ORIGINAL ARTICLE

Relaxation effects of *Eriobotrya japonica* toward tracheal smooth muscle via action mechanism on histamine-1 receptor and phosphodiesterase-5 enzyme

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ABSTRACT

The *Eriobotrya japonica* leaves have the activity to relax the smooth muscle in the respiratory tract. However, the mechanism of action due to that activity has never been carried out. This study aims to determine the relaxation effects of *E. japonica* leaves extract in the isolated trachea of the guinea pigs through the inhibition of the histamine-1 (H-1) receptor and the phosphodiesterase-5 (PDE-5) enzyme. The determination of the relaxation effects was carried out by using histamine to contract smooth muscle within the tracheal tract, followed by adding cumulative concentrations of extract. Michaelis–Menten kinetics equation was used to determine the antagonist type of extract toward H-1 receptor. The understanding of mechanism of action of the extract toward PDE-5 enzyme was performed by incubating the smooth muscle using sildenafil. The percentage value of responses, originated from the relaxation effect of the extract toward the trachea was analyzed by using the t-independent test. The result showed that the extract was able to relax the smooth muscle, which was contracted by histamine, and there was a positive correlation between concentration and relaxation effect ($P < 0.05$; $r = 0.973$). The extract also antagonized the histamine as a noncompetitive antagonist. The incubation within the trachea with sildenafil demonstrated equal relaxation effect, produced by the extract. It can be concluded that *E. japonica* extract had relaxation effect within the isolated trachea as antagonist noncompetitive toward H-1 receptor and inhibitor of the PDE-5 enzyme.

Key words: Anti-hypersensitivity, *Eriobotrya japonica* (Thunb.) Lindl, histamine-1 receptor, phosphodiesterase-5, relaxation, trachea

INTRODUCTION

Asthma is a chronic inflammation disorder in the respiratory tract, in which many cells and cellular elements are involved during the condition.[1] Based on the World Health Organization data, 235 million people have been reported to suffer asthma, and 80% of deaths from the population were caused due to asthma.[2] In Indonesia, asthma is in the top

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10 causes of morbidity and mortality. This issue remains a challenging health focus that is required to be solved.

The *Eriobotrya japonica* leaves are traditionally used to treat illness in the respiratory system such as cough, chronic bronchitis, inflammation, and diabetes.[3] Recent study has reported that the leaves also allow to relax the smooth muscle activities within the respiratory airway.[4] However, the mechanism of action toward the airway smooth muscle has never been carried out.

The stimulation of H-1 receptor and enhancement of cyclic adenosine monophosphate as well as the cyclic guanosine monophosphate (cGMP) concentrations in the cells have been contributed in the pathophysiology of asthma.[5,6] Therefore, inhibiting the activity of H-1 receptor and phosphodiesterase (PDE) may result the dilation of smooth muscle of the tract. The PDE is classified into 11 types which are commonly known as PDE-1 to PDE-11. Consequently, the inhibition of PDE-3, PDE-4, PDE-5, and PDE-7 has been suggested to have a relaxation response in the respiratory system.[7] Hence, it is necessary to conduct further research on the antagonism type toward H-1 receptor and type of PDE which could be inhibited by the extract of the *E. japonica* leaves.

MATERIALS AND METHODS

Materials

The *E. japonica* leaves were collected from the local area of Simalem Resort Park, on main highway of Merek, Sidikalang, Dairi, North Sumatra, Indonesia. The classification of the plant was conducted in the Herbarium Medanese (MEDA) Universitas Sumatera Utara (2352/MEDA/2018). Ethanol 96%, sodium chloride, potassium chloride, calcium chloride, magnesium sulfate, sodium bicarbonate, potassium dihydrophosphate, and glucose were supplied from the Merck corp., Germany. While the dimethyl sulfoxide, histamine, and sildenafil were purchased from Sigma-Aldrich, USA.

Extraction

The ethanol extract of *E. japonica* leaves was derived through the percolation method in accordance with previous study.[8] The yield extract was about 30.07%.

Phytochemical screening

The phytochemical screening of secondary metabolites in the ethanol extract of *E. japonica* was carried out to determine the alkaloids, flavonoids, tannins, glycosides, saponins, and triterpenoids/steroids, based on the standard methods.[9]

The measurements of the relaxation effects of *Eriobotrya japonica* leaves ethanol extract on isolated trachea smooth muscle of the guinea pig

The determination of effective concentration 80 (EC_{80}) of histamine in the study was approved by Animal Research Ethics Committees with given number of 0255/KEPH-FMIPA/2018. The trachea of guinea pigs was cut crosswise, then was suspended in an organ bath (PowerLab, ML0146/50 PanLab, AD Instrument, New Zealand), containing 40 mL Kreb's solution at 37°C in a condition 95% O_2 and 5% CO_2.[10] Inside the organ bath, amounts of histamine solution were added to obtain the concentrations of 10^{-8} –3 × 10^{-3} M (tracheal smooth muscle of guinea pig showed maximum contraction response). The contraction of tracheal guinea pig was recorded by a recorder.[11] Whilst, the determination of EC_{80} was calculated through the Eq. (1):

$$Log\ EC_{80} = \left[\frac{80 - Y_1}{Y_2 - Y_1} \times \left(X_2 - X_1\right)\right] + X_1 \quad (1)$$

Explanation:

X_1=*Log concentration with a response just below 80%*

X_2= *Log concentration with a response exactly above 80%*

Y_1=*% response exactly below 80%*

Y_2=*% response exactly above 80%*

The experimental results showed that the trachea was contracted in condition of 920 µl histamine 2 × 10^{-2} M. As a result, the concentration in the bath was found at 4.62 × 10^{-4} M (the EC_{80} of histamine concentration). After obtaining the expected conditions, amounts of extract were added gradually as much as 125 µl until 1000 µl, so that the concentration in the organ bath was accounted for 1–8 mg/ml. The contracted responses were recorded by the recorder.[12] Then, the relaxation effect was calculated by using the following Eq. (2):

$$Relaxation\ effect = \left[\frac{agonist\ contraction - agonist + antagonist\ contraction}{agonist\ contraction}\right] \quad (2)$$

The determination of antagonism type of *Eriobotrya japonica* extract toward histamine receptor

In determining an EC_{50} of histamine, amounts of histamine solutions with concentrations of 10^{-8} –3 × 10^{-3} M in organ bath (tracheal showed maximum contraction response) were added into the trachea that has already been equilibrated for 45 min. The contraction of the trachea that occurred were recorded by the recorder.[13] The determination of EC_{50} was calculated with the Eq. (3):

$$Log\ EC_{50} = \left[\frac{50 - Y_1}{Y_2 - Y_1} \times \left(X_2 - X_1\right)\right] + X_1 \quad (3)$$

Explanation:

X_1=*Log concentration with a response just below 50%*

X_2= *Log concentration with a response exactly above 50%*
Y_1=*% response exactly below 50%*
Y_2=*% response exactly above 50%*

In obtaining the antagonism type, the trachea that has been incubated with the extract at the doses of 1, 2, 3, 4 mg/ml as much as 1 ml for 20 min were experimentally conducted. Every concentration of extract was tested separately. Afterwards, the trachea contracted by adding serial concentration of histamine, amounted 10^{-8} –10^{-3} M. Then, the same procedure was applied exactly as it was conducted in determining the EC_{50}. The data from recorder were converted into percentage contraction. The graph was then plotted in form of contraction percentage versus log concentration of histamine. The antagonism type of *E. japonica* extract was carried out by using Michaelis-Menten equation through Lineweaver-Burk plot data analysis by using regression Eq. (4):[14]

$$y = a + bx \quad (4)$$

As x-axis is (1/histamine concentration) and as y-axis is (1/% contraction).

The determination of mechanism of action of *Eriobotrya japonica* extract on the trachea through inhibition of phosphodiesterase-5

The guinea pig trachea was incubated for 20 min with sildenafil with the concentration of 10^{-4} M (by adding 200 µl of sildenafil solution 2×10^{-2} M in to organ bath). Then, it was contracted with 920 µl histamine solution 2×10^{-2} M so that the concentration of histamine in the organ bath was 4.62×10^{-4} M (EC_{80} of histamine). After obtaining a stable maximum contraction, the extract was added gradually as much as 125 µl until 1000 µl to reach the concentration of the organ bath, which was accounted from 1 mg/ml to 8 mg/ml. The response was recorded by the recorder. Then, the correlation curve of extract and the percentages of relaxation response were plotted. The curve then compared with and without incubation with sildenafil.[15]

Data analysis

The response percentage value from the relaxation effect testing of *E. japonica* extract toward trachea was analyzed statistically by using the t-independent test.

RESULTS

Phytochemical constituents

The phytochemical screening revealed the presence of flavonoids, tannins, glycosides, saponins, and triterpenoids/steroids, as it is shown in Table 1.

The relaxation effects of *Eriobotrya japonica* leaves ethanol extract on isolated trachea smooth muscle of the guinea pig

The results can be seen in Figure 1.

Based on Figure 1, the administration of *E. japonica* extract toward contracted tracheal smooth muscle exerted relaxation effect. The concentration of 1 mg/ml revealed relaxation effect to the tracheal smooth muscle, accounted for 4.3634% ± 1.4362%, whereas at the concentration of 8 mg/ml, the relaxation demonstrated the highest effect, that was 73.8664% ± 15.3525%. Therefore, *E. japonica* extract was able to relax the tracheal smooth muscle contracted by histamine.

Based on the correlation and regression test, there was a significant correlation between *E. japonica* extract concentration and the relaxation effect within the tracheal smooth muscle ($P < 0.05$; $r = 0.973$). This result indicated the relaxation exerted was due to the effect of extract administration.

Antagonism type of *Eriobotrya japonica* extract toward histamine receptor

The effect of various concentration of *E. japonica* extract toward tracheal smooth muscle which was induced by gradual concentration of histamine compared to the contraction data from histamine control was plotted in to a graph, which is shown by Figure 2.

According to Figure 2, there was an increasing of contraction as a series of histamine concentrations rose. The administration of *E. japonica* extract at the initial experiment (incubation) caused the graph to shift to the right side. In other words, contraction percentages declined by the time of extract amounts were added. This phenomenon indicated the extract was able to antagonize the effect of

Table 1: Secondary metabolites in the ethanol extract of the *Eriobotrya japonica* leaves

Phytochemical compounds	Reagent	Presence
Alkaloid	Mayer	Negative
	Bouchardat	Negative
	Dragendorff	Negative
Flavonoids	Concentrated HCl	Positive
Tannins	Ferric chloride	Positive
Glycosides	Molisch and concentrated H_2SO_4	Positive
Saponins	Shaking and HCl 2N	Positive
Triterpenoids/steroids	Acetic acid anhydrous and concentrated H_2SO_4	Positive

histamine. Furthermore, the antagonism effect of the extract was evaluated through statistical analysis, given that the highest concentration of histamine was 3×10^{-3} M wasn't able to reach the 100% contraction because of administration of *E. japonica* extract. This finding suggested that the *E. japonica* extract antagonized the contraction caused by histamine, and the antagonist type was a noncompetitive antagonism. This result was also confirmed by the Michaelis-Menten kinetics analysis, as the contraction was converted into kinetics rate, as shown in Table 2. The data of kinetics rate and percentage of concentration were then plotted into a graph that is displayed in Figure 3.

Based on Figure 3, the linear lines from each regression equation touched different points on the y-axis. Thus, it can be concluded that the extract has a noncompetitive antagonistic activity.

The action mechanism of *Eriobotrya japonica* extract on trachea through phosphodiesterase-5 inhibition

The result can be seen in Figure 4.

In order to know *E. japonica* extract mechanism of action on trachea through PDE5 inhibition precisely, the area under curve calculation was conducted. The results can be seen in Table 2.

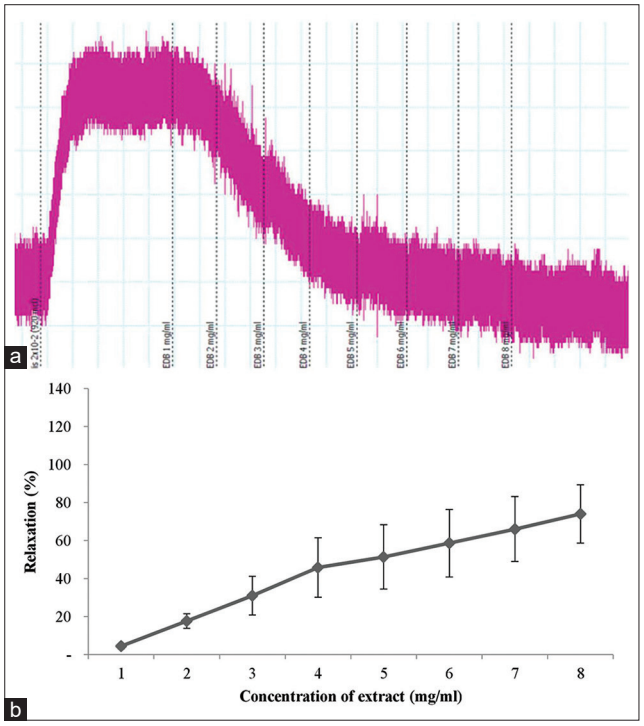

Figure 1: (a) Decreasing of tracheal smooth muscle contraction after administration series concentration of extract 1–8 mg/mL; (b) Relaxation effect of the trachea

Based on Table 2, there was no significant difference between the relaxation of tracheal smooth muscle with and without the incubation in sildenafil condition. Therefore, it can be obtained that the relaxation effect of *E. japonica* extract might be mediated with the same mechanism of sildenafil that was by the inhibition of PDE enzyme.

DISCUSSION

The histamine induction is an approach to the pathology of asthma caused by allergies. In human and guinea pig the histamine causes bronchoconstriction which occurs through histamine-1 (H-1) receptors.[5] The H-1 receptors are widely distributed in the tissues, found mainly in the respiratory pathway (smooth muscle). Histamine releases from the mast cell as long as the allergy period causes histamine binding to H-1 receptors in triggering the contraction within the airway smooth muscle.[16]

The sildenafil is a specific inhibitor of the PDE-5. An initial incubation of these compounds on trachea smooth muscle has been reported to aim at the inhibiting the activity of PDE-5.[17] Treatment with sildenafil at initial incubation was designed to proof whether the relaxation effect caused by *E. japonica* extract was mediated by PDE inhibition. Whilst, the PDE-5 inhibition causes an increase in cGMP within the cells, it is a potent vasodilator. The increase of cGMP would have activated cGMP-dependent kinase protein,

Table 2: Calculation of amount under curve value from relaxation caused by *E. japonica* extract toward tracheal smooth muscle with and without prior incubation

Treatment	Mean±SEM
E. japonica extract	309.2833±88.5727
Sildenafil + *E. japonica* extract	495.3494 ± 79.8752[a]

[a]Significantly different with another group. SEM: Standard error of the mean, E. japonica: *Eriobotrya japonica*

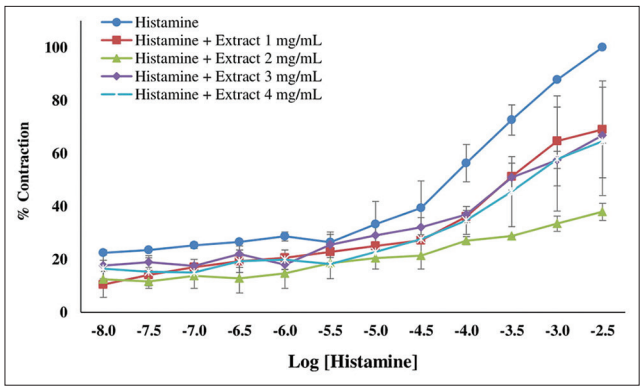

Figure 2: The effect of various concentration of *Eriobotrya japonica* extract toward tracheal smooth muscle which is induced by graded concentration of histamine

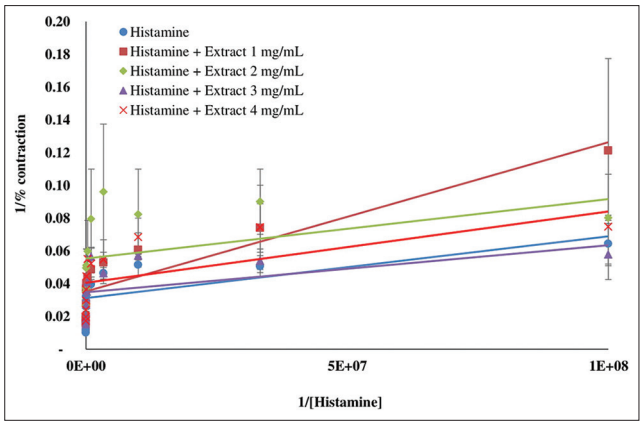

Figure 3: Michaelis-Menten kinetics analysis

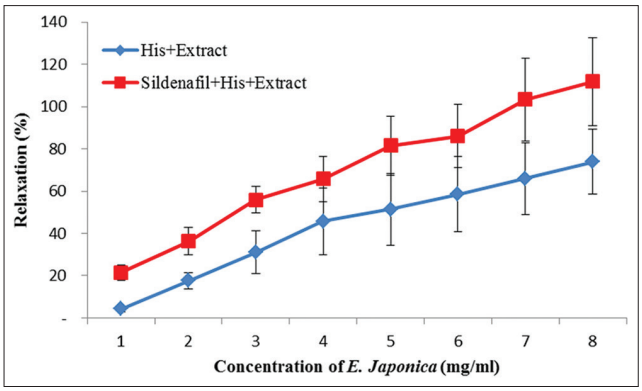

Figure 4: Relaxation of tracheal smooth muscle incubated with and without sildenafil

which causes a decrease Ca^{2+} in intracellular condition. An ultimately myosin light chain kinase can be no longer to be phosphorylate myosin molecules, leading to smooth muscle relaxation.[18]

It has been suggested that the natural antioxidants in the form of flavonoids could inhibit histamine release from mast cells, basophils as well as inhibiting the contraction, which was caused by histamine, acetylcholine, and PDE.[19] Another research has reported that terpenoid and steroid glycoside in the plant had a relaxant effect in the smooth muscle of trachea. This result has confirmed that the existence of flavonoid, steroid and triterpenoid in E. japonica extract contributed to the relaxation of trachea.[20,21]

The relaxation of the smooth muscle of the respiratory tract has been reported not only mediated by PDE, but also mediated by nitric oxide, prostaglandin E-2 (PGE2), cGMP, and adrenergic nerves through β2-adrenergic receptors.[12] Thus, further investigation regarding to these factors is required to evaluated.

CONCLUSIONS

The extract of *Eriobotrya japonica* leaves posed a relaxing effect on the isolated tracheal smooth muscle of the guinea pig induced by histamine. The mechanism behind the relaxing effect on the isolated muscle of the guinea pig was found to be mediated through the inhibition of the enzyme PDE-5.

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Conflicts of interest

There are no conflicts of interest.

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