

EFFECTS OF SKIMMIANINE, A QUINOLINE ALKALOID OF *AEGLE MARMELOS* CORREA ROOTS, ON THE HISTAMINE RELEASE FROM RAT MAST CELLS

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Abstract

Skimmianine is a quinoline alkaloid isolated from the roots of *Aegle marmelos* Correa. In the study, we studied the effects of skimmianine on the histamine release from rat mast cells. The study was performed by using two cell lines, rat basophilic leukemia (RBL-2H3) cell line, and rat peritoneal mast cells (RPMCs). DNP₂₄-BSA, thapsigargin, ionomycin, compound 48/80 and PMA were used as inducers for histamine release from rat mast cell. Skimmianine markedly inhibited the histamine release from RBL-2H3 cells induced by DNP₂₄-BSA, thapsigargin and ionomycin. The effect suggested is related to Ca²⁺ signaling since skimmianine showed strong effects when the histamine release induced by Ca²⁺ signal stimulants (thapsigargin and ionomycin). It is supported that skimmianine altered the influx of ⁴⁵Ca²⁺ into the cells. In RPMCs experiment, skimmianine also suppressed the histamine release induced by Ca²⁺ stimulants, and phorbol myristate acetate (PMA). However, skimmianine had no effect on the histamine release induced by compound 48/80. Based on the results, the inhibitory effects of skimmianine on the histamine release from mast cells might involve some mechanisms related to intracellular Ca²⁺ signaling events and protein kinase C signaling possessing a main role in granule exocytotic processes

Keywords: *Aegle marmelos*, skimmianine, histamine release, mast cell.

INTRODUCTION

Aegle marmelos Correa is a plant belonging to Rutaceae family. This plant grows widely in some areas of the Southeast and South Asia countries such as India, Sri Lanka, Indonesia, Malaysia and Vietnam. This plant has long been used as an ancient and modern traditional medicine to relieve dysentery, cholera, constipation (Manandhar *et al.*, 1978) and diabetes mellitus (Ohashi *et al.*, 1994). *Aegle marmelos* Correa has several pharmacological activities such as anti-inflammatory, antipyretic, analgesic (Arul *et al.*, 2005), antioxidant (Sabu *et al.*, 2004), and antidiabetes (Upadhya *et al.*, 2004). Moreover, several compounds of this plant have been isolated and evaluated for their pharmacological effects such as aegeline and skimmianine possessing hypoglycemic and insecticidal activities, respectively (Narender *et al.*, 2007; Samarasekera *et al.*, 2004). It is necessary to focus and develop these compounds to be effective drugs. Based on the phytochemical studies on *Aegle marmelos* Correa, the alcoholic root extract contains several compounds such as psoralen,

xanthotoxin, 6,7-dimethoxycoumarin, scopoletin, tembamide, skimmianine, marmesin, marmin and skimmianine (Shoeb *et al.*, 1973).

Skimmianine is a general component contained in some Rutaceae plants. Skimmianine is also named 4,7,8-trimethoxyfuro[2,3-b]quinoline, the chemical structure is shown in Figure 1. Skimmianine is reported possessing several biological activities such as inhibitory effect on spontaneous motor activity (Cheng, 1986), cardiovascular effect (Cheng *et al.*, 1990), antiplatelet aggregation activity (Chen *et al.*, 2000), and cytotoxic in HeLa cell line (Jansen *et al.*, 2006). Besides, the compound functions as 5-hydroxytryptamine receptors (Cheng *et al.*, 1994). In the study, the compound was isolated from the chloroform and methanolic extracts of the roots of *Aegle marmelos* Correa. In the preliminary study, skimmianine showed a promising effect to be investigated forward.

In the present study, we investigated the effect of skimmianine, a quinoline alkaloid of *Aegle marmelos* Correa leaves, on the histamine release from rat mast cells.

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In the study, we used two kinds of rat mast cells, rat basophilic leukemia (RBL-2H3) cells and rat peritoneal mast cells (RPMCs). DNP₂₄-BSA, thapsigargin, ionomycin, compound 48/80 and PMA were used as inducers for histamine release from mast cells.

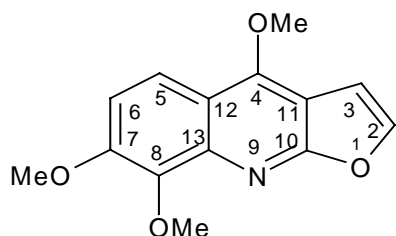


Figure 1. Chemical structure of skimmianine

MATERIALS AND METHODS

Preparation of skimmianine

Skimmianine or 4,7,8-trimethoxyfuro[2,3-b]quinoline was isolated from fresh root of *Aegle marmelos* Correa (Riyanto, 2003). The root was collected from area around Yogyakarta, Indonesia. This plant was identified by a botanist at Department of Pharmaceutical Biology, Gadjah Mada University, and the voucher specimen of the root was deposited in herbarium of the department.

In brief, dried ground powder of fresh root was extracted successively with chloroform and methanol. Chloroform extract was chromatographed over silica gel, and selected fractions were further chromatographed to yield several fractions. Selected fractions were combined and concentrated, and the solid obtained was crystallized to yield skimmianine. Methanol extract was triturated in water and subsequently shaken with ethyl acetate and *n*-butanol. Each ethyl acetate and *n*-butanol layer was dried with anhydrous sodium sulphate and concentrated to provide residues. Ethyl acetate extract from initial extraction of the roots was subjected to vacuum column chromatography which provided the compound (Riyanto, 2003).

Materials

The histamine release inducers used in the study were ionomycin, thapsigargin, compound 48/80, and phorbol myristate acetate (PMA) from Sigma Chemical Co. (St. Louis, MO, USA). Dinitro-phenylated bovine serum albumin (DNP₂₄-BSA) as an antigen and monoclonal IgE against DNP₂₄-BSA purified from supernatant in IgE producing hybridoma, were produced in our laboratory. Eagle's minimum essential medium (MEM) and antibiotics (combination of penicillin G sodium and streptomycin sulfate) were purchased from Gibco (Grand Island, NY, USA). Fetal calf serum was obtained from JRH Biosciences (Kansas, USA). Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was purchased from

Dojindo (Kumamoto, Japan), and *o*-phthalaldehyde was from Wako Pure Chemical Industries (Osaka, Japan).

Culture of RBL-2H3 cells

The RBL-2H3 cells were cultured in MEM containing 15% fetal calf serum and antibiotics (penicillin and streptomycin) in a flask in a humidified atmosphere (5% CO₂) at 37°C (Barsumian *et al.*, 1981). For the assay of histamine release, cells were seeded into 24-well culture plates at a density of 5 x 10⁵ cells/0.4 ml per each well. The cells were incubated overnight at 37°C. For DNP₂₄-BSA experiments, the cells were sensitized with 0.5 µg/ml of monoclonal IgE. On the second day, the medium was removed, and the cells were washed twice with 500 µl of PIPES buffer (119mM NaCl, 5mM KCl, 25mM PIPES, 5.6mM glucose, 0.4mM MgCl₂, 1mM CaCl₂, 40mM NaOH, and 0.1 % bovine serum albumin, pH 7.2), and pre-incubated for 10 min at 37°C after addition of 180 µl PIPES buffer either without (as a negative control) or with the drug. After 10 min pre-incubation, 20 µl of stimulant (200 ng/mL DNP₂₄-BSA, 5 µM thapsigargin, or 10 µM ionomycin) were added to each well and the plate was incubated at 37°C for 30 min.

Isolation of RPMCs

Male Wistar rats weighing between 250-300 g and aged 3-4 months were used. The animal experiments were conducted according to the guidelines of the Animal Care Committee of the Ehime University, and all experimental protocols had been approved by this Committee. Rats were killed by decapitation and exsanguination. RPMCs were isolated by injection of 25 ml phosphate buffered saline (PBS) pH 7.4 containing 5 IU/ml heparin and 0.1 % BSA into the peritoneal cavity and the abdomen was massaged for about 120 s. Afterwards, the peritoneal cavity was opened carefully, and the fluid containing mast cells were collected. The collected mast cells were centrifuged at 200 x g for 5 min at room temperature and then resuspended in 2 ml PBS buffer containing 0.1 % BSA. Peritoneal mast cells were separated from the other components (macrophages and lymphocytes) by layering on 4 ml of 38% BSA, and centrifuging at 800 x g for 20 minutes at 4°C. After the upper layer containing other components was aspirated and discarded, the remaining cell pellet was washed with 6 ml PBS buffer and resuspended in 1 ml of PIPES buffer. Mast cell preparations were about 95% pure as assessed by toluidine blue staining.

For the assay of histamine release, 120 µl of RPMCs suspension (2x10⁴ cells/ml) was preincubated for 10 min at 37°C after addition of 60 µl PIPES buffer either without (as a negative control) or with drugs at a range of concentrations (0.1-100 µM). After 10 min preincubation, 20 µl of stimulant (100 µg/ml compound 48/80, 5 µM thapsigargin, 10 µM ionomycin, or a combination of 100

nM PMA and 1 μ M ionomycin) was added to each well and the plates were incubated at 37°C for 30 min.

Assay of histamine release

Histamine released in the medium was measured by HPLC-fluorometry as described previously study (Yamatodani *et al.*, 1985). After a 30 min incubation, the plates were centrifuged at 1,800 x g for 5 min and 50 μ l of the supernatant was mixed with 250 μ l of 3% perchloric acid containing 5mM Na₂-EDTA. After addition of 30 μ l of 2 M KOH/1 M KH₂PO₄ and centrifugation at 10,000 x g for 15 min at 4°C, 50 μ l of the supernatant was injected directly onto a column packed with TSKgel SP-2SW cation exchanger (Tosoh, Tokyo). For measuring the total histamine content in cells, 350 μ l of PIPES buffer was added to 6 wells and the cells were then sonicated. Fifty microlitres 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, and post-labeled 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. The values were expressed as a percentage of net histamine release.

The percentage of net histamine release was calculated according to the following equation:

Net release (%) = (histamine content in the supernatant of cells stimulated – histamine content in the supernatant of unstimulated cells) / (total histamine content – histamine content in the supernatant of unstimulated cells) x 100.

Spontaneous release (%) = (histamine content in the supernatant of unstimulated cells/total histamine content) x 100.

The percent inhibition of histamine release was calculated according to the following equation.

Inhibition of histamine release (%) = [(histamine release in the absence of the test compound – histamine release in the presence of the test compound) / histamine release in the absence of the test compound] x 100.

Uptake of ⁴⁵Ca²⁺

This experiment was conducted using RBL-2H3 cells in a 24 well-plate. After overnight incubation at 37°C, the cells were washed twice with 500 μ l of PIPES buffer and then preincubated for 10 min at 37°C in 180 μ l PIPES buffer either without (as a negative control) or with the drug. After preincubation, PIPES buffer containing ⁴⁵Ca²⁺ (5 μ Ci/mL) and Ca²⁺ uptake stimulant (thapsigargin) was added into each well, and the plate was incubated at 37°C for 15 min. After this time, the reaction was stopped by washing with ice-cold Ca²⁺-free

buffer containing 100 μ M La³⁺. The cells were lysed with 0.3 ml of 0.1% Triton X, and 100 μ L of the solution was combined with 10 ml of scintillation cocktail for radioactivity counting. The values were expressed as the percentage of maximum uptake in the absence of inhibitor compounds.

Statistical analysis

All data were expressed as mean \pm SEM. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test were used for statistical analyses. *P*-values less than 0.05 were considered significant.

RESULTS

Effects on Histamine Release from RBL-2H3 Cells

In this study, we used DNP₂₄-BSA, thapsigargin and ionomycin to induce histamine release from mast cells. RBL-2H3 cells released 5.01 \pm 0.90 % (mean \pm SEM, n=3) of their total histamine content during a 30-min incubation at 37°C with the medium (the spontaneous release). Besides, we also observed the effect of skimmianine ranging 1-100 μ M on the histamine release from RBL-2H3 cells. The effect was considered significant if skimmianine caused spontaneous histamine release of more than 10 %. All concentrations of skimmianine showed low spontaneous histamine release, less than 10 % of the total histamine contained in RBL-2H3 cells (Fig. 2).

DNP₂₄-BSA was used as an antigen to stimulate histamine release from IgE-sensitized RBL-2H3 cells. In present study, 20 ng/mL DNP₂₄-BSA stimulated 39.57 \pm 1.39% (mean \pm SEM, n=3) of their total histamine content in RBL-2H3 cells. The alkaloid skimmianine suppressed successfully the histamine release in a concentration-dependent manner. The compound showed significant inhibitory effects at the concentration of 30 and 100 μ M by reducing the histamine release by 44.54 \pm 1.49% and 62.78 \pm 7.61%, respectively (Fig. 2a).

Besides, we also used Ca²⁺ signal-mediated histamine release stimulants to evaluate whether the inhibitory effect of skimmianine related to intracellular Ca²⁺ pathways. These are thapsigargin and ionomycin which act on both Ca²⁺ influx and intracellular calcium pool. RBL-2H3 cells released 43.27 \pm 6.31% and 30.73 \pm 1.65% (mean \pm SEM, n=3) of their total histamine content in response to 0.5 μ M thapsigargin and 1 μ M ionomycin, respectively (Fig. 2b and 2c). In this present study, skimmianine succeeded to suppress the histamine release from RBL-2H3 cells. Skimmianine showed a significant effect at the dose of 10 μ M and 30 μ M in ionomycin and thapsigargin experiments, respectively. At the highest dose (100 μ M), skimmianine suppressed 78.48 \pm 7.21%

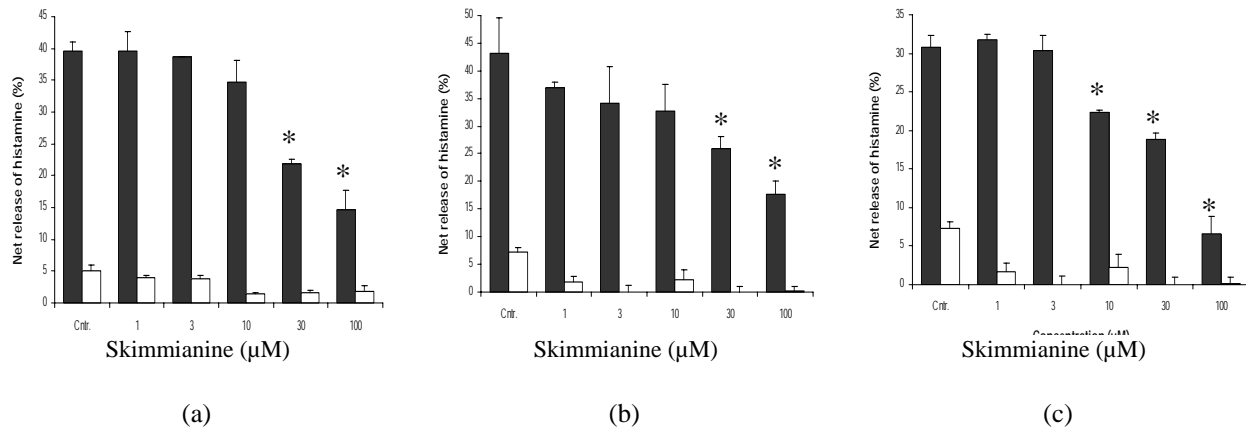


Figure 2. Effect of skimmianine on histamine release from RBL-2H3 cells in the presence (solid bar) or absence (open bar) of histamine stimulants i.e. DNP-BSA 20 ng/mL (a), thapsigargin 0.5 μ M (b), or ionomycin 1 μ M (c). Data represent mean \pm SEM, and are three independent experiments. * Significant difference $P < 0.05$ compared to the negative control value.

and $59.05 \pm 5.45\%$ of the histamine release from RBL-2H3 cells induced by ionomycin and thapsigargin, respectively. These strong effects of skimmianine were presumed to be related to intracellular Ca^{2+} signaling events in mast cells.

Table 1 shows the IC_{50} values of the inhibitory effects of skimmianine on the histamine release from RBL-2H3 cells induced by DNP-BSA, thapsigargin and ionomycin. These values represent potency of skimmianine effects in this study.

Effects on Histamine Release from RPMCs

In the present study, the stimulants for histamine release were compound 48/80, thapsigargin, ionomycin, and phorbol myristate acetate (PMA). RPMCs released $9.75 \pm 0.30\%$ (mean \pm SEM, $n=3$) of their total histamine content during a 30-min incubation at $37^\circ C$ with the medium. We also observed the effect of skimmianine ranging 1-100 μ M on the histamine release from RBL-2H3 cells. In this study, all concentrations of skimmianine showed low spontaneous histamine releases, less than 10% of the total histamine content (Fig. 3).

Compound 48/80, acting directly on G proteins in mast cells, stimulated the histamine release from RPMCs by $73.46 \pm 2.22\%$ of the total cellular content of histamine. In the study, skimmianine could not suppress the histamine release induced by 10 μ M compound 48/80 (Fig. 3a). The inhibitory effect of skimmianine was presumed to be not related to signaling events in G proteins activation pathways.

In the study, thapsigargin and ionomycin which stimulate the Ca^{2+} signaling in mast cell, increased histamine release from RPMCs by $46.49 \pm 0.78\%$; and $85.42 \pm 1.84\%$

(mean \pm SEM, $n=3$), respectively (Fig. 3b and 3c). Skimmianine also succeeded to suppress the histamine release from RPMCs induced by Ca^{2+} stimulants. These effects were concentration-dependent manner. This compound showed a significant effect at the dose of 30 μ M. At the highest dose (100 μ M), skimmianine inhibited $20.89 \pm 2.00\%$ and $55.23 \pm 0.46\%$ of the total histamine content induced by ionomycin and thapsigargin, respectively.

The combination of PMA (7.5 nM), a modulator of protein kinase C (PKC), and low-dose ionomycin (0.1 μ M) increased histamine release by $93.84 \pm 1.72\%$ in RPMCs (Fig. 3d). In this study, skimmianine showed a significant effect at the highest dose (100 μ M) by reducing the histamine release from RPMCs by $24.95 \pm 7.04\%$.

Table 4 shows the IC_{50} values of the inhibitory effects of skimmianine on the histamine release from RPMCs induced by compound 48/80, thapsigargin, ionomycin and PMA. These values represent potency of skimmianine effects in this study.

The effect on $^{45}Ca^{2+}$ influx

In our previous works, skimmianine showed promising effects when the histamine release induced by Ca^{2+} signaling stimulants. This study investigated the effect of skimmianine on intracellular Ca^{2+} signaling. The study was conducted by direct measurement of radiolabelled Ca^{2+} uptake in RBL-2H3 cells after stimulation with thapsigargin (Fig. 4). In the study, skimmianine suppressed the $^{45}Ca^{2+}$ uptake into intracellular side. At highest dose (100 μ M), skimmianine successfully suppressed intracellular accumulation of $^{45}Ca^{2+}$ by $21.87 \pm 3.19\%$.

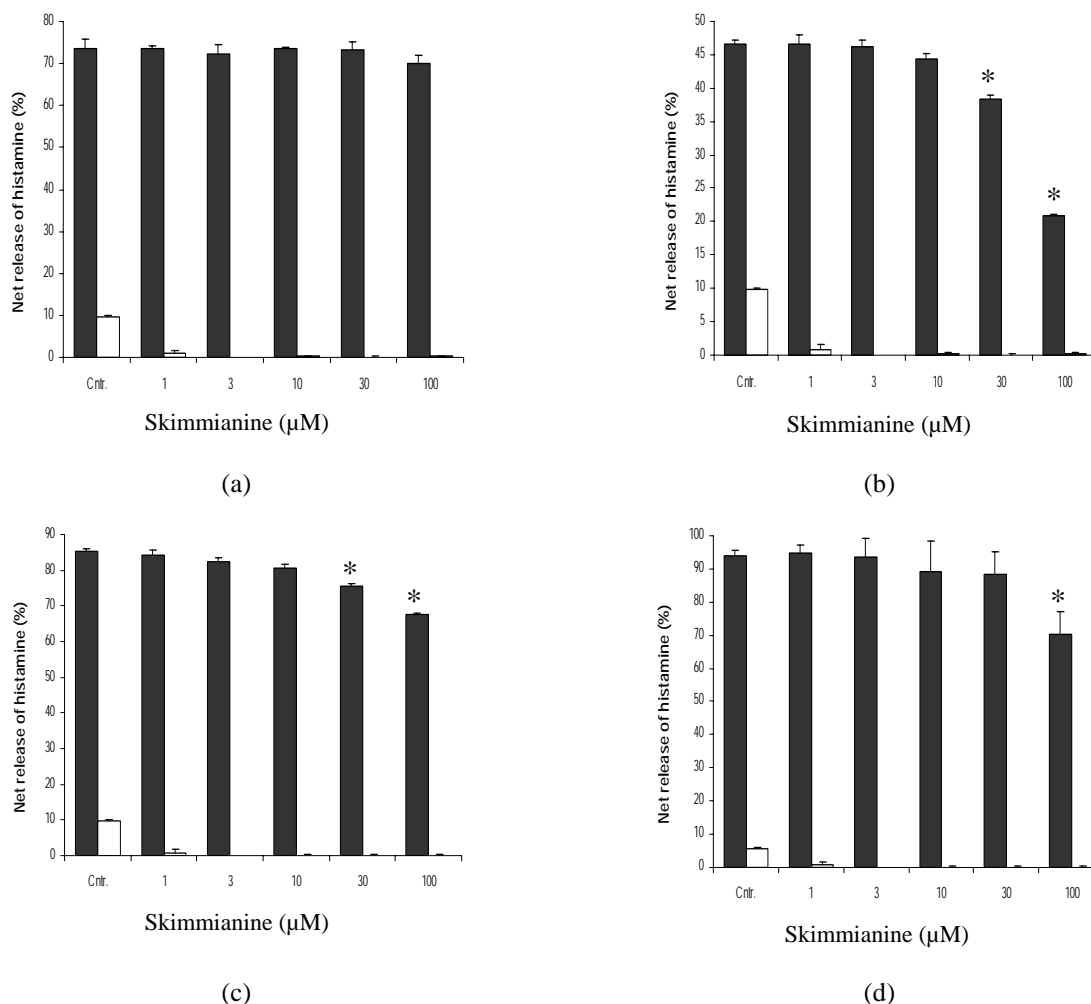


Figure 3. Effect of skimmianine on histamine release from rat peritoneal mast cells (RPMCs) in the presence (solid bar) or absence (open bar) of histamine stimulants i.e. compound 48/80 10 μM (a), thapsigargin 0.5 μM (b), ionomycin 1 μM (c); or phorbol myristate acetate 7.5 nM-ionomycin 0.1 μM (d). Data represent mean \pm SEM, and are three independent experiments. *Significant difference ($P < 0.05$) compared to the negative control value.

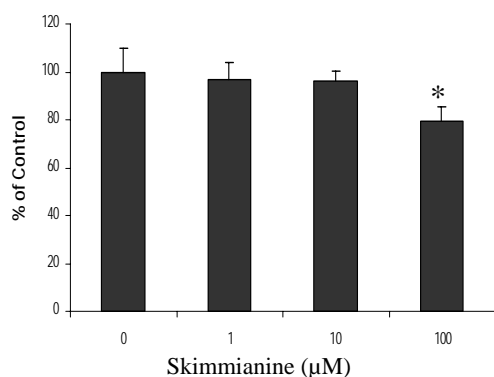


Figure 4. Inhibition of $^{45}\text{Ca}^{2+}$ uptake by skimmianine isolated from *Aegle marmelos* Correa in thapsigargin-stimulated RBL-2H3 cells. The data were representative of three independent experiments. *Significant difference ($P < 0.05$) compared to the negative control value.

DISCUSSION

Allergy reaction in our body can be triggered by allergens, such as grass pollen, dust mite, certain foodstuffs or some drugs, that evoke the production of IgE type then attach to mast cell via the high-affinity $\text{Fc}\epsilon\text{RI}$ receptors on the cell surface (Rang *et al.*, 2003). The cross-linkage of certain allergen into IgE antibody molecules on $\text{Fc}\epsilon\text{RI}$ receptors, and in turn can generate a series of intracellular signaling such as the activation of protein tyrosine kinases and an increase of intracellular Ca^{2+} levels. Finally, these subsequent signaling events trigger the granule exocytosis releasing histamine from mast cells (Metcalf *et al.*, 1997).

Skimmianine or 4,7,8-trimethoxyfuro[2,3-b]quinoline is a quinoline alkaloid isolated from Rutaceae plants. In this

study, skimmianine was isolated from the roots of *Aegle marmelos* Correa collected from Yogyakarta Indonesia. Chloroform and methanol were used for extracting the dried powder of fresh roots. Chloroform extract was purified by several steps of chromatography. The selected fractions were concentrated and recrystallized to yield skimmianine. Besides, methanol extract was triturated in water, and subsequently with ethyl acetate and *n*-butanol. Ethyl acetate extract was subjected to vacuum column chromatography to provide skimmianine (Riyanto, 2003). Histamine is a main mediator responsible for allergy reaction. The release of histamine from mast cells involves several signaling pathways. The signaling cascades involved in mast cell activation are interaction of an antigen with its specific IgE antibody on mast cell surface, tyrosines phosphorylation of phospholipase C- γ 1 (PLC- γ 1), hydrolysis of phosphatidyl inositol 4,5-biphosphate, formation of inositol triphosphate (IP₃) and diacylglycerol (DAG), activation of protein kinase C (PKC), intracellular Ca²⁺ mobilization by IP₃, increase of Ca²⁺ influx to cytoplasm, activation of mitogen-activated protein kinase (MAP kinases) and G-protein. The activation of Ca²⁺ signal by IP₃ and PKC signal by DAG interact synergistically to elicit exocytosis and in turn release the histamine from mast cells (Metcalf *et al.*, 1997; Beaven *et al.*, 1987).

DNP₂₄-BSA is a specific antigen for monoclonal IgE antibody. The antigen cross-link into IgE antibody molecules on Fc ϵ RI receptors to generate a series of intracellular signaling in mast cells. Finally, these phenomena trigger the granule exocytosis releasing histamine from mast cells (Metcalf *et al.* 1997; Liu *et al.*, 1980). In the study, skimmianine decreased the histamine release from RBL-2H3 cell successfully. This fact indicates that skimmianine influence the DNP₂₄-BSA effect on mast cells such as its interaction with IgE antibody attaching on the mast cell surface or subsequent intracellular signal transductions involved in mast cell degranulation.

Thapsigargin and ionomycin are histamine release stimulants acting on the intracellular Ca²⁺ signal. However, these drugs act on different targets. Thapsigargin, is a sesquiterpene lactone isolated from the plant *Thapsia garginica*. Its target is the ATP-dependent Ca²⁺ pump in the endoplasmic reticulum, and it can increase the concentration of cytosolic free calcium ion. Ca²⁺ release from intracellular store plays a major role in the opening of cell membrane Ca²⁺ channels to cause Ca²⁺ influx in mast cells (Metcalf *et al.*, 1997; Patkar *et al.*, 1979). Ionomycin, a selective Ca²⁺ ionophore, also induces histamine release from mast cells by increasing in intracellular Ca²⁺ concentration, both through the release from intracellular Ca²⁺ pools (endoplasmic reticulum) and via Ca²⁺ influx (Huang and Putney, 1998). In the present study, skimmianine succeeded to suppress the histamine

release from mast cells induced by thapsigargin and ionomycin. These effects in RBL-2H3 cells were more potent than that in RPMCs (Table 1). RBL-2H3 cell is totally dependent on the influx of external Ca²⁺. Besides, skimmianine also suppress alter the ⁴⁵Ca²⁺ uptake from extracellular side. Blockage of Ca²⁺ influx cause a rapid decline in intracellular Ca²⁺ concentration and then decrease the release of histamine from this cells (Ali *et al.*, 1994). RPMCs still release low histamine in the absence of influx of external Ca²⁺ (Truneh *et al.*, 1982; Barrett and Pearce, 1983). In this case, the intracellular Ca²⁺ pool has an important role in histamine secretion (Ennis *et al.*, 1980). These results indicate that skimmianine influence the Ca²⁺ signaling-mediated histamine release in mast cells.

Table 1. The mean of IC₅₀ of skimmianine effects on the histamine release from RBL-2H3 cells and RPMCs with several histamine release inducers.

Histamine inducer	IC ₅₀ (μ M)	% inhibition of histamine release at 100 μ M
1. RBL-2H3 cell lines		
DNP-BSA	50.96	62.78 \pm 7.61
Thapsigargin	66.31	59.05 \pm 5.45
Ionomycine	49.81	78.48 \pm 7.21
2. RPMCs		
Compound 48/80	-	-
Thapsigargin	90.33	55.23 \pm 0.46
Ionomycine	> 100	20.89 \pm 2.00
PMA-low dose of ionomycine	> 100	24.95 \pm 7.04

Compound 48/80 is a substance that activate mast cell secretory processes by increasing the rate of GTP S binding to G-proteins (Go/Gi mixture) (Mousli *et al.*, 1990; Palomaki and Laitinen, 2006). The activation of G-proteins can trigger intracellular signaling events such as activation of phospholipase C, protein kinase C, and Ca²⁺ signaling, which ultimately results in the release of histamine from these cells. In the study, skimmianine did not influence the histamine release from RPMCs induced by compound 48/80. This finding indicates that the inhibitory effect of skimmianine is not related to signaling events in G protein activation pathway.

Phorbol myristate acetate (PMA) is also a histamine secretagogue, but does not elicit histamine release to the same extent as other inducers of histamine release (Okano *et al.*, 1985). PMA activates protein kinase C (PKC) signaling event in mast cells (Okano *et al.*, 1986; Bergstrand *et al.*, 1992). Since the activity of PKC in promoting granule exocytosis and inflammatory mediator release from mast cells is dependent on the intracellular Ca²⁺ concentration (Metcalf *et al.*, 1997), a sub-effective

dose of calcium ionophore is often used concomitantly with PMA to stimulate histamine release (Yen *et al.*, 1992; Shin *et al.*, 2004). In RPMCs, skimmianine also inhibited the histamine release induced by PMA and ionomycin in combination. It suggests that this compound might influence the interaction between PKC and intracellular Ca^{2+} during granule exocytotic processes.

In conclusion, skimmianine isolated from the fresh roots extract of *Aegle marmelos* Correa inhibited histamine release and might involve some intracellular signaling mechanisms such as intracellular Ca^{2+} signaling and PKC signaling having a main role in granule exocytotic process. Nevertheless, further study is required to investigate the detail mechanism of skimmianine in mast cells. Moreover, a further *in vivo* study should be useful to provide information to further explain drug action.

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