Malaysian Journal of Science **18**: 17-20 (1999)

Chemical components and anti-inflammatory activity of the aqueous extract of the rhizomes of *Kaempferia galanga* L.

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Abstract. This study reports the effects of the aqueous extract of the rhizomes of *Kaempferia galanga* L. on rats administered intraperitoneally. The extract showed anti-inflammatory activity at doses of 30, 60 and 125mg kg^{-1} in carrageenin-induced paw oedema, The extract at a dose of 125 mg kg^{-1} also inhibited histamine-induced paw oedema. In peroxide-induced paw oedema, the extract at doses of 125 and 250 mg kg^{-1} inhibited the oedema when the doses were given 20 minutes before the injection of glucose oxidase. The percentage suppression of oedema was higher at 450 mg kg^{-1}. As the aqueous extract was also shown to have irritant property that can exert anti-inflammatory effects through a 'counter-irritant' mechanism, the the irritant nature of the extract could contibute to the observed anti-inflammatory effects.

Abstrak. Tujuan kajian melaporkan kesan ekstrak akueus rizom *Kaempferia galanga* L. ke atas tikus secara pemberian intraperitoneal. Ekstrak pada dos 30, 60 dan 125 mg kg^{-1} menunjukkan aktiviti anti-inflamasi ke atas edema yang diinduksikan oleh karagenin. Ekstrak pada dos 125 mg kg^{-1} merencat edema yang diinduksikan oleh histamin. Untuk edema yang diinduksikan oleh peroksida, ekstrak pada dos 125 dan 250 mg kg^{-1} menunjukkan perencatan apabila ekstrak diberikan 20 minit sebelum penyuntikan glukos oksidase. Peratus perencatan edema adalah lebih tinggi apabila 459 mg kg^{-1} diberikan. Oleh kerana ekstrak akueus uga menunjukkan sifat iritan yang boleh menyebabkan kesan anti-inflamasi melalui mekanisme 'lawan-iritan', sifat iritan ini mungkin menyumbang kepada aktiviti anti-inflamasi ekstrak yang diperhatikan.

Introduction

The genus *Kaempferia* (Zingiberaceae) comprises small herbs having fleshy rhizomes and tuberous roots. Of the four species of *Kaempferia* reported for Peninsular Malaysia [1], *Kaempferia galanga* L. is perhaps the most widely cultivated throughout South-East Asia. In Malaysia, *K. galanga* can be found as a common village plant locally known as *cekur*, which is consumed as food and is used in traditional medicine for rheumatism, cough, inflammation, eczema and asthma. In Indonesia, it is sold as a health tonic. The aqueous extract of the rhizomes of *K. galanga* have anti-asthmatic activity, but the oral administration of the extract did not show anti-inflammatory activity in mice [2]. The present study reports the effects of the aqueous extract when administered intraperitoneally to rats. Three models of acute inflammation (carrageenin-induced paw oedema, histamine-induced paw oedema and peroxide-induced oedema) was used as models of inflammation model. The extract was also tested for irritancy.

Experimental

Preparation of aqueous extract of the rhizomes. Samples of *K. galanga* rhizomes were obtained from the herbs garden of the University of Malaya. The rhizomes were cleaned, chopped into small pieces and then homogenised in water by using a blender. This mixture was filtered to remove insoluble particles. The filtrate was centrifuged at 100,000 G for 15 minutes. The supernatant obtained was then freeze-dried.

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Sprague-Dawley rats by using the method of Winter *et al.* [3. The extract was injected in the left hind paw of the rats; 0.05 mL of 2% carrageenin solution in normal saline was used. The paw oedema was evaluated by measuring the change in thickness of the paw with a caliper immediately before and after 3 and 4.5 hours after the carrageenin injection. The percentage inhibition of oedema was assessed by comparison with a control group. The statistical difference between test groups was calculated by the use of Student's t-test. Results were considered significant if $P < 0.05$.

In the histamine- and peroxide-induced inflammation, 0.1 mL of 3% solution of histamine and 0.1 mL of 4% solution of glucose oxidase in normal saline were each injected into the hind paw of each rat. The assessment of anti-inflammatory activity was the same as for carrageenin oedema, but measurements were taken at 30 and 60 minutes after the injection of histamine, and at 40 minutes, 1.5 hours and 3 hours after the injection of glucose oxidase.

Testing for anti-inflammatory activity. In the carrageenin-induced oedema, rats were given 1 mL of the extract intraperitoneally at doses of 30, 60 and 125mg kg^{-1} an hour before the injection of carrageenin. In the histamine-induced oedema, 1 mL of the extract at a dose of 125mg kg^{-1} was injected intraperitoneally to the rats 30 minutes before the injection of histamine and in the peroxide-induced inflammation, the extract at doses of 125, 250 and 450mg kg^{-1} was given intraperitoneally at 20 or 40 minutes before the injection of glucose oxidase. Control rats were injected intraperitoneally with distilled water.

Test for irritancy of the aqueous extract. The irritant activity of the extract at doses of 30, 60 and 125mg kg^{-1} was assessed by measuring the increase in paw diameter folowing the injection of 0. 1 mL of the extract into the hind paws. Controls were treated with the same volume of 0.9% saline.

Results and Discussion

Carrageenin-induced paw oedema is a commonly used acute inflammatory reaction for screening anti-inflammatory drugs. The oedema that develops after carrageenin injection is a biphasic event [4]. The initial phase is attributed to the release of histamine and serotonin. The oedema is then maintained by kinin-like substances and the second phase (3 - 5 hour) and is believed to be promoted by prostaglandin-like substances. The aqueous extract of *K. galanag* showed anti-inflaminatory activity in the carrageenin-induced oedema when it was given intraperitoneally to the rats at doses of 30, 60 and 125mg kg^{-1} (Table 1). At these doses, the percentage suppression of oedema when measured at 3 hours after the injection of carrageenin was 19%, 42% and 82%, and 16%, 32% and 79% when foot measurements were taken at 4.5 hours after the injection of carrageenin. The aqueous extract at a dose of 125mg kg^{-1} also inhibited histamine-induced oedema (Table 2). The percentage suppression of oedema when measured at 30 and 60 minutres. after the histamine injection was 30% and 41%.

The intraplantar injection of glucose oxidase produce an inflammatory response in rats. The glucose oxidase reacts with endogenous glucose to generate gluconic acid and hydrogen peroxide (H_2O_2). Hydrogen peroxide can react to produce hydroxy (OH) radicals that are responsible for tissue damage and for the accompanying inflammation changes.

For such inflammation, the anti-inflammatory effect of the aqueous extract was observed to be short acting because at doses of 125 and 250 mg kg^{-1}. The anti- inflammatory effect was observed only when the doses were given 20 minutes before the injection of glucose oxidase (Table 3) but not when they were given 40 minutes before the injection of glucose oxidase. At a dose of 250 mg kg^{-1}, the percentage suppression of oedema when measured at 0.67, 1.5 and 3 hours after the injection of glucose oxidase was 25, 22 and 10%. At a dose of 125mg kg^{-1}, the extract only inhibited the oedema when paw measurements were taken at 40 minutes after the injection of glucose oxidase (percentage suppression of oedema was about 10%). At a higher dose (450 mg kg^{-1}), the anti-inflammatory effect of the extract on the peroxide-induced inflammation was stronger. The percentage suppression of oedema at 0.67, 1.5 and 3 hours after the injection of glucose oxidase was 49, 39 and 22% (Table 4).

The anti-inflanimatory action of a substance can also be the result of the substance being an irritant. Irritants have long been known to exert anti-inflammatory effects [5,6]. The mechanism by which irritants exert anti-inflammatory effect is unknown; various hypothesis have been put forward to explain the 'counter- irritant' effect [7]. The aqueous extract has irritant action (Table 5), i.e., it induces paw swelling following its subplantar injection. Because the irritancy and the fact that the extract is not effective orally (unrecorded observation) would suggest that counter-irritation had reduced the paw oedema in a non- specific manner, the possibility cannot be ruled out that the anti-inflammatory effect of the extract is the result of both counter-irritant and other mechanisms of action.

Acknowledgments

We would like to thank the University of Malaya for financial support (PJP 25188).

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Table 1. Anti-inflammatory action of aqueous extract of *K. galanga* on carrageenin-induced oedema

Treatment	Increase in paw diameter (mm) Afer 3 hours	Afer 4.5 hours
Control	4.67±0.17	4.67±0.17
30 mg kg^{-1}	3.78±0.11**	3.91±0.11*
60 mg kg^{-1}	2.71±0.17***	3.18±0.11***
125 mg kg^{-1}	0.83±0.11***	1.00±0.13 ***

Each result represents the mean of 6 values ±SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 2. Anti-inflammatory action of aqueous extract of *K. galanga* on histamine-induced oedema

Treatment	Increase in paw diameter (mm) Afer 0.5 hour	Afer 1 hour
Control	2.50±0.22	2.25±0.17
125 mg kg^{-1}	1.75±0.11*	1.33±0.11**

Each result represents the mean of 6 values ±SEM. $*P < 0.05$; $**P < 0.01$.

Table 3. Effect of aqueous extract of *K. galanga* given before the injection of glucose oxidase

Treatment	Increase in paw diameter (mm) Afer 2/3 hour	Afer 1.5 hours	After 3 hours
Control	4.42±0.02	4.83±0.11	5.17±0.11
125 mg kg^{-1}	4.00±0.02*	4.50±0.13	4.02±0.15
250 mg kg^{-1}	3.33±0.17***	3.75±0.17***	4.67±0.11*

Each result represents the mean of 6 values ±SEM. $*P < 0.05$; $***P < 0.001$.

Table 4. Anti-inflammatory action of aqueous extract of *K. galanga* on peroxide-induced oedema

Treatment	Increase in paw diameter (mm) Afer 2/3 hour	Afer 1 hour	After 3 hours
Control	5.08±0.02	5.75±0.17	6.33±0.11
125 mg kg^{-1}	2.58±0.15***	3.50±0.13***	4/02±0.15***

Each result represents the mean of 6 values ±SEM. $**P < 0.01$.

Table 5 Anti-inflammatory action of aqueous extract of *K. galanga* on peroxide-induced oedema

Treatment	Increase in paw diameter (mm) Afer 1 hour	Afer 3 hour	After 5 hours
Control	0.17±0.11	0.00±0.00	0.00±0.00
30 mg kg^{-1}	1.00±0.13	0.92±0.02	0.67±0.11
60 mg kg^{-1}	1.75±0.11	1.75±0.11	1.33±0.11
125 mg kg^{-1}	2.17±0.17	2.17±0.17	2.00±0.13