Research Article

The relaxation effect of ethanolic extract of Biwa leaves (*Eriobotrya japonica*) on the histamine-1 receptor in isolated guinea pigs tracheal

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ABSTRACT

Background: *Eriobotrya japonica* or Biwa (family of Rosaceae) is one of the traditional medicines that used for bronchitis and cough. **Objective:** The aims of the study were to investigate the relaxation effect of the ethanolic extract of *E. japonica* leaves on the isolated guinea pig tracheal induced by histamine and its mechanism on phosphodiesterase-3 (PDE-3), nitric oxide, and prostaglandin. **Materials and Methods:** The study of the relaxation effect of the ethanolic extract (1–8 mg/mL) on the contraction of histamine 4.6×10^{-4} M was conducted *in vitro* using isolated guinea pig tracheal organ in the Krebs solution. Mechanistic evaluation was carried out by organ pre-incubation using theophylline, L-NAME, and acetosal. **Results:** The results showed that the ethanolic extract of *E. japonica* leaves produced a relaxation effect on histamine-induced contraction on guinea pig isolated tracheal and it was concentration-dependent manner. Relaxation percentages at the extract concentration of 8 mg/ml reached 43.24 ± 7.09%. The mechanism of the relaxation effect of the extract is mediated through PDE-3 inhibition, the prostaglandin pathway, and the nitric oxide pathway. **Conclusion:** The ethanolic extract of *E. japonica* leaves showed a relaxation effect on histamine induced-contraction mediated through PDE-3 inhibition, prostaglandin, and nitric oxide pathway.

KEY WORDS: *Eriobotrya japonica*, Phosphodiesterase, Nitric oxide, Prostaglandin, Relaxation

INTRODUCTION

Asthma is blockages in airflow in the airways caused by chronic inflammatory.[1] Asthma in Indonesia is included in the top ten diseases that cause pain and death. The highest incidence of asthma reached among men.[2,3]

Eriobotrya japonica (Thunb.) Lindl. or Biwa (family of Rosaceae) is one of the native Chinese fruit trees known as Loquat in Japan and used as a traditional medicine called Kampo.[4,5] Biwa has been used as an effective herb to treat respiratory diseases and several biological effects such as antioxidants,[6] antihyperglycemic,[7] anti-inflammatory,[8] antiobesity,[9] cough,[8] and anticancer effects.[10] Phytochemical studies on Biwa leaves revealed the presence of various triterpenes, flavonoids, sesquiterpenes, and tannins.[10-12]

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This study aims to determine the relaxation effect of ethanolic extract of Biwa leaves on histamine-induced contraction on isolated guinea pig tracheal as a model for pathological conditions of asthma caused by allergies.

MATERIALS AND METHODS

Materials

The chemicals used in this study were ethanol, sodium chloride, potassium chloride, calcium chloride, magnesium sulfate, sodium bicarbonate, potassium dihydrophosphate, glucose, dimethyl sulfoxide, histamine, theophylline, N-Nitroarginine methyl ester (L-NAME), and acetosal (Sigma-Aldrich).

Sample Preparation and Extraction

Biwa leaves were obtained from Simalem Resort Park, Sidikalang, North Sumatra and have been identified by the Herbarium Medanense (MEDA) Faculty of Mathematics and Science, Universitas Sumatra Utara

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(No. 2353/MEDA/2018). The leaves were washed, dried, and then grinded until the dried powder was obtained. One hundred grams of powder were extracted with ethanol (96%) using the percolation method. The extract was then concentrated with a rotary evaporator until a thick extract was obtained.

Phytochemical Testing

The secondary metabolite content in extracts was tested by standard phytochemical screening test methods.[13]

Animals and Organ Preparation

Male guinea pigs weighing between 300 and 500 g at the age of 3–4 months were acclimatized for a week and fasted for 24 h before the experiment and sacrificed by cervical dislocation. The research protocol was approved by the Animal Research Ethics Committee, Universitas Sumatra Utara (No. 0267/KEPH/FMIPA/2018). The trachea was cleared from the connective tissue, the tip of the trachea was attached to the organ bath (PowerLab ML0146/50), and the transducer (MLT0201) connected with PowerLab T15-0676 (PanLab, ADInstrument, New Zealand). The experiment conducted using 40 ml of Krebs solution with gas O_2:CO_2 (95:5%).[14]

Relaxation Effect Evaluation of the Ethanolic Extract of Biwa Leaves on Isolated Guinea Pig Tracheal

The isolated guinea pig tracheal has been equilibrated for 45 min (with 3 times change of Kreb's solution) contracted by the administration of histamine solution of 4.62×10^{-4} M. After obtaining a stable of maximum contraction condition then given the ethanolic extract of Biwa leaves from 1 mg/mL to 8 mg/mL. The response that occurs will be recorded in the recorder.[15]

Mechanistic Evaluation of the Ethanolic Extract of Biwa Leaves through Phosphodiesterase-3 (PDE-3) Enzymes, Nitric Oxide, and Prostaglandin

The mechanism of action evaluation of the ethanolic extract of Biwa leaves on isolated guinea pig tracheal through inhibition of PDE-3 enzymes (PDE), nitric oxide, and prostaglandin carried out in the same procedure for the relaxation effect of the extract on isolated guinea pig tracheal but the organs were pre-incubated with theophylline 10^{-4} M, L-NAME 10^{-4} M and acetosal 10^{-4} M for 20 min after equilibration.[14]

RESULTS AND DISCUSSION

The ethanolic extract of Biwa leaves contains tannins, saponins, triterpenes/steroids, flavonoids, and glycosides [Table 1]. This result is consistent with the previous report that the ethanolic extract of Biwa leaves contains flavonoids, tannins, glycosides, saponins, and triterpenes/steroids.[14]

The Relaxation Effect of the Ethanolic Extract of Biwa Leaves in Isolated Tracheal

The administration of the extract at a concentration of 1–8 mg/mL has shown a relaxation effect on tracheal smooth muscle [Figure 1]. The extract at a concentration of 8 mg/mL showed the highest relaxation effect reaching 40% [Figure 2]. The relaxation effect of extracts may be due to the complexity of the secondary metabolic contained, and the main contributor is ursolic acid.[16]

Table 1: Phytochemical contents of the ethanol extract of Biwa leaves

Secondary metabolite	Result
Alkaloid	−
Tannins	+
Saponins	+
Triterpenes/Steroids	+
Flavonoids	+
Glycosides	+

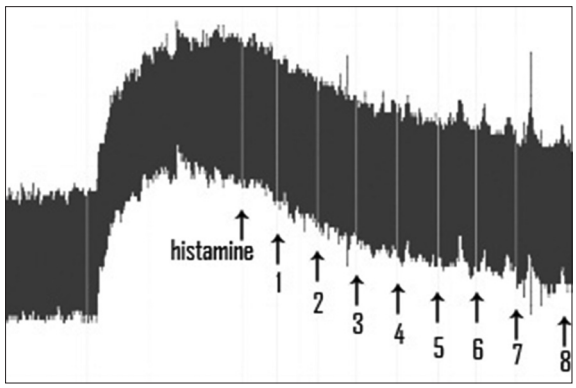

Figure 1: Relaxation of isolated guinea pig tracheal smooth muscle after the administration of concentration series of extract (1–8 mg/mL). Single contraction by histamine of 4.62×10^{-4} M. Data represent mean ± SEM, $n = 4$

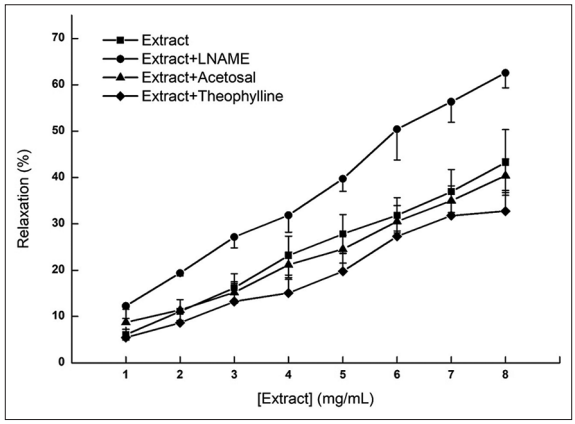

Figure 2: Relaxation effect of the ethanolic extract of Biwa leaves on isolated guinea pig tracheal smooth muscle. Single contraction by histamine of 4.62×10^{-4} M. Data represent mean±SEM, $n = 4$

The Evaluation of the Mechanism of Action of the Ethanolic Extract of Biwa Leaves on Isolated Guinea Pig Tracheal

PDE-3 enzymes

The mechanism of action of the relaxation effect of the extract on tracheal smooth muscle showed that in the initial pre-incubation with theophylline 10^{-4} M there was no significant difference in the relaxation effect compared to the relaxation effect of the extract alone (as control). This result is due to the inhibition of PDE by theophylline caused the extract no longer relaxing the tracheal through its mechanism. It can be concluded that the relaxation effect of extract related to the inhibition of the PDE activity. Some extracts from plants have been reported to have inhibitory effects on PDE, such as *Sceletium tortuosum,*[17] *Aloe barbadensis,*[18] *Pistacia integerrima*[19] *Toddalia asiatica,*[20] *Lepidium sativum,*[21] and black pepper.[22] Active metabolite compounds from fungus, such as benzoquinones and terphenyl, have been shown as PDE-4B inhibitors.[23]

Pre-incubation of tracheal smooth muscle with theophylline aimed to inhibit the activity of PDE.[24] Inhibition of PDE will increase cellular cyclic adenosine monophosphate (cAMP) concentration that it will be caused bronchodilatation.[25,26] The cAMP causes smooth muscle relaxation through contractile protein phosphorylation and lower intracellular calcium levels.[15,20,27,28]

Nitric oxide

Pre-incubation with L-NAME produced a relaxation effect that was significantly different compared with without pre-incubation treatment. Figure 2 shows that the relaxation effect of pre-incubation treatment with L-NAME was higher than without pre-incubation. These results indicate that the relaxation effect of the extract is possible through the NO pathway. The extract being able to trigger an increase in NO compound levels by affecting the nitric oxide synthase (NOS) enzyme. Some extracts from plants have been reported to have the effect of increasing NO levels. Garlic extract was able to increase NO levels through the activation of nNOS and eNOS.[29-31] L-NAME was an eNOS and nNOS inhibitor[32,33] so it can be assumed that extract was able to increase NO compounds through other pathways such as iNOS so that the relaxation effect increases.

NO has been an important role in airway smooth muscle relaxation, as a bronchodilator. Smooth muscle relaxation by NO occurs through the NO-GC-cGMP pathway. NO activates guanylate cyclase which will convert GTP to cGMP. Relaxation was obtained through the activation of protein kinase G (PKG) by cGMP[15] Initial incubation with L-NAME aims to inhibit NO to produce bronchoconstriction effects.[16]

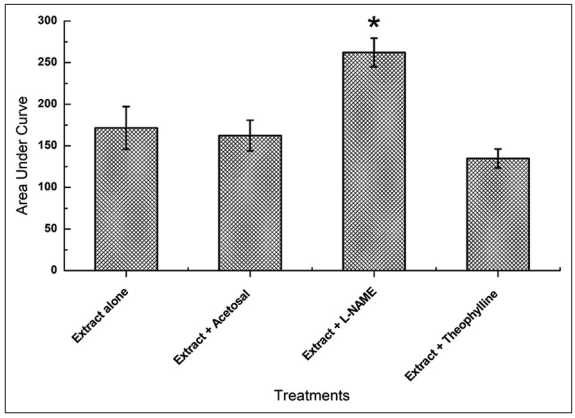

Figure 3: The area under the curve from the relaxation effect of the ethanolic extract of *E. japonica* leaves. *Significant difference $P < 0.05$ compared to the values of the extract alone group. Data represent mean±SEM, $n = 4$

Prostaglandin

The isolated guinea pig tracheal pre-incubation with acetosal before administration of the extract showed no different relaxation effect from the extract alone [Figure 3]. These findings indicate that the relaxation effect of the extract can be through the prostaglandin inhibition pathway. The smooth muscle airways contracted by the derivatives of eicosanoids derivate such as PGF2α, TXA2, and PGD2.[34-37] The acetosal was a COX inhibitor that inhibits prostaglandin formation.[38] The results concluded that the ethanolic extract of *E. japonica* leaves possessed relaxation effect through the PDE-3 inhibition pathway, prostaglandin, and the NO pathway.

CONCLUSION

The results showed that the ethanolic extract of *E. japonica* leaves showed a relaxation effect on histamine induced-contraction mediated through PDE-3 inhibition, prostaglandin, and nitric oxide pathway. Further studies are needed to determine the content of secondary metabolites from *E. japonica* that responsible for its mechanism of action.

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