EFFECTS OF MARMIN, A COMPOUND ISOLATED FROM *AEGLE MARMELOS* CORREA, ON CONTRACTION OF THE GUINEA PIG-ISOLATED TRACHEA

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ABSTRACT

Marmin or 7-(6',7'-dihydroxygeranyl-oxy)coumarin is a compound isolated from *Aegle marmelos* Correa. In the study, we examined the effects of marmin on the contraction of guinea pig-isolated trachea stimulated by several inducers, namely histamine, metacholine, compound 48/80. We also evaluated its action against contraction induced by extracellular or intracellular calcium ion. The possibility of marmin to potentiate the relaxation effect of isoprenaline was also studied. Marmin added in the organ bath at 10 min prior to the agonist inhibited the contraction elicited by histamine and metacholine in a concentration-dependent manner. Moreover, marmin antagonized the histamine-induced contraction in competitive manner. Marmin mildly potentiated the relaxation effect of isoprenaline. In the study, marmin abrogated the contraction of tracheal smooth muscle induced by compound 48/80, an inducer of histamine release. Besides, marmin successfully inhibited $CaCl_2$-induced contraction in Ca^{2+}-free Krebs solution. Marmin also inhibited two phases of contraction which were consecutively induced by metacholine and $CaCl_2$ in Ca^{2+}-free Krebs solution. Based on the results we concluded that marmin could inhibit contraction of the guinea-pig tracheal smooth muscle, especially by interfering histamine receptor, inhibiting the histamine release from mast, inhibiting intracellular Ca^{2+} release from the intracellular store and the Ca^{2+} influx through voltage-dependent Ca^{2+} channels.

Keywords: *Aegle marmelos* Correa, marmin, trachea smooth muscle, guinea pig.

INTRODUCTION

Indonesia is the second larger country in the world after Brazil in terms of biodiversity including medicinal plants. One of Indonesian medicinal plant is *Aegle marmelos* Correa (Rutaceae). 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. *Aegle marmelos* Correa has been reported having several pharmacological activities such as antifungal (Rana *et al.*, 1997), anti-inflammatory, analgesic, antipyretic (Arul *et al*., 2005), antioxidant (Upadhya *et al.*, 2004), antidiabetes (Sabu and Kuttan, 2004), antiproliferative (Lampronti *et al*., 2003).

Several compounds of this plant have been isolated and evaluated for their pharmacological effects. 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). It is necessary to focus and develop these compounds to be more effective drugs. In the previous study, there were three active compounds (aegeline, skimmianine, and marmin) which inhibited the histamine release from rat mast cells potentially. Among them, marmin was most potent compound, and could inhibit the histamine release induced by DNP-BSA (an antigen) or thapsigargin (an intracellular Ca^{2+} stimulant) by 60 %. Besides, marmin potently inhibited $^{45}Ca^{2+}$ influx induced by thapsigargin. The effect of marmin on inhibiting the histamine release from mast cells highly depended on the type of mast cell and also involved mechanisms related to intracellular Ca^{2+} signaling events by blocking Ca^{2+} influx into mast cells (Nugroho *et al.*, 2008).

Marmin is a coumarine compound, and can be isolated from roots and stem bark of *Aegle marmelos* Correa. In present study, marmin was isolated from petroleum ether, chloroform and methanol extracts of the roots and stem bark of this plant. The extracts were fractionated using chromatography (vacuum column, gravity column) and developed by gradient elution. The solid material obtained was recrystallized to yield the compound (Riyanto, 2003). In addition, marmin was reported to be isolated from *Aurantii fructus immaratus* (Satoh *et al*., 1996).

The present study was designed to elucidate the action of marmin isolated from *Aegle marmelos* Correa on contraction of the guinea pig-isolated trachea induced by receptor-activating compounds that involve in the etiology of respiratory disease such as histamine and metacholine. Both compounds are agonists for histamine

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and muscarinic acetylcholine receptors, respectively (Rang *et al.*, 2003). Also, we evaluated its effect on contraction induced by the histamine release by using compound 48/80, an inducer of histamine release from mast cells. Besides, its action has been evaluated against contraction induced by extracellular or intracellular calcium ions. Finally, we assessed the influence of marmin on relaxation effect of isoprenaline, a β adrenoreceptor agonist in tracheal smooth muscle. In the airways system, β_2 adrenoreceptor participate in regulation of smooth muscle relaxation (Rang *et al.*, 2003).

MATERIALS AND METHODS

Preparation of marmin

Marmin or (7-(6',7'-dihydroxygeranyl-oxy)coumarin was isolated from *Aegle marmelos* Corrêa collected from area around Yogyakarta, Indonesia. The chemical structures of the compounds are shown in fig. 1. *Aegle marmelos* was identified by a botanist at Pharmaceutical Biology Department, University of Gadjah Mada, and the voucher specimen was stored in herbarium of the department.

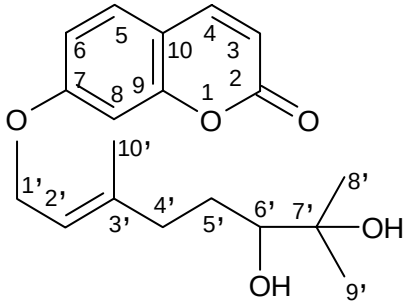

Fig. 1: Chemical structure of marmin.

In brief, dried ground powder of bark and fresh root were extracted in consecutive with petroleum ether, chloroform and methanol. Chloroform extract of both were chromatographed over silica gel, and selected fractions were further chromatographed using mini column to yield several fractions. Selected fractions were combined and concentrated, and the solid obtained was recrystallized to yield marmin. Methanol extract of dried ground powder of bark or petroleum ether extract of dried ground powder of fresh root were separated using vacuum column chromatography with gradient elution to yield several fractions. Selected fractions were combined and evaporated, then separated by using gravity column chomatography to provide marmin (Riyanto, 2003).

Materials

The following materials were used : histamine, serotonin, metacholine, isoprenaline, phentolamine, ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The Krebs solution with this following composition (mM) : NaCl 118.0; KCl 4.4; $CaCl_2$ 2.5; $NaHCO_3$ 25.0; $MgSO_4$ 1.1; KH_2PO_4 1.2; glucose 11.0 (Sayah *et al.*, 1997), were purchased from Merck & Co., Inc. (New Jersey, USA). The solution must be made freshly, and continuously bubbled with 95% O_2 and 5% CO_2. Composition of Ca^{2+}-free Krebs solution was the same as that of previous Krebs except $CaCl_2$ was replaced by editic acid 0.1 mmol/L (Pang *et al.*, 2002).

Animals and tissue preparation

Male guinea pigs weighing 300-450 g were supplied by Experimental Animal Center of Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy Gadjah Mada University, Indonesia. The animals were sacrificed by stunning and exsanguination. The trachea was gently removed and dissected out from connective tissues. The trachea was cut transversely into six rings, and the rings were cut longitudinally into strips. The strips of isolated trachea smooth muscle were then mounted in organ bath containing 20 mL Krebs solution (pH 7.4) aerated with 95% O_2 and 5% CO_2; and maintained at 37°C. The tissue strips were equilibrated for at least 60 min under a resting tension of 1 g, and the Krebs solution was replaced with the fresh solution every 15 min. Isotonic contractions were recorded by a level transducer (type 368 HSE, Germany) connected to a bridge amplifier (type 336 HSS, Germany) and a recorder (Kipp & Zonen BBD 41, The Netherlands).

The contraction of tracheal smooth muscle induced by histamine or metacholine

Following 60-min equilibration, the tracheal smooth muscle strips were contracted with a single concentration (3.10^{-4} M) of histamine or metacholine (agonists). After the contraction reached a plateau, the tissue strips were washed by fresh Krebs solution in the same condition for 60 min (with replacement of the Krebs solution every 15 min). Then, cumulative concentrations of the agonist (10^{-8}-10^{-3} M) were added in the organ bath. After the maximum contraction was reached, the tissue strips were washed again and the procedure repeated to get two contraction effects of the agonist. Following a 60 min washing period, marmin (10 μM) was administered at 10 min prior to cumulative concentrations of the agonist administration. After washing the tissue, the procedure was repeated for the next concentration of marmin (100 μM).

The contraction of tracheal smooth muscle induced by Compound 48/80

In the study, compound 48/80 was used for stimulating mast cells to release histamine. Subsequently, histamine endogen interacts with histamine receptor to trigger contraction of tracheal smooth muscle. Because the source of histamine originates from endogenous mast cells so that only one complete cumulative concentration-response curve was employed in the study. In addition, control and test tissues were separated in different organ baths.

Following 60 min equilibration, a single concentration (3.10^{-4} M) of histamine was administered to contract the trachea smooth muscle tissue in both organ baths. After the contraction reached a plateau, the tissue strips were washed. In control tissue bath, cumulative concentrations of compound 48/80 (1-100 μg/ml) were added in the bath. Whereas, in test tissue bath, marmin (100 μM) was administered 10 min prior to addition of compound 48/80.

The relaxation of tracheal smooth muscle induced by isoprenaline

In the experiment, the Krebs solution contained ascorbic acid to prevent degradation of isoprenaline. Following the equilibration period of 60 min, phentolamine, an α adrenereceptor blocking agent, was added in the strips-containing organ bath, and a single concentration (3.10^{-4} M) of metacholine was administered to contract the trachea smooth muscle. After the contraction reached a plateau, cumulative concentrations of isoprenaline (10^{-7}-10^{-3} M) were added. After the maximum relaxation was obtained, the tissue strips were washed again and the procedure repeated to get two relaxation effects of isoprenaline. Then, the strips were washed for the next step. After the contraction effect of metacholine reached a plateau, the first concentration of marmin (10 μM) was administered at 10 min prior to cumulative concentrations of the agonist administration. After washing the tissue, the procedure was repeated for the next concentration of marmin (100 μM).

The contraction of tracheal smooth muscle induced by $CaCl_2$

The procedure was conducted to evaluate the marmin effect on contraction of the tracheal smooth muscle induced by intracellular Ca^{2+} influx (Pang *et al*., 2002). In the experiment, the tissue strips were 60-min equilibrated in Ca^{2+}-free Krebs solution. KCl solution (80 mmol/L) was administered in the strips for 5 min to depolarize the membrane of smooth muscle cells in order to activate voltage-dependent Ca^{2+} channels. Subsequently, $CaCl_2$ solution (10mM) was added to contract the strips. Then, the tissue strips were washed and equilibrated. After administration of KCl for 5 min, the tissue strips were incubated with marmin (100 μM) for 10 min, and then followed by $CaCl_2$ to contract the strips.

The two phase of contraction induced by metacholine and $CaCl_2$

The procedure was conducted to determine the marmin effect on contraction of the tracheal smooth muscle induced by intracellular Ca^{2+} release from Ca^{2+} store (Pang *et al*., 2002). The tissue strips in this experiment were also equilibrated in Ca^{2+}-free Krebs solution for 60 min. Metacholine (3.10^{-4} M) was added in the tissue strips to induce a short contraction (first phase of contraction). After the contraction response reached a plateau, $CaCl_2$ (10 mM) was immediately administered to induce the second phase of contraction. Then, the tissue strips were washed and equilibrated. The procedure was repeated, however, marmin (100 μM) was administered in the tissue strips at 10 min prior to metacholine administration.

STATISTICAL ANALYSIS

All data were presented as mean ± the standard error of the mean (SEM). The pD_2 values are derived from the negative logarithm to base 10 of the agonists concentration which cause half maximal response in the form of either contraction or relaxation (Waldron *et al*., 1999), calculated by interpolation from semi-logarithmic plots of individual concentration-response curve. This value represents potency of the effect of agonist on the tracheal smooth muscle. Unpaired t test and one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test were used for statistical analyses. *P*-values less than 0.05 were considered significant.

RESULTS

Effects on the contraction of trachea induced by histamine

In the study, cumulative concentrations of histamine (10^{-8}-10^{-3} M) obviously produced concentration-dependent contraction of the guinea-pig tracheal smooth muscle (fig. 2). pD_2 value of histamine effect on the guinea-pig trachea was 6.06 ± 0.06. Pretreatment with marmin (10 and 100 μM) markedly suppressed the contraction to histamine, and significantly decreased the pD_2 value of histamine effect (6.06 ± 0.06) to 5.83 ± 0.07 and 4.95 ± 0.05, respectively (table 1). Marmin shifted the concentration-response curve of histamine to the right in a paralleled manner. However, marmin could not decrease the maximum contraction effect of histamine.

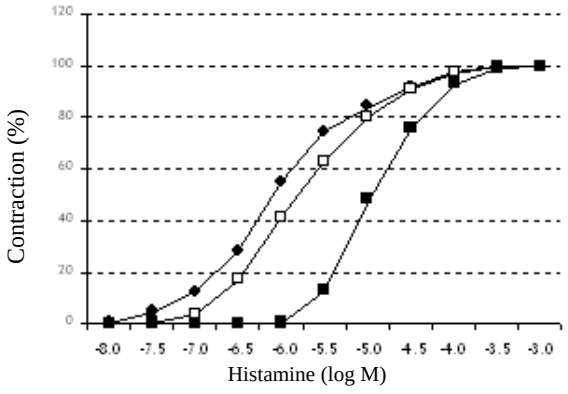

Fig. 2: Concentration-response curves to histamine in the absence (●) or presence of marmin at concentration of 10 μM (□) and 100 μM (■) in guinea-pig tracheal smooth muscle (Data represent mean±SEM, n=5-10).

Effects on the contraction of trachea induced by metacholine

Metacholine (10^{-8}-10^{-3} M) also obviously produced concentration-dependent contraction of the guinea-pig

tracheal smooth muscle (fig. 3). pD_2 value of metacholine effect on the guinea-pig trachea was 6.11 ± 0.09. Pretreatment with marmin at concentration of 10 and 100 μM inhibited the contraction induced by metacholine, and significantly decreased the pD_2 value of metacholine effect (6.11 ± 0.09) to 5.71 ± 5.59 and 5.30 ± 0.03, respectively (table 1). Marmin shifted the concentration-response curve of metacholine to the right in an unparalleled manner. In the study, marmin also could not decrease the maximum contraction effect of metacholine.

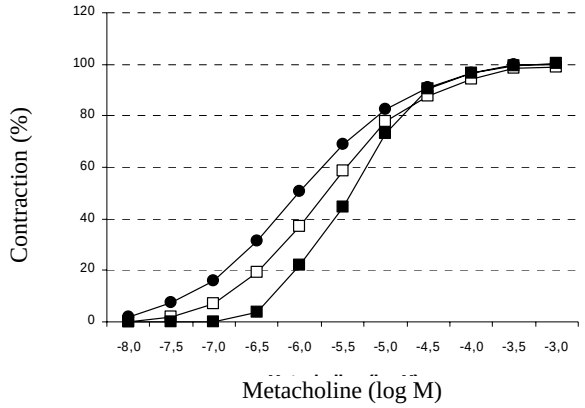

Fig. 3: Concentration-response curves to metacholine in the absence (●) or presence of marmin at concentration of 10 μM (□) and 100 μM (■) in guinea-pig tracheal smooth muscle (Data represent mean±SEM, n=5-10).

Effect on the contraction of trachea induced by Compound 48/80

In the study, compound 48/80 (1-100 μg/ml) also stimulated concentration-dependent contraction of the guinea-pig tracheal smooth muscle. At concentration of 100 μM, marmin abrogated the contraction of trachea smooth muscle induced by compound 48/80. The incubation of marmin for 10 min markedly decreased the concentration-response curve of compound 48/80 (fig. 4).

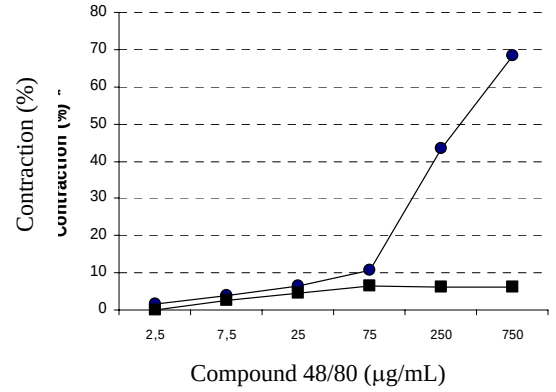

Fig. 4: Concentration-response curves to compound 48/80 in the absence (●) or presence of marmin at concentration of 100 μM (■) in guinea-pig tracheal smooth muscle (Data represent mean±SEM, n=4).

*compare to the maximum contraction of tracheal smooth muscle induced by histamine

Effect on the relaxation of tracheal induced by isoprenaline

In the study, cumulative concentration of isoprenaline (10^{-7}-10^{-3} M) produced concentration-dependent relaxation of the guinea-pig tracheal smooth muscle (fig. 5). pD_2 value of isoprenaline effect on guinea-pig trachea was 6.05 ± 0.08. Pretreatment with marmin at concentration of 10 μM could not influence the relaxation effect of isoprenaline. However, marmin at concentration of 100 μM mildly potentiated the relaxation to isoprenaline, and significantly increased the pD_2 value of histamine effect (6.05 ± 0.08) to 6.38 ± 0.15 (table 1). The incubation of marmin for 10 min mildly shifted the concentration-response curve of histamine to the left. Marmin also could not influence the maximum relaxation effect of isoprenaline.

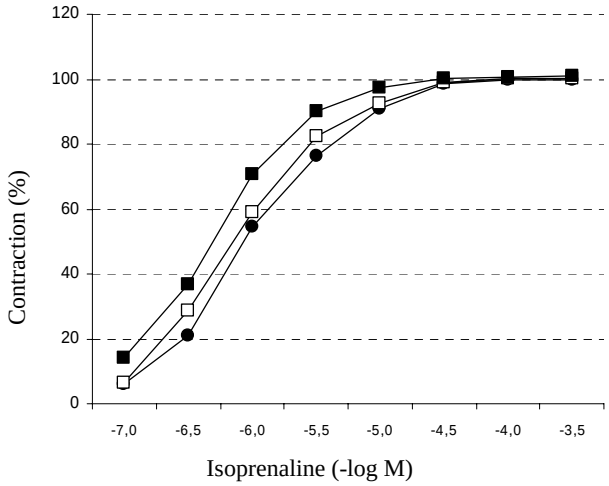

Fig. 5: Concentration-response curves to isoprenaline in the absence (●) or presence of marmin at concentration of 10 μM (□) and 100 μM (■) in guinea-pig tracheal smooth muscle (Data represent mean±SEM, n=4-8).

Effect on the contraction of trachea induced by $CaCl_2$

After depolarizing membrane of tracheal smooth muscle cells by adding KCl (80 mmol/L), the administration of $CaCl_2$ (10mM) obviously stimulated contraction of tracheal smooth muscle in Ca^{2+}-free Krebs solution. Pretreatment with marmin (100 μM) inhibited the contraction effect of $CaCl_2$. The incubation of marmin at 10 min prior to $CaCl_2$ markedly decreased the observation time-response curve of $CaCl_2$ (fig. 6).

Effects on the two phase of contraction induced by metacholine and $CaCl_2$

Metacholine (3.10^{-4} M) could induce contraction of tracheal smooth muscle in Ca^{2+}-free Krebs solution (first phase). The first phase of contraction was caused by the release of intracellular Ca^{2+} from intracellular Ca^{2+} store especially from sarcoplasmic reticulum. Subsequently, $CaCl_2$ obviously induced contraction of trachea (second

phase). The second phase contraction was caused by Ca^{2+} influx from intracellular through the store-operated calcium channels (SOC channels). Pretreatment with marmin (100 μM) obviously inhibited the first phase of contraction, but mildly inhibited the second phase of contraction (fig. 7).

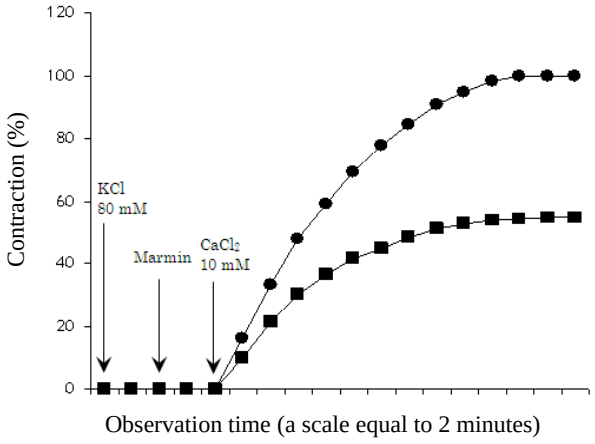

Fig. 6: Time observation-response curves to $CaCl_2$ in the absence (●) or presence of marmin at concentration of 100 μM (■) in Ca^{2+}-free Krebs solution (Data represent mean±SEM, n=4-5).

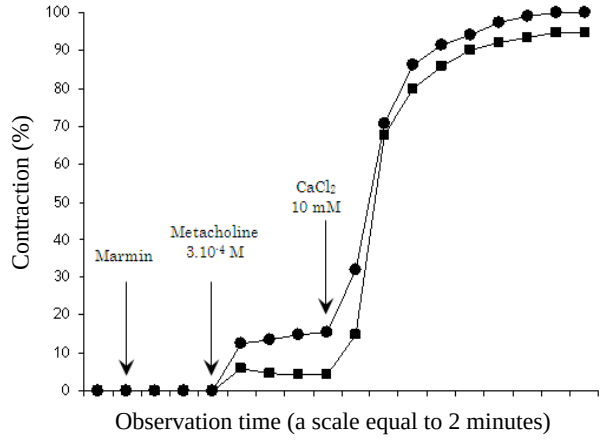

Figure 7. Two phase of contraction of tracheal smooth muscle consecutively induced by metacholine (3.10^{-4} M) and CaCl2 (10 mM) in the absence (●) or presence of marmin at concentration of 100 μM (■) in Ca^{2+}-free Krebs solution (Data represent mean±SEM, n=4-6).

DISCUSSION

Marmin is a coumarine compound isolated from roots and stem bark of *Aegle marmelos* Correa. The compound was isolated from petroleum ether, chloroform and methanol extracts of the roots and stem bark of the plant. The extracts were fractionated using chromatography (vacuum column, gravity column) and developed by gradient elution. The solid material obtained was recrystallized to yield the compound.

Previous study, three active compounds isolated from *Aegle marmelos* Correa, namely aegeline, skimmianine, and marmin. These compounds potently inhibited the histamine release from rat mast cells. Among them, marmin possessed most potential inhibitory effect. The compound strongly suppressed the histamine release from rat mast cells induced by either DNP-BSA, an antigen, or thapsigargin, an intracellular Ca^{2+} stimulant, by 90 %. Marmin potently inhibited $^{45}Ca^{2+}$ influx into mast cells induced by thapsigargin. However, marmin could not influence the histamine release induced by a protein kinase C (PKC) activator namely phorbol myristate acetate (PMA). This data indicated that the effect of marmin on inhibiting the histamine release from mast cells involved mechanisms related to intracellular Ca^{2+} signaling events by blocking Ca^{2+} influx into mast cells (Nugroho *et al.*, 2008).

In this study, we found that marmin inhibited contraction of the tracheal smooth muscle induced by histamine or metacholine. Besides, marmin also mildly potentiated the relaxation effect of isoprenaline on the tracheal smooth muscle. It was interesting that marmin shifted the concentration-response curve of histamine to the right in a paralleled manner without decreasing maximum contraction effect of histamine. In other hand, marmin shifted the curve of metacholine to the right in an unparalleled manner. The finding suggests that marmin inhibited the contraction of trachea by interfering the histamine receptor in the tracheal smooth muscle competitively. Marmin isolated from *Aurantii fructus immaturus* was reported exhibiting concentration-dependent relaxations of contraction of guinea pig ileum induced by acetylcholin or histamine (Takase *et al.*, 1994).

Table 1: The pD_2 values of several agonist-induced responses in the guinea-pig isolated trachea in the absence or presence of marmin.

Agonist	Control	Marmin (μM)	
		10	100
Histamine	6,06 ± 0,06	5,83 ± 0,07*	4,95 ± 0,05*
Metacholine	6,11 ± 0,09	5,71 ± 0,06*	5,59 ± 0,10*
Isoprenaline	6,05 ± 0,08	6,16 ± 0,12	6,38 ± 0,15*

Data represent mean±SEM, and are three independent experiments.
* Significant difference P<0.05 compared to the control value.

In the study, marmin abrogated contraction of the tracheal smooth muscle induced by compound 48/80, a substance which was capable of degranulating mast cells to release histamine. Subsequently, histamine endogen interacts with histamine receptor to trigger contraction of the tracheal smooth muscle. Compound 48/80 is a substance that activates secretory processes of mast cells through the rate of GTPγS binding to G-proteins (Go/Gi mixture) (Mousli *et al*., 1990; Palomaki *et al*., 2006). The activation of G-proteins can trigger intracellular signaling events involving the activation of phospolipase C, protein kinase C, and Ca^{2+} signaling, which end up with the release of histamine from mast cells (Metcalfe *et al*., 1997). Based on the results, marmin potently suppressed the contraction induced compound 48/80 through two ways, (1) by inhibiting the histamine release from mast cells, and (2) by competitively disrupting the histamine receptor in tracheal smooth muscle.

In the contraction of smooth muscle, intracellular Ca^{2+} has an important role Rang *et al.*, 2003). The intracellular Ca^{2+} originated from the release of intracellular Ca^{2+} from sarcoplasmic reticulum (SR), or the inflx of Ca^{2+} through cells membrane calcium channels (Scharenberg *et al*., 2007; Alonso *et al*., 2008). The release of intracellular Ca^{2+} can be through SR (Ca^{2+} store) when inositol triphosphat (IP_3) receptor on SR is activated by IP_3. The IP_3 is generated by activation of many type of G-protein coupled receptor such as acetylcholin muscarinic receptor (M_3 receptor). Activation of this M_3 receptor through G-protein then stimulates phospholipase C inducing the formation of two intracellular messengers, IP_3 and diacylglycerol (DAG). The contraction of smooth muscle is triggered when phosphorylation of myosin light chain (MLC) occurs by myosin-light-chain kinase (MLCK). This kinase is activated by binding to the complex of intracellular Ca^{2+}-calmodulin (Rang *et al*, 2003).

In the study, we found that marmin inhibited the contraction of tracheal smooth muscle induced by $CaCl_2$ in Ca^{2+}-free Krebs solution. KCl depolarized the membrane of smooth muscle cells to activate voltage-dependent Ca^{2+} channels. This condition allowed Ca^{2+} influx from intracellular through these channels. The finding indicates that marmin inhibits the intracellular Ca^{2+} influx through voltage-dependent Ca^{2+} channels.

Marmin also inhibited two phases of contraction consecutively induced by metacholine and $CaCl_2$ in Ca^{2+}-free Krebs solution. The first phase of contraction was caused by the release of intracellular Ca^{2+} from intracellular Ca^{2+} store especially from sarcoplasmic reticulum. The second phase of contraction was caused by Ca^{2+} influx from intracellular through the store-operated calcium channels (SOC channels). Pretreatment with marmin (100 μM) obviously inhibited the first phase of contraction, but mildly inhibited the second phase of contraction. The finding indicates that marmin tends to inhibit the release of intracellular Ca^{2+} from intracellular Ca^{2+} store.

CONCLUSION

Marmin isolated from *Aegle marmelos* Correa could inhibit the contraction of guinea-pig tracheal smooth muscle, in particular by interfering histamine receptor, inhibiting the histamine release from mast, inhibiting intracellular Ca^{2+} release from intracellular Ca^{2+} store and the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels.

REFERENCES

Alonso E, Alfonso A, Lober K and Botana LM (2008). The effect of rottlerin in calcium regulation in HMC-1(560) cells is mediated by a PKC-delta independent effect. *J. Cell Biochem.*, **105**(1): 255-261.

Arul V, Miyazaki S and Dhananjayan R (2005). Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. *J. Ethnopharmacol.*, **96**(1-2): 159-163.

Lampronti I, Martello D, Bianchi N, Borgatti M, Lambertini E, Piva R, Jabbar S, Choudhuri MS, Khan MT and Gambari R (2003). *In vitro* antiproliferative effects on human tumor cell lines of extracts from the Bangladeshi medicinal plant *Aegle marmelos* Correa. *Phytomedicine*, **10**(4): 300-308.

Metcalfe DD, Baram D and Mekori YA. (1997) Mast Cells. *Physiol Rev*., **77**(4): 1033-1064

Mousli M, Bronner C, Landry Y, Bockaert J and Rouot B (1990). Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. *FEBS Lett*., **259**(2): 260-262.

Nugroho AE, Riyanto S, Sukari MA and Maeyama K (2008). The Effects of compounds isolated from *Aegle marmelos* Correa on the histamine release from mast cells. The 81st Annual Meeting of the Japanese Pharmacological Society, Yokohama, Japan, pp.189P.

Palomaki VA and Laitinen JT (2006). The basic secretagogue compound 48/80 activates G proteins indirectly via stimulation of phospholipase D-lysophosphatidic acid receptor axis and $5\text{-}HT_{1A}$ receptors in rat brain sections. *Br. J. Pharmacol*., **147**(6): 596-606.

Pang JJ, Xu XB, Li HF, Zhang XY, Zheng TZ and Qu SY (2002). Inhibition of β-estradiol on trachea smooth muscle contraction *in vitro* and *in vivo*. *Acta Pharmacol. Sin.*, **23**(3): 271-277.

Rang HP, Dale MM, and Ritter JM (2003). Pharmacology, 4th ed., Churchill Livingstone, Melbourne, pp. 62-63, 136-148.

Rana BK, Singh UP and Taneja V (1997). Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos. J. Ethnopharmacol*., **57**(1): 29-34.

Riyanto S. (2003) Phytochemical Studies and Bioactivity Tests of *Murraya paniculata* Jack, *Aegle marmelos* Correa, and *Zingiber amaricans* Blume. Dissertation, Universiti Putra, Malaysia, pp 58-67, 179-188.

Sabu MC and Kuttan R (2004). Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. *Indian J. Physiol. Pharmacol*., **48**(1): 81-88.

Satoh Y, Tashiro S, Satoh M, Fujimoto Y, Xu JY and Ikekawa T (1996). Studies on the bioactive constituents of *Aurantii Fructus Immaturus*. *Yakugaku Zasshi*., **116**(3): 244-250.

Sayah ME, Filho VC, Yunes RA and Calixto JB (1997). Action of the extract of *Drymis winteri* on contraction induced by inflammatory mediators, compound 48/80 and ovalbumin of the guinea-pig trachea *in vitro*. *Gen. Pharmac*., **28**(5): 699-704.

Scharenberg AM, Humphries LA and Rawlings DJ (2007). Calcium signalling and cell-fate choice in B cells. *Nat. Rev. Immunol.*, **7**(10): 778-789.

Shoeb A, Kapil RS and Popli SP (1973). Coumarins and Alkaloids of *Aegle marmelos*. *Phytochemistry*, **12**: 2071-2073.

Takase H, Yamamoto K, Hirano H, Saito Y and Yamashita A (1994). Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine, *Aurantii fructus immaturus*. *Jpn. J. Pharmacol*., **66**(1):139-147.

Upadhya S, Shanbhag KK, Suneetha G, Balachandra Naidu M and Upadhya S (2004). A study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. *Indian J. Physiol. Pharmacol*., **48**(4): 476-480.

Waldron GJ, Ding H, Lovren F, Kubes P and Triggle R (1999). Acetylcholine-induced relaxation of peripheral arteries isolated from mice lacking endothelial nitric oxide synthase. *Br. J. Pharmacol.*, **128**(3): 653-658.