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ANTI-ALLERGIC EFFECTS OF 1,5-BIS(4′-HYDROXY-3′-METHOXYPHENYL)-1,4-PENTADIENE-3-ONE ON MAST CELL-MEDIATED ALLERGY MODEL

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1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one is a 1,5-diphenyl-1,4-pentadiene-3-one analogue of curcumin that is produced by modifying the middle site of curcumin leading to 1,4-pentadiene-3-ones to maintain the hydroxy moiety at the aromatic rings that are responsible for its biological activities. Curcumin has been reported to have anti-allergic effects and can inhibit the release of histamine from mast cells. In the present study, we evaluated the anti-allergic effects of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one in a mast cell-mediated allergy mode in order to provide information about a newly synthesised-compound for an alternative allergy drug. The study was performed using (1) a rat basophilic leukaemia (RBL-2H3) cell line, which is a tumour analogue of mast cells, with DNP_{24}-BSA, thapsigargin and ionomycin as inducers for secretory markers from mast cells, and (2) an active cutaneous anaphylaxis (ACA) reaction, with ovalbumin as an inductor of mast cell degranulation. Treatment with 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one strongly inhibited the DNP_{24}-BSA, thapsigargin and ionomycin-mediated release of histamine and β-hexosaminidase from the RBL-2H3 cell line. The results indicated that this compound influenced the activation processes of FcεRI by antigen and intracellular Ca^{2+} signalling events in mast cells. In type 1 allergy model, this compound also inhibited the active cutaneous anaphylactic reaction on rat dorsal skins generated by ovalbumin. We conclude that the compound 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one showed anti-allergic activities mediated by mechanisms related to intracellular signalling events in mast cells.

Keywords: *1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one, RBL-2H3, Anti-allergic Activity, Histamine, β-hexosaminidase*

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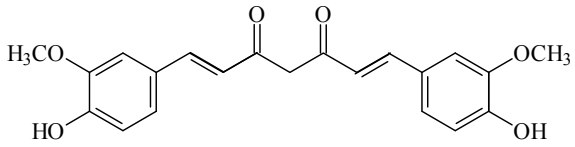
INTRODUCTION

The compound 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one is a 1,5-diphenyl-1,4-pentadiene-3-one analogue of curcumin (Sardjiman *et al.* 1997). It is naturally isolated from the rhizomes of *Curcuma domestica* and *Curcuma longa* L. (Masuda *et al.* 1993; Park and Kim 2002). Its chemical structure is similar to that of curcumin; both compounds have hydroxy and methoxy moieties at their aromatic rings. The chemical difference occurs in the middle site where curcumin possesses 1,7-diphenyl-1,6-heptadiene-3,5-dione, whereas its analogue possesses 1,5-diphenyl-1,4-pentadiene-3-one (Fig. 1). Although curcumin has several beneficial pharmacological effects, its use is limited by its instability, which is strongly affected by factors such as pH and exposure to light. The instability of curcumin at alkaline pH is caused by the active methylene moiety. The decomposition of curcumin can also occur as a result of light exposure, a process that is also mediated by the active methylene moiety (Tonnesen and Karlsen 1985; van der Goot 1995). Compounds such as the 1,4-pentadiene-3-ones, which lack the active methylene moiety and 1 carbonyl moiety of curcumin, display increased stability. The modification of the middle site of curcumin leading to 1,4-pentadiene-3-ones still maintains the hydroxy moiety at the aromatic rings that are responsible for its biological activities. Additionally, the synthesis of 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one is a simple and easy method (Sardjiman *et al.* 1997).

Several studies have examined the effects of curcumin on allergic diseases. An ethyl acetate extract of *Curcuma longa* L., which contains curcuminoids, has been shown to exhibit a preventive effect on type I and IV allergy models (Yano *et al.* 2000a). Furthermore, both the ethyl acetate extract of *Curcuma longa* L. and curcumin were found to decrease histamine release from mast cells by blocking intracellular signalling events in mast cells. However, the ethyl acetate extract of *Curcuma longa* L. had a somewhat stronger activity than that of curcumin, which may have been due to the presence of curcumin derivatives (Yano *et al.* 2000b). Another study has previously examined the anti-allergy activities of curcumin and curcumin-related compounds in relation to their antioxidant activities. Most of these compounds were shown to inhibit histamine release from RBL-2H3 cells induced by concanavalin A or a calcium ionophore. In the study, hydroxy moieties of curcumin were shown to have significant anti-oxidative and anti-allergic activities. Most

of the compounds showed anti-allergic activities through mechanisms related to anti-oxidative activities, although others display anti-allergic mechanisms unrelated to their antioxidative activity. These results suggest that the hydroxy moiety of curcumin is responsible for its antioxidant activity and may also contribute to the inhibition of histamine release (Suzuki *et al.* 2005). In a guinea pig model of airway hyper-responsiveness, 20 mg/kg body weight of curcumin significantly inhibited ovalbumin (OVA)-induced airway constriction and airway hyperactivity (Ram, Dass and Ghosh 2003).

In the present study, we investigated the anti-allergic effects of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one. In the *in vitro* study, we examined the effect of this compound on the release of histamine and β-hexosaminidase from RBL-2H3 (rat basophilic leukaemia) cells induced by mediator releasing agents such as DNP_{24}-BSA, thapsigargin and ionomycin. In the *in vivo* study, we determined the effect of this compound on an active cutaneous anaphylaxis reaction on the dorsal skin of rats, which was generated by ovalbumin. This study may provide information about discovery of a cheap and easily synthesised compound for an alternative allergy drug.



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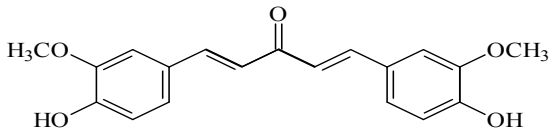


(b)

Fig. 1: Chemical structure of curcumin (a) and 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one (b).

METHODS

Materials

1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one was synthesised according to methods described previously (Sardjiman *et al.* 1997). Histamine release inducers used were ionomycin, thapsigargin, and ovalbumin (Sigma Chemical Co., St. Louis, MO, USA). Dinitrophenylated bovine serum albumin (DNP_{24}-BSA), which was used as an antigen, and monoclonal IgE against DNP_{24}-BSA (purified from supernatants of an IgE producing hybridoma) were purchased from the Department of Pharmacology, Ehime University, Japan. Eagle's minimum essential medium (MEM) and antibiotics were purchased from Gibco (Grand Island, NY, USA). Foetal calf serum was obtained from JRH Biosciences (Kansas, USA). Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was purchased from Dosindo (Kumamoto, Japan). Evans blue, Alcian blue and Nuclear fast red were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were high-grade qualified materials.

Preparation of Drug Solution

The drug was dissolved in DMSO to provide a 25 mM stock solution and diluted with PIPES buffer to provide a series of drug concentrations (0.1–100 μM). Serial dilutions of the drug were prepared immediately before the *in vitro* assays were performed. In our preliminary study, 0.4% DMSO did not influence the histamine release from rat mast cells. In the *in vitro* assays, we used PIPES buffer solution for the control group treatment.

Animals

Male Wistar rats (250-300 g) were housed in a room with controlled temperature and lighting and allowed free access to chow and tap water. The animal handling protocols of this study were in accordance with the guidelines of the animal care of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University, Indonesia, and approved by the committee for animal research.

Culture of RBL-2H3 Cells

RBL-2H3 cells were cultured in MEM medium containing 15% foetal calf serum and antibiotics (penicillin and streptomycin) in a humidified atmosphere (5% CO_2) at 37°C (Barsumian *et al.* 1981). The cells were seeded into 24-well culture plates at a density of 5 x 10^5 cells/0.4 mL per each well and then incubated overnight at 37°C. For DNP_{24}-BSA experiments, the cells were sensitised with 0.5 μg/mL of monoclonal IgE. On the second day, the medium was removed, and the cells were washed twice with 0.5 mL PIPES buffer (25 mM PIPES, 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 40 mM NaOH, and 0.1% bovine serum albumin at pH 7.2) and pre-incubated for 10 min at 37°C after addition of 180 μL PIPES either without (as a negative control) or with the drug (0,1–100 μM). After incubation, 20 μL of stimulant (200 ng/mL DNP_{24}-BSA, 5 μM thapsigargin, or 10 μM ionomycin) was added to each well, and the plate was incubated at 37°C for 30 min.

Assay of Histamine Release

Histamine released into the medium was measured by HPLC-fluorometry, as described previously (Yamatodani *et al.* 1985). After a 30-min incubation, the plates were centrifuged at 3,000 rpm for 5 min and 50 μL of the supernatant was mixed with 250 μL of 3% perchloric acid containing 5 mM Na_2-EDTA. After addition of 30 μL of 2 M KOH/1 M KH_2PO_4 and centrifugation at 10,000 x g for 15 min at 4°C, 50 μL of the supernatant was injected directly onto a column packed with a TSKgel SP-2SW cation exchanger (Tosoh, Tokyo). To measure the total histamine content in cells, 350 μL of PIPES buffer was added to 6 wells, and the cells were then sonicated. 50 μL of cell homogenate was used for the histamine assay described above. Histamine was eluted with 0.25 M potassium phosphate at a flow rate of 0.6 mL/min, post-labelled with *o*-phthalaldehyde under alkaline conditions and detected using a F1080 Fluorometer (Hitachi, Tokyo) at excitation and emission wavelengths of 360 and 450 nm, respectively.

Assay of β-Hexosaminidase Enzyme Release

For the β-hexosaminidase enzyme assay, 50 μL of supernatant was incubated with *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranocide (100 μL, 2.5 mM in 50 mM sodium citrate buffer, pH 4.5) for 1 h at 37°C. 20 μL of 2 M KOH/ 1 M KH_2PO_4 was then added, and the β-hexosaminidase enzyme activity was determined by a colorimetric analysis method using a microplate reader at 405 nm.

Active Cutaneous Anaphylaxis (ACA) Reaction

The rats were sensitised on days 1 and 7 with 0.1% ovalbumin and 10% $Al(OH)_3$ (1 mL per 200 g body weight BW) at the dorsal site, subcutaneously (SC). On day 14, the anaphylaxis reaction was generated with 52.5 mg per 200 g BW of ovalbumin, given SC. To determine vascular permeability, the rats were injected with 1% Evans blue via the tail vein, and the dorsal pigmentation areas were observed for 8 h after ovalbumin challenge. The drugs were orally administered 15 min prior to ovalbumin challenge. Cromolyn sodium (2.16 mg/kg BW, SC) was used as a reference drug. The area under the curve of observed Evans blue dye was calculated to evaluate the inhibitory effect.

Histological Observation

The dorsal skins were removed and fixed in Carnoy's solution (ethanol, chloroform and glacial acetic acid in a ratio of 60:30:10 by volume) for 12 h, changing to fresh solution every 3 h. To detect mast cells, the specimens were stained with 0.1% Alcian Blue (pH 0.3) and Nuclear fast red (NFR). They were rinsed in distilled water and gradually dehydrated in a series of alcohol concentrations. The slides were cleared in xylene and mounted with mounting medium. Under light microscopy, mast cells were qualitatively identified as blue granules against a pale red background.

Statistical Analysis

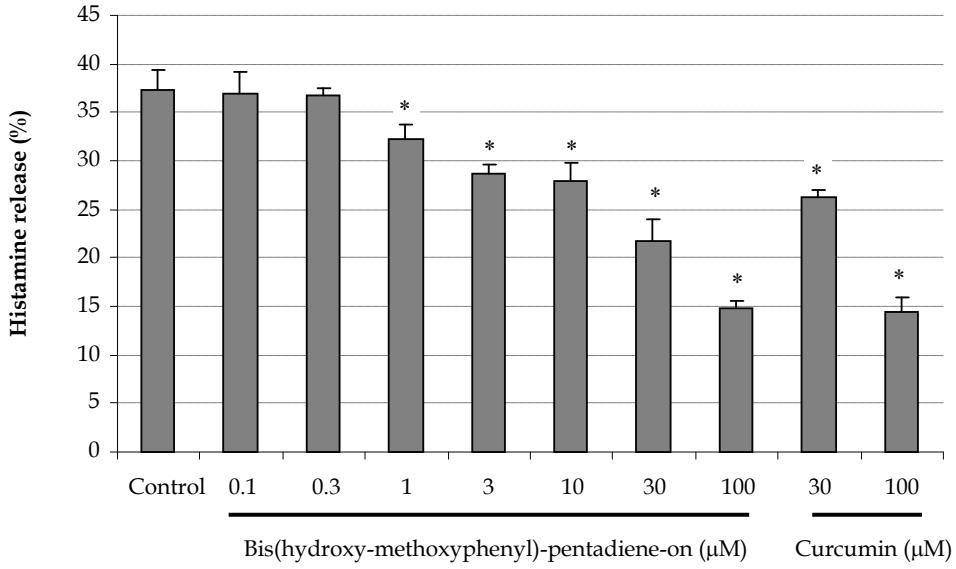
All data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was used

for statistical analyses. P-values of less than 0.05 were considered significant.

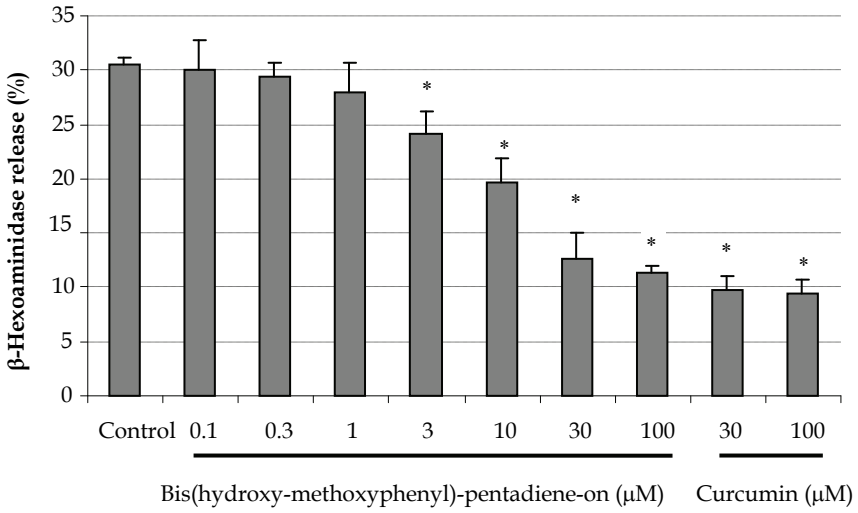
RESULTS AND DISCUSSION

In our preliminary study, 180 μL of DMSO solution diluted 250x in PIPES buffer to yield 0.4% DMSO did not have a significant effect on the release of histamine from rat mast cells when compared to controls (48.54 ± 1.22 vs. 48.24 ± 0.84, n= 3 to 5). DMSO can greatly influence the effect of various histamine inducers at high concentrations (> 3 or 5% DMSO); however, at low concentrations, the effect of DMSO on the histamine release from mast cells was not considered significant (Moldt, Andersen and Christensen 1988). In our study, the highest concentration of DMSO was 0.4%.

DNP_{24}-BSA (20 ng/mL) stimulated the release of histamine and β-hexosaminidase from RBL-2H3 cells by 37.23 ± 2.16% and 30.50 ± 0.58%, respectively (Fig. 2). Pre-incubation of RBL-2H3 cells with 1,5-bis (4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one for 30 min signify-cantly inhibited the DNP_{24}-BSA-induced release of histamine and β-hexosaminidase in a dose-dependent manner. At the highest dose (100 μM), the compound inhibited the release of histamine and β-hexosaminidase from RBL-2H3 cells by 60.17 ± 1.90% and 30.50 ± 0.58%, respectively (Table 1). The IC_{50} value of the inhibitory effect of 1,5-bis (4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one was used as the parameter of potency. The IC_{50} values of the inhibitory effects of the compound on DNP_{24}-BSA-mediated histamine and β-hexosaminidase release were 62.09 μM and 22.64 μM, respectively.



(a)



(b)

Fig. 2: Effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on **(a)** histamine and **(b)** β-hexoaminidase release from RBL-2H3 cells in the presence of DNP-BSA 20 ng/mL. Data represent mean±SEM, and are three independent experiments. * $P<0.05$ compared to the negative control value.

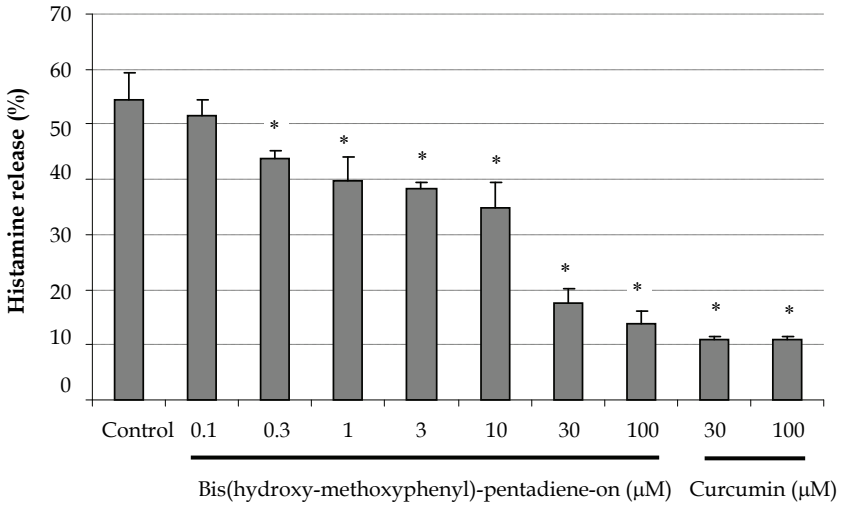
Table 1: The inhibitory effects of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on the histamine release from RBL-2H3 cells and and its spontaneous histamine and β-Hexosaminidase enzyme release.

No.	Conc. (μM)	Histamine release inhibition (%)			Spontaneous histamine release (%)
		DNP_{24}-BSA	Thapsigargin	Ionomycin	
1.	0.1	0.75±5.94	5.49±5.34	12.05±6.07	4.80±0.38
2.	0.3	1.45±2.42	19.75±2.36	14.38±10.67	0.56±0.76
3.	1	13.43±4.14	27.06±8.05	26.31±7.30	0.76±0.86
4.	3	23.06±2.38	29.69±2.12	29.10±1.74	2.20±0.52
5.	10	25.03±5.28	36.08±8.41	39.06±6.58	3.50±0.87
6.	30	41.39±5.95	67.97±4.91	61.18±2.01	3.99±0.96
7.	100	60.17±1.90	74.45±3.90	72.61±3.46	4.52±0.90

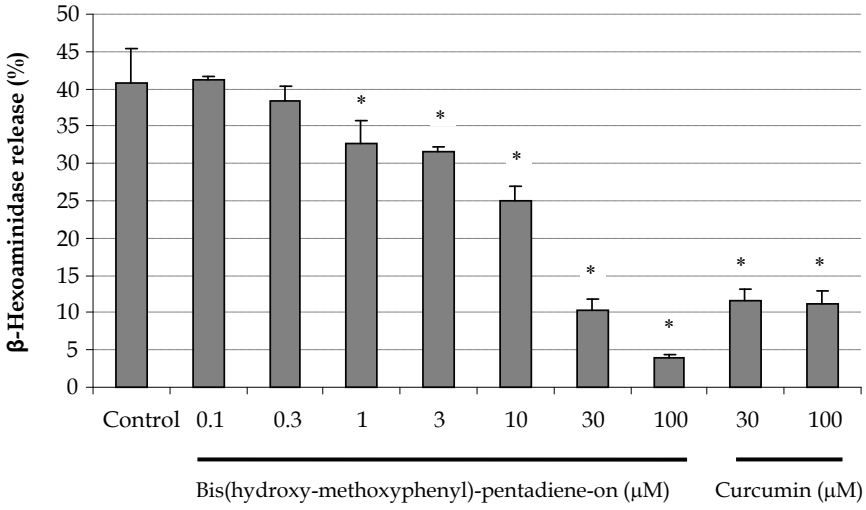
No.	Conc. (μM)	β-Hexosaminidase release inhibition (%)			Spontaneous β-hexosaminidase release (%)
		DNP_{24}-BSA	Thapsigargin	Ionomycin	
1.	0.1	1.51±8.99	-0.70±1.12	10.28±3.19	0.15±0.84
2.	0.3	3.46±3.80	6.06±4.79	11.47±0.36	-0.18±0.55
3.	1	8.31±8.75	20.30±7.56	21.99±2.31	-1.14±0.56
4.	3	21.04±6.83	22.90±1.62	21.31±2.60	0.70±0.87
5.	10	35.43±7.08	39.05±4.84	30.51±7.64	1.68±1.46
6.	30	58.49±7.83	74.63±3.38	53.11±6.22	-1.82±0.27
7.	100	62.97±2.45	90.45±1.02	76.04±5.31	1.04±1.58

Concentration of DNP_{24}-BSA, thapsigargin and ionomycin were 20 ng/mL, 0.5 μM and 1 μM, respectively. Data represent mean ±SEM of three independent experiments.

Thapsigargin (0.5 μM), which acts on both the influx of calcium ions and intracellular calcium pathways (Brayden *et al.* 1989), stimulated the release of histamine and β-hexosaminidase by 54.57 ± 4.80%; and 40.87 ± 4.42%, respectively (Fig. 3). 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one significantly inhibited the thapsigargin-induced release of histamine and β-hexosaminidase in a dose-dependent manner. At a dose of 100 μM, the compound potently inhibited the release of histamine and β-hexosaminidase from RBL-2H3 cells by 74.45 ± 3.90% and 90.45 ± 1.02%, respectively (Table 1). The respective IC_{50} values of the



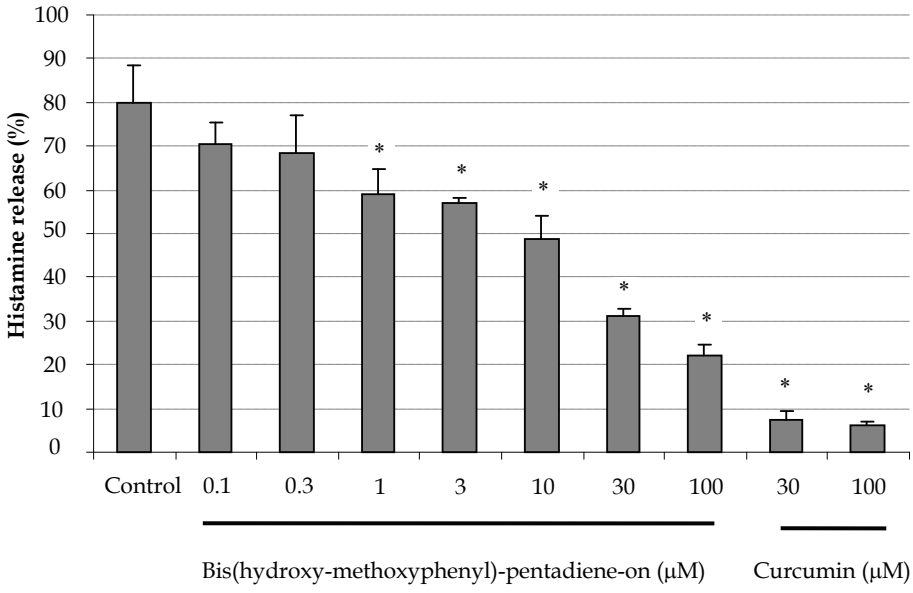
(a)



(b)

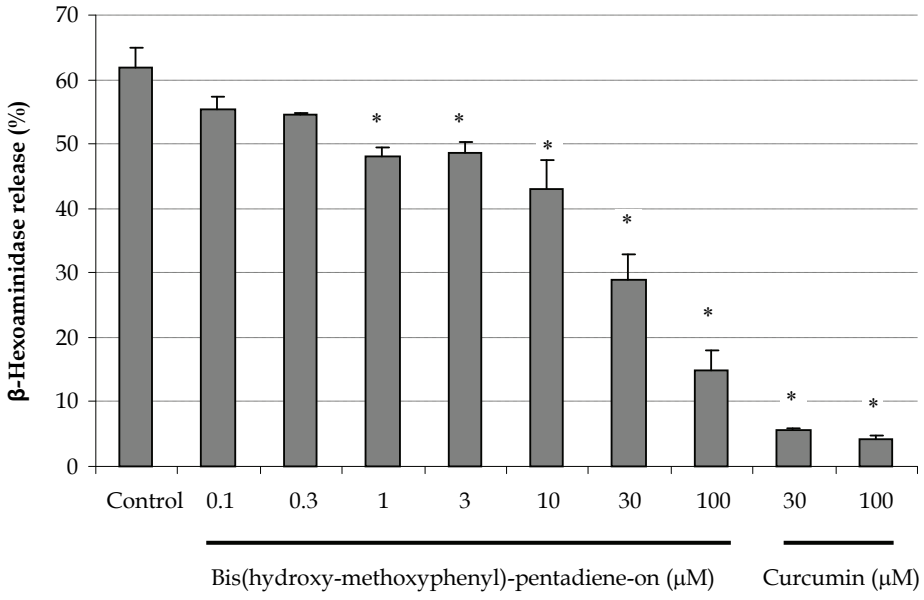
Fig. 3: Effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on histamine **(a)** and β-hexoaminidase **(b)** release from RBL-2H3 cells in the presence of thapsigargin 0.5 μM. Data represent mean±SEM, and are three independent experiments. * $P<0.05$ compared to the negative control value.

inhibitory effects of the compound on thapsigargin-mediated release of histamine and β-hexosaminidase were 18.73 μM and 16.16 μM. Ionomycin (1 μM), which acts as a calcium ion ionophore (Huang and Putney 1998), stimulated the release of histamine and β-hexosaminidase by 80.10±8.43% and 61.73 ± 3.13%, respectively (Fig. 4). 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one significantly inhibited the ionomycin-induced release of histamine and β-hexosaminidase in a dose-dependent manner. At a dose of 100 μM, the compound inhibited the release of histamine and β-hexosaminidase from RBL-2H3 cells by 72.61 ± 3.46% and 76.04 ± 5.31%, respectively (Table 1). The respective IC_{50} values of the inhibitory effects of the compound on thapsigargin-mediated histamine and β-hexosaminidase release were 19.89 μM and 27.25 μM.



(a)

Fig. 4: Effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on histamine **(a)** and β-hexoaminidase **(b)** release from RBL-2H3 cells in the presence of ionomycin 1 μM. Data represent mean±SEM, and are three independent experiments. *P<0.05 compared to the negative control value *(continued on next page)*.



(b)

Fig. 4: *(continued)*

Another way to evaluate the anti-allergic property of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one is to study its effect on the ACA reaction. Ovalbumin (OVA) and $Al(OH)_3$ were injected subcutaneously into the dorsal skin of rats on the day 1 and 7. On day 14, a cutaneous anaphylactic reaction was generated by a subcutaneous injection of ovalbumin and visualised after an intravenous injection of Evans blue dye via the tail vein. 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one was orally administered to the rats 15 min prior to ovalbumin challenge, and the ACA reaction was evaluated as shown in Figure 5 and Table 2. Since pharmacokinetic data of the drug is not yet available, the 15 min oral treatment of the drug prior to OVA challenge was performed according to our group's previous studies using other analogues of curcumin, such as Gamavuton-0 potassium (Nugroho *et al.* 2007) and Pentagamavunon-0 and its potassium salt (Ikawati *et al.* 2006). We assumed that analogues of curcumin have similar pharmacokinetic patterns. As seen in Table 2, this compound inhibited vascular permeability, which is an indication of cutaneous anaphylactic reaction.

Cromolyn sodium, a stabiliser of mast cell membranes, also clearly reduced the anaphylactic reaction.

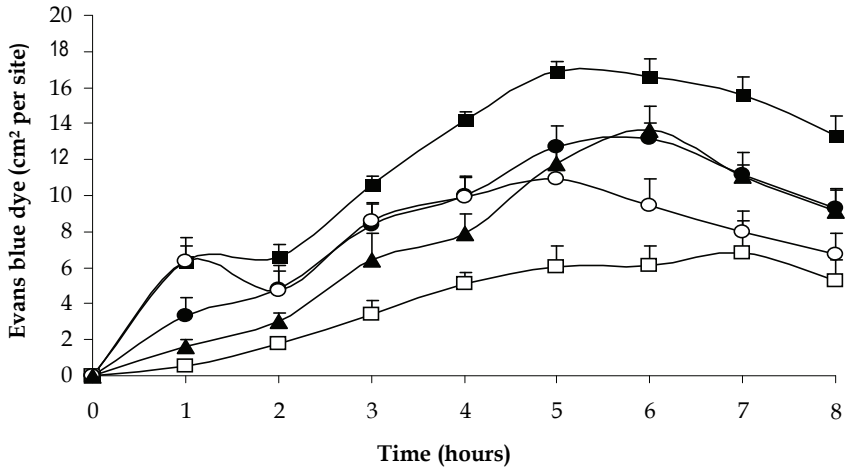


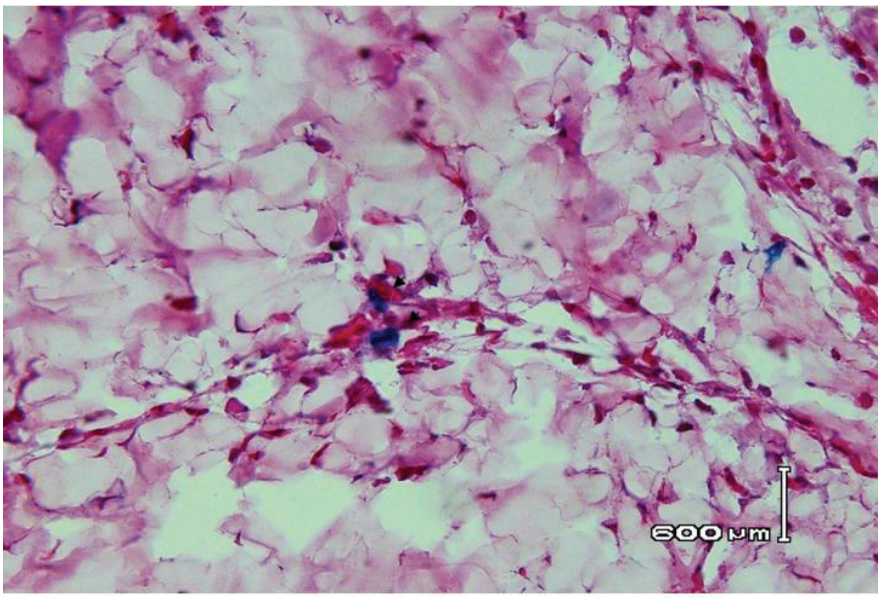
Fig. 5: Effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on ovalbumin-induced active cutaneous anaphylaxis in rat dorsal skin. Data represent mean ± SEM, and are five independent experiments. ■=control (vehicle), □= treatment of cromolyn sodium 2,16 mg/kg BW, ●= treatment of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one 10 mg/kg BW, ○= 20 mg/kg BW, ▲= 40 mg/kg BW.

Table 2: The inhibitory effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on ovalbumin-induced active cutaneous anaphylaxis in rat dorsal skin.

Treatment (mg/kg BW)	AUC_{0-8} (cm^2.hours)	Inhibition (%)
Vehicle	93.53 ± 2.50	
10	32.55 ± 4.45 *	65,20 ± 4,76
20	68.22 ± 3.90 *	27.06 ± 4.17
40	61.35 ± 7.78 *	34.41 ± 8.32
Cromolyn sodium (2.16 mg/kg BW)	59.97 ± 5.94 *	35.89 ± 6.35

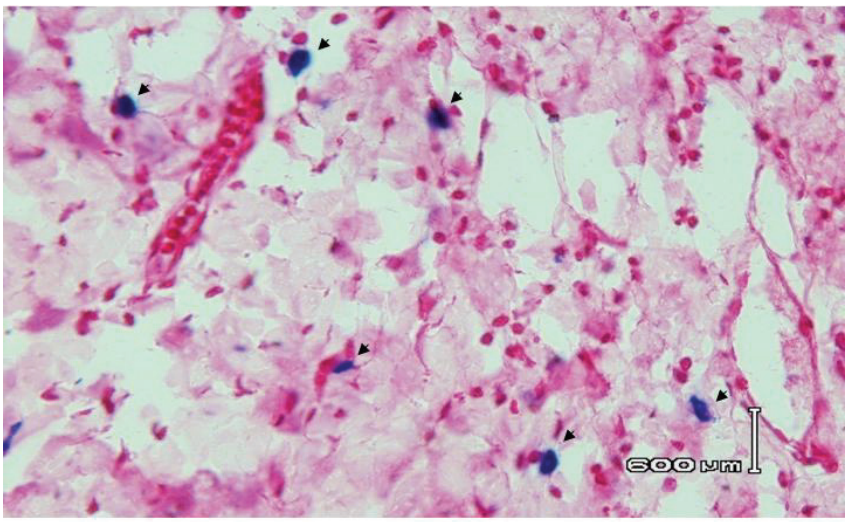
The drug was administered 15 min prior to challenge with ovalbumin. Each value represents the mean ±SEM of five independent experiments. *P<0.05, significantly different from the vehicle value.

Mast cells in the rat dorsal site stained by Alcian blue and NFR are shown in Figure 6. The control group, which was treated with ovalbumin, contained only a few mast cells. However, the number of stained mast cells increased after treatment with 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one 15 min prior to ovalbumin challenge (Fig. 5). In the present study, the histological observation provided qualitative data to support the active cutaneous anaphylactic reaction data (*in vivo* study). Representative photographs of skin sections in the study are shown, however, quantification of mast cell numbers is needed in further studies.



(a)

Fig. 6: The histopathology of alcian blue-NFR stained mast cells from dorsal skin of type I allergy models, **(a)** control (vehicle), **(b)** treatment of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one 20 mg/kg BW, and **(c)** treatment of cromolyn sodium 2.16 mg/kg BW. Magnifications were 200x. Mast cells were stained blue indicated by arrow *(continued on next page).*



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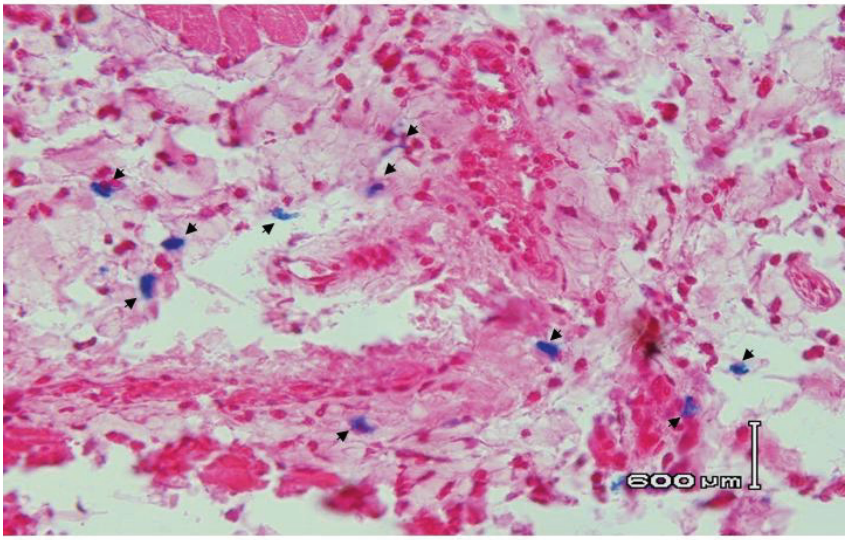

(c)

Fig. 6: *(continued)*

1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one is a 1,5-diphenyl-1,4-pentadiene-3-one analogue of curcumin (Sardjiman *et al.* 1997). The compound can be naturally isolated from the rhizomes of *Curcuma domestica* and *Curcuma longa* (Masuda *et al.* 1993; Park and Kim 2002). This compound can be easily synthesised in acidic conditions using vanillin and acetone as starting materials (Sardjiman *et al.* 1997). As a curcumin analogue, this compound is expected to have curcumin-like effects, such as anti-allergy and anti-inflammation properties. Curcumin was previously reported to show potent anti-allergy activities in both *in vitro* and *in vivo* studies (Yano *et al.* 2000a; Yano *et al.* 2000b). The hydroxy moieties of curcumin, which are responsible for its anti-oxidative effects, also play a significant role in its anti-allergy activity (Suzuki *et al* 2005). In the present study, we determined the anti-allergy effects of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on RBL-2H3 cells (a tumour analogue of mast cells) and in an *in vivo* active cutaneous anaphylaxis (ACA) reaction. Histamine release inducers used in the *in vitro* study were DNP_{24}-BSA, thapsigargin or ionomycin, whereas ovalbumin was used for the *in vivo* study.

Dinitrophenylated bovine serum albumin (DNP_{24}-BSA) is a specific antigen for monoclonal IgE antibodies (Bottcher *et al.* 1980; Liu *et al.* 1980). The cross-linkage of antigen to IgE antibody molecules on FcεRI receptors can in turn, generate a series of intracellular signalling events such as the activation of protein tyrosine kinases and an increase of intracellular Ca^{2+} levels. These subsequent signalling events trigger granule exocytosis, which releases histamine from mast cells (Metcalfe *et al.* 1997). In the present study, 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one was able to inhibit the DNP_{24}-BSA-induced release of histamine and β-hexosaminidase from RBL-2H3 cells. These findings indicate that the curcumin analogue might alter the effect of DNP_{24}-BSA on mast cells by affecting its interaction with IgE on the surface of mast cells or by altering the intracellular signal transduction that is involved in mast cell degranulation.

To investigate whether the inhibitory effect of 1,5-bis(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one is related to intracellular Ca^{2+} signalling, we used thapsigargin and ionomycin. Thapsigargin, a sesquiterpene lactone isolated from plant *Thapsia garginica*, is able to induce the release of mediators from isolated rat mast cells (Patkar, Rasmussen and Diamant 1979; Brayden *et al.* 1989). Thapsigargin prevents Ca^{2+} store refilling by targeting the sarco/endoplasmic reticulum Ca^{2+}

ATPase (SERCA). The RE-store becomes depleted because intracellular Ca^{2+} released from RE plays a major role in the opening of cell membrane Ca^{2+} channels, which causes Ca^{2+} influx into mast cells (Thastrup *et al.* 1990; Metcalfe *et al.* 1997). This phenomenon is called calcium release-activated calcium current (CRAC current or ICRAC)(Hoth and Penner 1992). SERCA functions to maintain ER stores of Ca^{2+} by pumping Ca^{2+} from the cytosol to the ER (Scharenberg, Humphries and Rawlings 2007). Ionomycin is a polyether antibiotic with a high affinity for calcium ions and can be obtained in pure form from fermentation broths of *Streptomyces conglobatus* sp. nov. Trejo by solvent extraction (Liu *et al.* 1978; Westley *et al.* 1979). It functions as a selective Ca^{2+} ionophore and increases intracellular Ca^{2+}. Ionophores are hydrophobic molecules that dissolve in lipid bilayers and increase membrane permeability to specific inorganic ions (Albert *et al.* 1994). Ionomycin can induce the release of histamine from mast cells by increasing Ca^{2+} from both intracellular Ca^{2+} pools and Ca^{2+} influx (Huang and Putney 1998). In our study, 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one successfully inhibited both the thapsigargin and ionomycin-induced release of histamine and β-hexosaminidase from RBL-2H3. This finding indicates that the effect of this compound on the release of mast cells mediators may involve mechanisms related to intracellular Ca^{2+} signalling events or downstream processes of intracellular Ca^{2+} signalling in mast cells.

In general, the inhibitory effect of 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one on RBL-2H3 cells was potent, but slightly less potent than that of curcumin, especially in the ionomycin experiments (Fig. 4). One study has reported that the anti-allergy effect of curcumin is closely related to its anti-oxidative effect, and hydroxy moieties of curcumin play a significant role in both effects of curcumin (Suzuki *et al.* 2005). In another study, the antioxidant activity of curcumin was shown to be mainly due to the hydroxy group, although a small fraction may be due to the methylene group (Priyadarsini *et al.* 2003). Additionally, the anti-oxidative effect of curcumin was shown to be a bit more potent than that of 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one (Sardjiman *et al.* 1997). It has been suggested that omission of the active methylene group and one carbonyl group leading to 1,4-pentadiene-3-one alters its biological activity.

Cutaneous anaphylactic reaction is a type I mast cell-mediated allergy model (Yano *et al.* 2000a). Mast cells play a role in the pathogenesis of some type I allergic reactions, such as hay fever, the

initial phase of asthma, and urticaria. In some cases, the reaction is more severe and can produce anaphylactic shock (Rang *et al.* 2003). In the present study, we used ovalbumin to sensitise and generate the anaphylactic reaction. The effect of the drug at 20 mg/kg BW appeared higher than at 40 mg/kg BW, although both effects were statistically the same. This finding indicates that the maximum effect of the drug was reached at a dose of 20 mg/kg BW. At this dose, the drug can work with all possible targets in the body. Increasing the dose will not increase the effect and may perhaps decrease the effect of the drug. Histopathologically, the treatment of ovalbumin results in few mast cells or causes mast degranulation. Vascular permeability, an indication of cutaneous anaphylactic reaction, was observed by intravenous injection of Evans blue dye via the tail vein. In this study, 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one significantly inhibited the anaphylactic reaction and prevented mast cell degranulation. We can therefore, suggest that inhibition on mast cell degranulation underlies its inhibitory effect on the anaphylactic reaction.

CONCLUSION

The compound 1,5-bis(4'-hydroxy-3'-methoxyphenyl)-1,4-pentadiene-3-one showed anti-allergic activities in mast cell-mediated allergy models through mechanisms related to intracellular signalling events, such as intracellular Ca^{2+} signalling or its subsequent downstream processes within mast cells. Nevertheless, further studies are required to investigate the detailed mechanisms of the anti-allergic effect of this compound.

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