonstrated that **1** at similar doses significantly suppressed granulomatous tissue formation in cotton pellet-induced granuloma test.

1. Introduction

Zerumbone (**1**), is a natural cyclic sesquiterpene found to be the main bioactive compound in the rhizome of *Zingiber zerumbet* Smith (Zingiberaceae) [1–3]. In Malaysia, the rhizome of the plant is commonly used as a condiment for flavoring food and have antispasmodic, analgesic, antirheumatic and carminative effects in folk medicine [4,5]. A great number of pharmacological activities have been attributed for **1**, with much interest has been given particularly to its *in vitro* anti-cancer, anti-inflammatory and chemopreventive potentials [6–12]. It has been demonstrated that **1** attenuated the cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression via modulation of nuclear factor (NF)-kappa B activation in cell culture systems [8,13,14]. However, as far as anti-inflammatory effects of **1** are concerned, there are limited reports on its pharmacological activity in *in vivo* study. Therefore, the present study was conducted to investigate the anti-inflammatory effect of **1** in acute and chronic models of inflammation in mice, namely carrageenan-induced anti-inflammatory and cotton pellet-induced granuloma tests, respectively.

2. Experimental

2.1. Plant material and isolation of compound

Zerumbone (**1**) was isolated from the rhizomes of *Z. zerumbet* Smith as previously reported (purity more than 99%) [15].

2.2. Preparation of test sample and drugs

Acetylsalicylic acid (ASA) and lambda (λ)-carrageenan was purchased from Sigma Chemicals Co. (St. Louis, MO, USA) and dissolved in saline solution (0.9% NaCl), while **1** was dissolved in a mixture of saline and dimethylsulfoxide (DMSO). The final concentration of DMSO did not exceed 1% and caused no detectable effect '*per se*'.

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2.3. Animals

Experiments were conducted using adult male ICR mice (25–35 g) purchased from the Institute for Medical Research, Kuala Lumpur. They were housed at 22 ± 2 °C under a 12-h light/12-h dark cycle and with access to food and water ad libitum. The animals were acclimatized and habituated to the laboratory for at least a week before testing and were used only once throughout the experiments. Experiments reported in this study were performed in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals [16].

2.4. Carrageenan-induced mice paw edema

Inflammation of the paw in mice was induced as described previously with slight modification [17]. Mice ($n = 6$ per group) received i.p. injection of **1** (5, 10, 50 and 100 mg/kg) and ASA (100 mg/kg, as positive control) or a similar volume of vehicle (10 ml/kg) 30 min prior to subplantar injection of 0.02 ml of λ-carrageenan (dissolved in 0.9% NaCl) into the left hind paw of each mouse. Paw volume was measured with a plethysmometer (Ugo Basile, Italy) immediately prior to the injection of carrageenan and thereafter at an interval of 1 h for a period of 5 h. Edema inhibitory activity was calculated according to the following formula [18]:

$$\text{Percentage inhibition} = \frac{\left[(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}\right]}{\left[(C_t - C_0)_{\text{control}}\right]} \times 100$$

where, C_t = mean paw volume for each group at time t, and C_0 = mean paw volume for each group before carrageenan injection.

2.5. Cotton pellet-induced granuloma

Cotton pellet-induced granuloma in mice was conducted according to the method as previously described [19]. Granulomatous lesions were induced by surgically implanting sterile cotton pellet (10 ± 0.5 mg) subcutaneously in the dorsal region of the mice. **1** (10, 50 and 100 mg/kg) and ASA (100 mg/kg) or vehicle (10 ml/kg) were given i.p. once daily for 7 consecutive days. On day 8, the mice were anesthetized, and the pellets covered by granulomatous tissue were dissected out and dried at 60 °C to a constant weight. The mean weights for different groups were determined, and compared to the control group.

2.6. Toxicity test

The toxicity test of **1** was investigated according to the procedures described previously with slight modifications [2]. Mice were divided into four groups of 6 mice each. They were fasted for 2 h before and then were given i.p. administration of **1** at the doses of 10, 50, 100 and 1000 mg/kg on a daily basis for 7 days. The control group only received a similar volume of vehicle (10 ml/kg). The mice were observed for any abnormal behavior such as diarrhea, salivation, respiratory distress, motor impairment and hyperexcitability for 3 h following administration of **1** daily. Furthermore, the incidence of mortality for each group was recorded after administration. At the end of the seventh day, the animals were sacrificed and the internal organs (stomach, small intestine, kidneys and liver) were observed for any abnormal lesions macroscopically and microscopically.

2.7. Statistical analysis

The data obtained was statistically analyzed using one-way ANOVA, followed by Dunnett's multiple comparison tests. The results were expressed as the mean ± S.E.M. to show variation in groups. Differences are considered significant when $P \leq 0.05$.

3. Results and discussion

The present study was carried out to assess the anti-inflammatory effect of **1** in acute exudative and chronic proliferative models of inflammation in mice. Results obtained from the present study provided evidence that **1** possessed an anti-inflammatory activity in both acute and chronic inflammatory models. In the present study it was demonstrated that i.p. administration of **1** at the doses of 5, 10, 50 and 100 mg/kg, significantly produced dose-dependent inhibition of paw oedema induced by carrageenan in mice over a period of 5 h, with percentage of inhibition at 3 h post carrageenan injection of 33.3, 66.7, 83.3, and 83.3%, respectively (Table 1). ASA (100 mg/kg, i.p.) showed an inhibition of 66.7%. The carrageenan-induced paw edema formation is a classical model of acute inflammation and it is believed to involve a biphasic event. It is well known that the early phase (1–2 h) is mediated by the release of histamine and serotonin [20], while the second phase (3–5 h) is the result of the release of kinins and mainly prostaglandins [20,21]. In general, development of edema induced by carrageenan is correlated with the early exudative stage of inflammation, one of the

Table 1
Effect of Zerumbone on the carrageenan-induced mice paw oedema. Each value is the mean of paw volume ± S.E.M. in ml. ($n = 6$); (% inhibition of paw oedema).

Paw volume, ml (% of Inhibition)							
Group	Dose (mg/kg)	Time interval (h)					
		0	1	2	3	4	5
Control	–	0.23 ± 0.02	0.31 ± 0.02	0.33 ± 0.03	0.35 ± 0.03	0.37 ± 0.04	0.34 ± 0.05
ZER	5	0.21 ± 0.02	0.27 ± 0.02 (25.0)	0.28 ± 0.01 (30.0)	0.29 ± 0.02 (33.3)*	0.29 ± 0.02 (42.9)#	0.27 ± 0.02 (45.5)#
	10	0.20 ± 0.02	0.25 ± 0.01 (37.5)*	0.24 ± 0.03 (60.0)#	0.24 ± 0.02 (66.7)#	0.25 ± 0.01 (64.3)#	0.24 ± 0.01 (63.6)#
	50	0.21 ± 0.01	0.25 ± 0.01 (50.0)*	0.25 ± 0.02 (60.0)#	0.23 ± 0.01 (83.3)#	0.22 ± 0.01 (92.9)#	0.22 ± 0.01 (90.9)#
	100	0.23 ± 0.01	0.27 ± 0.02 (50.0)*	0.27 ± 0.02 (60.0)#	0.25 ± 0.02 (83.3)#	0.24 ± 0.02 (92.9)#	0.23 ± 0.02 (100)#
ASA	100	0.22 ± 0.02	0.25 ± 0.03 (62.5)*	0.26 ± 0.04 (60.0)#	0.26 ± 0.03 (66.7)#	0.27 ± 0.03 (64.3)#	0.26 ± 0.02 (63.6)#

*$p < 0.05$ and #$p < 0.001$ when compared with control (ANOVA followed by Dunnett's test).

Table 2
Effect of Zerumbone on granuloma tissue formation in mice.

Group	Dose (mg/kg)	Granuloma dry weight[a] (mg)	Inhibition (%)
Control	–	25.61 ± 0.012	–
ZER	10	16.70 ± 0.005	34.8
	50	10.08 ± 0.001*	60.6
	100	7.533 ± 0.002#	70.6
ASA	100	11.60 ± 0.005*	54.7

*$p<0.05$ and #$p<0.001$ when compared with control (ANOVA followed by Dunnett's test).

[a] Each value is the mean weight (mg) ± S.E.M. ($n=6$).

important processes of inflammation [22]. Based on the present results it can be suggested that the inhibitory effect of **1** on carrageenan-induced paw edema in mice may be due to the suppression of the release of mediators responsible for inflammation including histamine, serotonin, bradykinin and prostaglandin.

The cotton pellet-induced granuloma is an established chronic inflammatory model [23]. The present results showed that **1** at the doses of 10, 50 and 100 mg/kg (i.p), produced a significant reduction on the granulomatous tissue formation on implanted cotton pellets with inhibition of 34.8, 60.6 and 70.6%, respectively as compared with ASA (100 mg/kg, i.p.) which produced significant inhibition of 54.7% (Table 2). The cotton pellet-induced granuloma has been widely used to evaluate the transudative and proliferative components of chronic inflammation and the dried weight of the pellets correlates well with the amount of granulomatous tissue formed [24]. Our results revealed the efficacy of **1** to inhibit the activity of fibroblasts as well as synthesis of collagen with mucopolysaccharide, which are natural proliferative events of granulation tissue formation [25] and it is also possible that, in this inflammatory model, **1** acts by inhibiting neutrophils and macrophages migration [18,26,27]. In addition, it appears relevant to point out that the toxicity test obtained shows no occurrence of death over the period of 7 days of treatment even with the highest dose of **1** (1 g/kg). Furthermore, macroscopic and microscopic observations of the organs appeared normal and did not show any treatment-related organ abnormality (data not shown). These results indicating that **1** might have a reasonable safety margin with regards to acute toxicity.

Based on the present study, it can be concluded that **1** possessed anti-inflammatory effect against both exudative and proliferative phases of inflammation. Although the present data do not clearly indicate the mechanism of action, the observed anti-inflammatory effect of **1** in both carrageenan-induced paw edema and cotton pellet-induced granuloma is in agreement with the *in vitro* models [8,10,14,28] and justifies the wide use of *Z. zerumbet* for the treatment of inflammatory conditions. The exact mechanism(s) that underlie the anti-inflammatory effects of **1** observed in this study are currently under investigation.

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