EFFECTS OF AEGELINE, A MAIN ALKALOID OF *AEGLE MARMELOS* CORREA LEAVES, ON THE HISTAMINE RELEASE FROM MAST CELLS

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ABSTRACT

Aegeline or *N*-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide is a main alkaloid isolated from *Aegle marmelos* Correa collected in Yogyakarta Indonesia. In our study, we investigated the effects of aegeline on the histamine release from mast cell. The study was performed by using (1) rat basophilic leukemia (RBL-2H3) cell line, and (2) rat peritoneal mast cells (RPMCs). DNP_{24}-BSA, thapsigargin, ionomycin, compound 48/80 and PMA were used as inducers for histamine release from mast cell. In our study, aegeline inhibited the histamine release from RBL-2H3 cells induced by DNP_{24}-BSA. Indeed, aegeline showed strong inhibition when RBL-2H3 cells induced by Ca^{2+} stimulants such as thapsigargin and ionomycin. Aegeline is suggested to influence the intracellular Ca^{2+} pool only since could not inhibit the $^{45}Ca^{2+}$ influx into RBL-2H3 cells. Aegeline showed weak inhibitory effects on the histamine release from RPMCs, even though still succeed to inhibit when the histamine release induced by thapsigargin. These findings indicate that aegeline altered the signaling pathway related to the intracellular Ca^{2+} pool in which thapsigargin acts. Based on the results, the inhibitory effects of aegeline on the histamine release from mast cells depended on the type of mast cell and also involved some mechanisms related to intracellular Ca^{2+} signaling events via the same target of the action of thapsigargin or downstream process of intracellular Ca^{2+} signaling in mast cells.

Keywords: *Aegle marmelos* Correa, aegeline, histamine release, mast cell.

INTRODUCTION

Aegle marmelos Correa, also named as Crataeva marmelos *Linn.* and Crataeva religiosa *Ainslie*, is a plant belonging to Rutaceae family. This plant originates from and grows widely in some areas of the Southeast and South Asia countries such as India, Sri Lanka, Indonesia, Malaysia and Vietnam. Furthermore, studies on *A. marmelos* were mostly conducted in South Asia countries (India and Sri Lanka), because the plants are more abundant. In there, *A. marmelos* Correa is named locally as "bael". To date, there are only few investigations of *A. marmelos* from the Southeast Asia countries (especially Indonesia and Malaysia). In this region, *A. marmelos* Correa is named locally as "maja", and used as ancient and modern traditional medicines for treating various disorders (Backer and van den Brink, 1965; Bentley and Trimen, 1983).

Aegle marmelos Correa has several pharmacological activities such as antiproliferative (Lampronti *et al*., 2003), anti-inflammatory, antipyretic, analgesic (Arul *et al*., 2005), antioxidant (Upadhya *et al*., 2004), antifungal (Rana *et al*., 1997), hypoglycemic (Sachdewa *et al*., 2001), and antidiabetes (Sabu and Kuttan, 2004). Moreover, several compounds of this plant have been isolated and evaluated for their pharmacological effects (Shoeb *et al*., 1973; Basu and Sen, 1974; Srivastava *et al*., 1996). It is necessary to focus and develop the 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, skimmin, marmesin, marmin and skimmianine (Shoeb *et al*., 1973). Methanolic extraction of the leaves, and partitioned in hexane and chromatograped yield some alkaloids and aegeline. These isolated alkaloids were classified as aegeline type. Aegeline is a main compound of leaves extract of *Aegle marmelos* Correa. Aegeline is also named as *N*-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide (Manandhar *et al*., 1978; Govindachari *et al*., 1983; Narender *et al*., 2007), the chemical structure is shown in Figure 1. Nevertheless, aegeline was also found in methanolic extract of *Sarcorhachis naranjoana* as an active compound after following bioassay-guided fractionation (Williams *et al*., 2003). In our preliminary study about the screening of the inhibitory effects on histamine release using rat basophilic leukemia (RBL-2H3), aegeline showed a promised-inhibitory effect on the histamine release.

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Mast cells have long been considered to be involved in many different acute and chronic inflammatory processes including acting in delayed and immediate hypersensitivity reactions (Bienenstock *et al*., 1987; Galli, 1993). Type I hypersensitivity reaction or allergy can be developed in present of allergen such as grass pollen, product from dead house dust mites, foodstuffs or some drug by evoking the production of antibodies of the IgE type that bind to mast cell and eoshinophils. Subsequently, the mast cells release some allergy mediators such as histamine, eicosanoids and cytokines (Rang *et al*., 2003). Many antiallergy drugs have been developed from several sources including from natural products. Therefore it is urgent and promising to discover new potential antiallergy drugs isolated from natural products (Clark, 1996).

In the study, we investigated the effect of aegeline, a main compound of *Aegle marmelos* Corr*e*a, on the histamine release from mast cells. Aegeline could be isolated especially from the methanolic leaves extract of this plant (Manandhar *et al*., 1978; Govindachari *et al*., 1983). In our study, we used two kinds of rat mast cell, rat basophilic leukemia (RBL-2H3) cells and rat peritoneal mast cells (RPMCs). DNP_{24}-BSA, thapsigargin, ionomycin, compound 48/80 and phorbol myristate acetate (PMA) were used as inducers for histamine release from mast cell. The result of these studies may provide useful information for further discovering pharmacologically traditional plants isolated-active compounds for treatment of some diseases related to histamine or mast cells.

MATERIALS AND METHODS

Preparation of aegeline

Aegeline or *N*-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide was isolated from *Aegle marmelos* Corr*e*a by Dr. Sugeng Riyanto from Gadjah Mada University, Indonesia when he completed his PhD thesis in Universiti Putra Malaysia. The molecular structure was confirmed in Faculty of Science and Environmental Studies, Universiti Putra Malaysia. The chemical structures of the compounds are shown in fig. 1. *Aegle marmelos* Corr*e*a was collected from area around Yogyakarta, Indonesia. *Aegle marmelos* Corr*e*a was identified by a botanist at Pharmaceutical Biology Department, University of Gadjah Mada, and the voucher specimen was deposited in herbarium of the department.

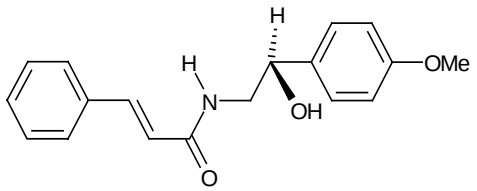

Fig. 1: Chemical structure of aegeline

In brief, dried ground powder of fresh leaves was extracted successively with petroleum ether, chloroform and methanol. Each of the extracts was collected and evaporated under reduced pressure to give of petroleum ether extract, chloroform extract and methanol extract. All extract were separated using vacuum column chromatography and developed by gradient elution to yield several fractions. The selected fractions were further purified using column chromatography to yield several fractions. Selected fractions were combined and concentrated, and the solid obtained was recrystallized to yield aegeline.

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 Industires (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% CO_2) at 37°C as described by Barsumian *et al*. (1981). For the assay of histamine release, cells were seeded into 24-well culture plates at a density of 5 x 10^5 cells/0.4 ml per each well. The cells were incubated overnight at 37°C. For DNP_{24}-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 $MgCl_2$, 1mM $CaCl_2$, 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 DNP_{24}-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 (2×10^4 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 µM 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 µl of the supernatant was mixed with 250 µl of 3% perchloric acid containing 5mM 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 TSKgel SP-2SW cation exchanger (Tosoh, Tokyo). For measuring the total histamine content in cells, 350 µl 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) × 100.

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

Uptake of $^{45}Ca^{2+}$

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 µl of PIPES buffer and then preincubated for 10 min at 37°C in 180 µl PIPES buffer either without (as a negative control) or with the drug. After preincubation, PIPES buffer containing $^{45}Ca^{2+}$ (5µCi/mL) and Ca^{2+} 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 Ca^{2+}-free buffer containing 100 µM La^{3+}. The cells were lysed with 0.3 ml of 0.1% Triton X, and 100 µL 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 ± 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

To investigate the effects of aegeline on the histamine release from RBL-2H3 cells, we used several histamine activators such as DNP_{24}-BSA, thapsigargin and ionomycin. In the study, RBL-2H3 cells released 5.39±0.28 % (mean±SEM, n=3) of their total histamine content during a 30-min incubation at 37°C with the medium (the spontaneous release). We also observed the possibility of the spontaneous release from RBL-2H3 cells by aegeline with a series of concentration (1-100 µM). The effect was considered significant if aegeline caused spontaneous histamine release of more than 10 %. All concentrations of aegeline showed low spontaneous histamine release, less than 10 % of the total histamine contained in RBL-2H3 Cells (fig. 2).

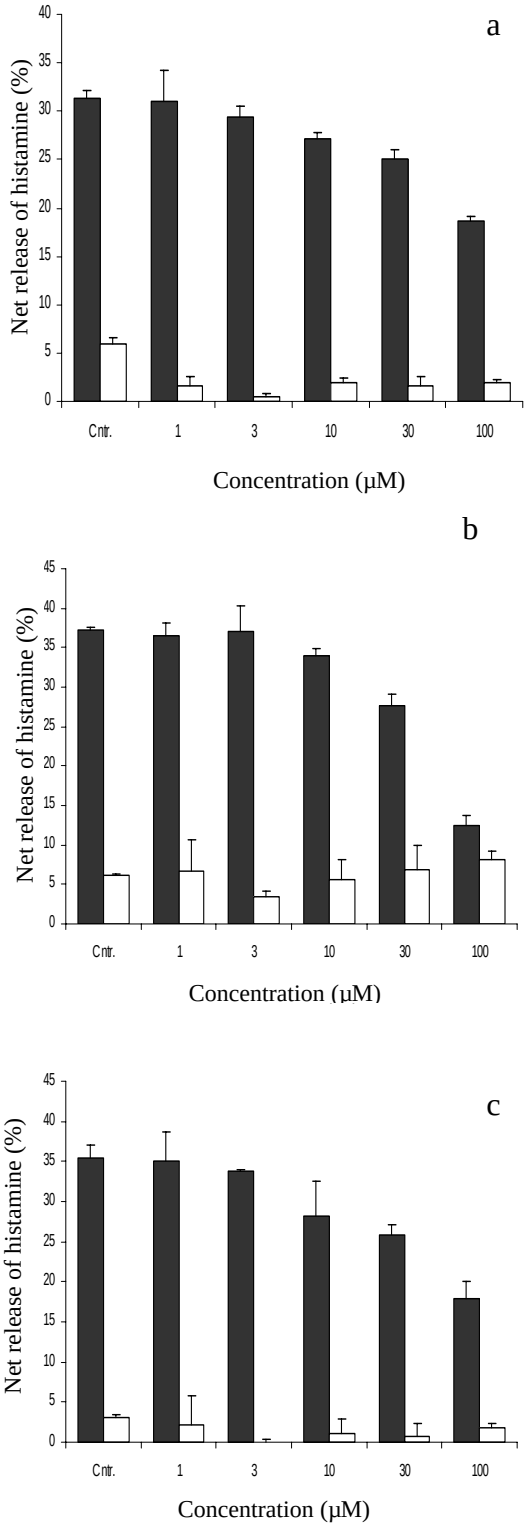

Fig. 2: Effect of aegeline on histamine release from RBL-2H3 cells in the presence (solid bar) or absence (open bar) of histamine stimulants i.e. DNP-BSA 20 ng/mL (a), thapsigargin 0.5 μM (b), or ionomycin 1 μM (c). Data represent mean±SEM, and are three independent experiments. * $P<0.05$ compared to the negative control value. ** $P<0.1$ compared to the negative control value.

DNP_{24}-BSA was used as an antigen to stimulate histamine release from IgE-sensitized RBL-2H3 cells. RBL-2H3 cells release 31.38±0.78% (mean±SEM, n=3) of their total histamine content in response to 20 ng/mL DNP_{24}-BSA (fig. 2a). The alkaloid aegeline markedly suppressed the histamine release in a concentration-dependent manner. Aegeline showed a significant effect at the dose of 10 μM by reducing 13% of the histamine release. At the highest dose (100 μM), aegeline inhibited the histamine release by 40%.

To assess the effect of aegeline on intracellular Ca^{2+} pathways mediating the histamine release from mast cell, we used thapsigargin and ionomycin which act on both Ca^{2+} influx and intracellular calcium pool. RBL-2H3 cells released 38.95±2.79% and 35.42±1.60% (mean±SEM, n=3) of their total histamine content in response to 0.5 μM thapsigargin and 1 μM ionomycin, respectively (Fig. 2b and 2c). In the study, aegeline also suppressed the histamine release from RBL-2H3 cells successfully. Aegeline showed a significant effect at the dose of 10 μM and 30 μM in ionomycin and thapsigargin experiments, respectively. At the maximum dose (100 μM), aegeline suppressed the histamine release by 49.46±6.24% and 65.36±3.61% in ionomycin and thapsigargin experiments, respectively. The effect of aegeline was presumed to be related to intracellular Ca^{2+} signaling events in mast cells. Table 4 show the IC_{50} values of the inhibitory effects of aegeline on the histamine release from RBL-2H3 cells induced by DNP-BSA, thapsigargin and ionomycin. These values represent potency of aegeline effect in this study.

Effects on Histamine Release from RPMCs

Compound 48/80, thapsigargin, ionomycin, and PMA were used for stimulating the histamine release rat peritoneal mast cells (RPMCs). RPMCs released 8.56±0.13 % (mean±SEM, n=3) of their total histamine content during a 30-min incubation at 37°C in the absence of histamine stimulant (the spontaneous release). Aegeline ranging 1-100 μM showed low spontaneous histamine release, less than 10 % of the total histamine contained in RPMCs (fig. 3).

To investigate the effect of aegeline on G proteins mediating the histamine release from mast cell, we used compound 48/80 which acts directly on G proteins in mast cells. RPMCs released 73.76±1.88% (mean±SEM, n=3) of their total histamine content in response to 10 μM compound 48/80 (fig. 3a). In this study, aegeline showed inhibitory effect on histamine release only at the highest dose (100 μM) by 9.97±2.34%.

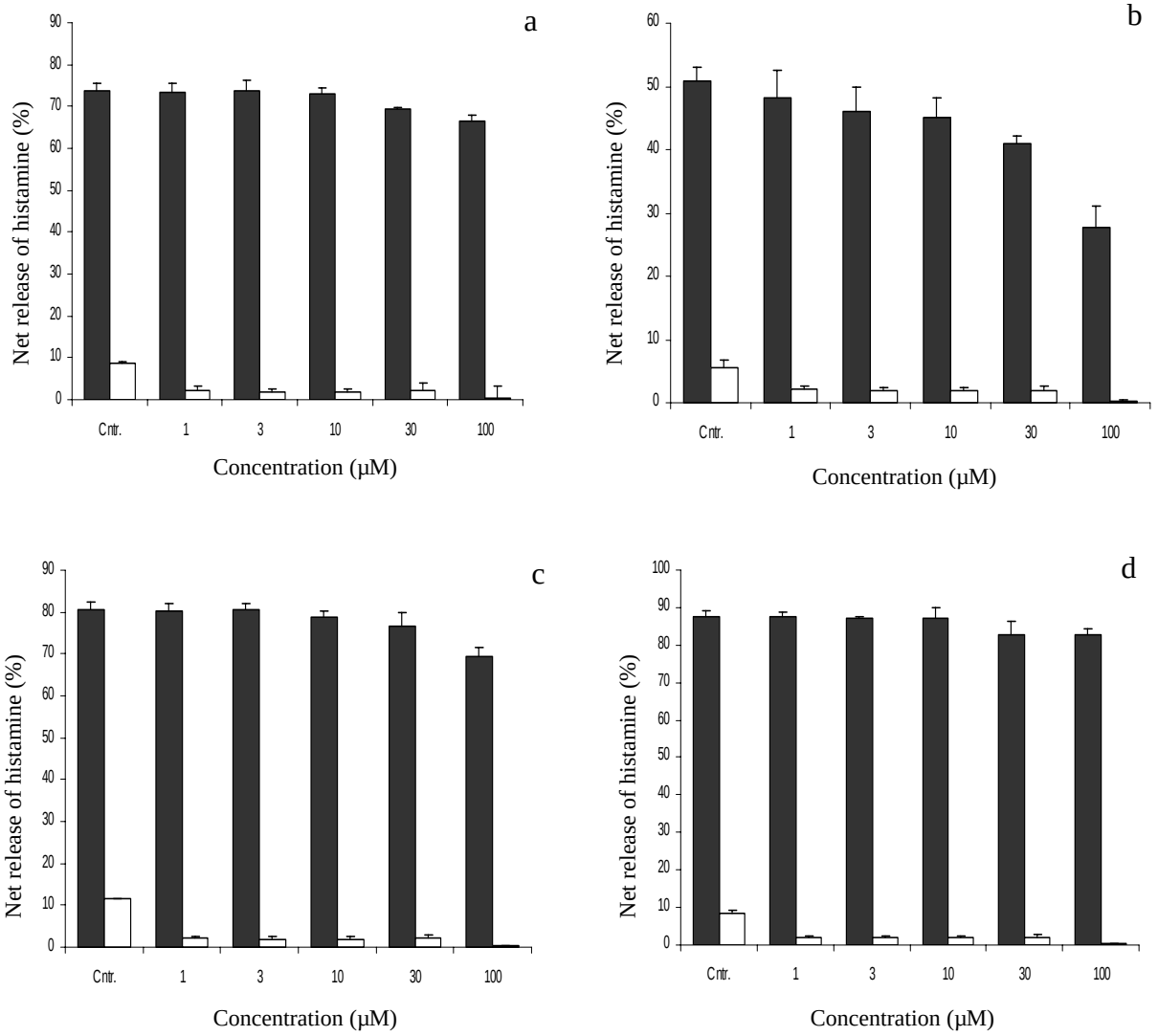

Fig. 3: Effect of aegeline on histamine release from rat peritoneal mast cells (RPMCs) in the presence (solid bar) or absence (open bar) of histamine stimulants i.e. compound 48/80 10 μM (a), thapsigargin 0.5 μM (b), ionomycin 1 μM (c); or phorbol myristate acetate 10 nM-ionomycin 0.1 μM (d).Data represent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

Thapsigargin and ionomycin were used to investigate the effect of aegeline on intracellular Ca^{2+} pathways mediating the histamine release from RPMCs. Thapsigargin and ionomycin increased the histamine release from RMPCs by 50.81±2.11%; and 80.66±1.92% (mean±SEM, n=3), respectively (fig. 3b and 3c). In the study, aegeline succeeded to suppress the histamine release induced by thapsigargin. Aegeline showed a gradual inhibitory effect, and finally at the highest dose suppressed the histamine release by 45.67±6.97%. Nevertheless, this effect is still less potent than this on RBL-2H3 cells. Whereas aegeline had little effects on the histamine release from RPMCs induced by ionomycin. At the highest dose (100μM), aegeline inhibited 13.82±2.41% of the total histamine content.

To assess involvement of aegeline on protein kinase C-mediated histamine release, we used compound combination of PMA, a modulator of PKC, and low-dose ionomycin. RPMCs released 87.57±1.42% (mean±SEM, n=3) of their total histamine content in response to combination 10 nM PMA and 0.1 μM ionomycin (Fig. 2a). In the study, aegeline did not suppress the histamine release induced by combination PMA and low-dose ionomycin. The inhibitory effect of aegeline was presumed to be not related to signaling events in PKC pathway.

Table 1 showed the IC_{50} values of the inhibitory effects of aegeline on the histamine release from RPMCs induced by compound 48/80, thapsigargin, ionomycin and PMA. These values represent potency of aegeline effect in this study.

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

Following the previous works that aegeline showed promising effects in both RBL-2H3 cells and RPMCs when induced by thapsigargin, we studied the effect of aegeline on Ca^{2+} influx. The study was conducted by direct measurement of radiolabelled Ca^{2+} uptake in RBL-2H3 cells after stimulation with thapsigargin. The result showed that aegeline did not suppress the $^{45}Ca^{2+}$ uptake into the mast cells (fig. 4). The finding is not parallel with the aegeline's effect on the histamine release.

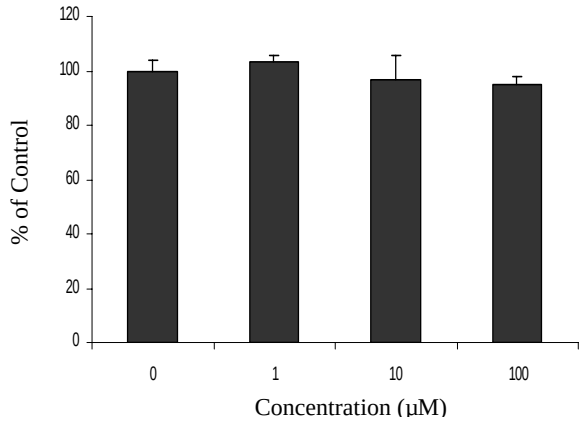

Fig. 4: Inhibition of $^{45}Ca^{2+}$ uptake by aegeline isolated from *Aegle marmelos Correa* in thapsigargin-stimulated RBL-2H3 cells. The data were representative of 3 independent experiments. *Significant difference ($P<0.05$) compared to the negative control value.

DISCUSSION

Aegeline or *N*-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide is an alkaloid compound that can be isolated mainly from the leaves of *Aegle marmelos* Corr*e*a. This plant originates from the Southeast and South Asia countries (Backer and van den Brink, 1965; Bentley and Trimen, 1983). In the study, *Aegle marmelos* Corr*e*a was collected from Yogyakarta Indonesia. Extraction of dried ground powder of fresh leaves by petroleum ether, chloroform and methanol provided crude extracts. The compound was obtained after the crude extracts were subjected to column chromatography (Riyanto, 2003).

The mechanism of histamine release from mast cell 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 phosphophorylation of phospholipase C-γ1 (PLC-γ1), hydrolysis of phosphatidyl inositol 4,5-biphosphate, formation of inositol triphosphate (IP_3) and diacylglycerol (DAG), activation of protein kinase C (PKC), intracellular Ca^{2+} mobilization by IP_3, increase of Ca^{2+} influx to cytoplasma, activation of mitogen-activated protein kinase (MAP kinases) and G-protein. The activation of Ca^{2+} signal by IP_3 and PKC signal by DAG interact synergistically to elicit exocytosis and in turn release the histamine from mast cells (Metcalfe *et al*., 1997; Beaven *et al*., 1987).

In the study, we investigated the effect of aegeline on the histamine release from mast cells. We used two kinds of mast cell models, rat basophilic leukemia (RBL-2H3) cell line, a tumor analog of mast cells; and rat peritoneal mast cell (RPMC). RBL-2H3 cell, a kind of mucosal mast cell, is totally dependent on the influx of external Ca^{2+}. Inhibition on Ca^{2+} influx cause a rapid decline in intracellular Ca^{2+} concentration and then decrease the release of histamine from this cells (Ali *et al*., 1994). Whereas, RMPCs still release low histamine in the absence of influx of external Ca^{2+} (Truneh *et al*., 1982; Barret and Pearce, 1983). In this case, the intracellular Ca^{2+} pool has an important role in histamine secretion (Ennis *et al*., 1980).

Table 1: The mean of IC_{50} of inhibitory effects of aegeline on the histamine release from RBL-2H3 cells and RPMCs with several histamine release inducers

Histamine inducer	IC50 (μM)	% inhibition of histamine release at 100 μM
1. RBL-2H3 cell lines		
DNP-BSA	> 100	40.68 ± 1.46
Thapsigargin	77.16	58.63 ± 7.40
Ionomycine	42.22	65.36 ± 3.61
2. RPMCs		
Compound 48/80	>> 100	9.97 ± 2.34
Thapsigargin	> 100	46.80 ± 7.15
Ionomycine	>> 100	13.82 ± 2.41
PMA-low dose of ionomycine	-	5.53 ± 1.90

In the present study, aegeline inhibited histamine release from RBL-2H3 cells induced by DNP_{24}-BSA, an specific antigen for monoclonal IgE antibody (Bottcher *et al*., 1980; Liu *et al*., 1980). This result indicates that aegeline might alter the effect of DNP_{24}-BSA on mast cells by affecting its interaction with IgE antibody attaching on the mast cell surface or by altering subsequent intracellular signal transductions involved in mast cell degranulation.
To investigate whether the inhibitory effect of aegeline is related to intracellular Ca^{2+} signal, we used thapsigargin and ionomycin. Thapsigargin, a sesquiterpene lactone isolated from the plant *Thapsia garginica*, is a stimulant for histamine release from mast cells. Its target is the ATP-dependent Ca^{2+} pump in the endoplasmic reticulum, and it can increase the concentration of cytosolic free calcium ion (Patkar *et al*., 1979; Brayden *et al*., 1989). Ca^{2+} release from intracellular store plays a major role in the opening of cell membrane Ca^{2+} channels to cause Ca^{2+} influx in mast cells (Metcalfe *et al*., 1997). Ionomycin, a selective Ca^{2+} ionophore, also induces histamine release from mast cells by increasing in intracellular Ca^{2+} concentration, both through the release from intracellular Ca^{2+} pools (endoplasmic reticulum) and via Ca^{2+} influx (Huang and Putney, 1998). In the study study, aegeline succeeded to inhibit the histamine release from RBL-2H3 cells induced by thapsigargin and ionomycin. However, aegeline did not alter the $^{45}Ca^{2+}$ uptake from intracellular side. Its fact indicates that aegeline effect involve the intracellular Ca^{2+} signaling by altering intracellular Ca^{2+} signal from intracellular Ca^{2+} pool rather than from Ca^{2+} influx.

Besides, aegeline could suppress the histamine release from RPMCs induced by thapsigargin, even though the effect was lower than this in RBL-2H3 cells. Aegeline had little effect when RMPCs induced by ionomycin. As mentioned above, RMPCs still release low histamine in the absence of influx of external Ca^{2+} so that intracellular Ca^{2+} pool have important role in histamine secretion. Figure 4, aegeline did not alter the $^{45}Ca^{2+}$ influx from intracellular side to intracellular side. It suggests that aegeline has possible action : 1). alters the Ca^{2+} signal via the same target of the action of thapsigargin in the mast cells, 2) inhibits the downstream process of intracellular Ca^{2+} signaling in mast cells, then decreases exocytosis process and releases histamine from mast cells.

Compound 48/80 is known to 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). Activation of G-proteins can trigger intracellular signaling events such as activation of phospolipase C, protein kinase C, and Ca^{2+} signaling, which ultimately results in the release of histamine from these cells. Compound 48/80 also stimulates histamine release from RPMCs in both the presence and absence of extracellular calcium (Metcalfe *et al*., 1997). In this study, aegeline had little effect on the histamine release from RPMCs induced by compound 48/80. It suggests that the inhibitory effect of aegeline is not related to signaling events in G protein activation pathway.

PMA is a histamine secretagogue that activate protein kinase C (PKC) signaling event in mast cells (Okano *et al*., 1986; Bergstrand *et al*., 1992). However, PMA does not elicit histamine release to the same extent as other inducers of histamine release (Okano *et al*., 1985). Since the activity of PKC in promoting granule exocytosis and inflammatory mediator release from mast cells, PMA is dependent on the intracellular Ca^{2+} concentration (Katakami *et al*., 1984; Chakravarty, 1990). 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). Aegeline did not affect the histamine release from RMPCs induced by PMA and ionomycin in combination. It suggests that this compound did not alter the interaction between PKC and intracellular Ca^{2+} during granule exocytotic processes.

In conclusion, aegeline isolated from the leave extract of *Aegle marmelos* Correa inhibited histamine release and might involve mechanisms related to intracellular Ca^{2+} signaling events. Nevertheless, further study is required to investigate the detail mechanism of aegeline in mast cells. Moreover, the further in vivo study is very useful to provide information to explain drug action.

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