INHIBITORY EFFECT OF ETHANOLIC EXTRACT OF ERIobotrya Japonica LEAVES PRE-INCUBATED WITH THEOPHYLLINE AND ASPIRIN ON ISOLATED GUINEA PIG TRACHEAL

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ABSTRACT

Objectives: The objectives of the study were to examine the inhibitory effect of ethanol extract of Eriobotrya japonica leaves (EEEL) pre-incubated with theophylline and aspirin on isolated guinea pig tracheal smooth muscle contracting acetylcholine (ACh)-induced contraction.

Methods: The effect of EEEL (1-8 mg/Ml) on the isolated tracheal strips was tested in vitro. Furthermore, the mechanism of relaxant effects of EEEL was evaluated in the presence of theophylline and aspirin.

Results: The contractile response evoked by ACh (1.25 × 10⁻³ M) was decreased by EEEL (effective concentration ₃₀ = 1.36 mg/ml) and has no significant difference of relaxant effect to that of EEEL pre-incubated with theophylline and aspirin (p>0.05).

Conclusion: The EEEL decreased the ACh-induced contraction through the inhibition of PDE and the protective effect on prostaglandin E2.

Keywords: Eriobotrya japonica, Biwa, ACh-muscarinic-3 receptor, Theophylline, Aspirin.

INTRODUCTION

Asthma is a chronic airway inflammatory disease characterized by obstruction, hyperresponsiveness, and remodeling of airway [1]. It is considered as a serious health problem and represents a major cause of public health in both developed and developing countries. According to the World Health Organization, there are around 300 million annual cases of asthma worldwide. The incidence of asthma has scaled dramatically, the World Health Organization, there are around 300 million annual cases of asthma worldwide. The incidence of asthma has scaled dramatically, despite increased use of medications that suppress airway inflammation and repress contraction of smooth muscle that surrounds the airways. Asthmatic patients display episodes of airway inflammation that potentiates reversible airway smooth muscle contraction [2]. Recent pharmacological management for controlling asthma is mainly based on corticoids as anti-inflammatory agents in combination with β2-adrenergic agonists as bronchodilators [3]. However, there is an increasing demand for finding new medicines in asthma therapy, as these drugs cause serious side effects.

Natural products offer a remarkable pharmacological perspective for drug development relating to their widespread acceptability and broad-spectrum activities. Hence, it is valuable to search for effective medicines for treating asthma using plant resources. Folium eriobotryae (FE), the dry leaf of Eriobotrya japonica (Thunb.) Lindl. (Rosaceae), commonly known as Biwa in Indonesia, has been used traditionally to treat diseases of the respiratory system, cough, chronic bronchitis, inflammation, and diabetes. In addition, this plant is also useful to treat diarrhea, stress relievers, cholesterol, nicotin e neutralizer, pain reliever, and to smooth the skin. FE has several other biological activities such as anti-diabetes [4], neuroprotective [5], antioxidant [6], and anticancer effects [7]. In the previous studies, FE was demonstrated to exert a noticeable effect on ovalbumin-induced asthma [8] and relieve the symptoms of chronic bronchitis rats [9]. The phytochemical studies on FE shown the presence of various triterpenes, flavonoids, sesquiterpenes, and tannis [10,11]. Nevertheless, despite the many studies performed on the biological effects of FE, there are no scientific data about its bronchodilatory effects. Therefore, the present study aimed to investigate the pharmacological action and possible mechanisms of the ethanol extract of FE isolated tracheal strips.

METHODS

Plant and extracts

E. japonica leaves were collected from Simalem Resort garden, Merek, Sidikalang, North Sumatera, and dried at room temperature in the absence of sunlight. The plant was identified by botanists in the Herbarium Medanese, Universitas Sumatera Utara; the specimen number of the plant is 1443/MEDA/2017. The ethanol extract was prepared as follows: 500 g of the chopped, dried plant was macerated with 300 mL ethanol for 48 h. The solvent of the extract was then removed under reduced pressure until the extract volumes reached 20 mL. Plant ingredient concentration in the final extract was 14.74 W/W.

Tissue preparations

Guinea pigs (300-400 g) were killed by cervical dislocation, and the tracheas were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle and sutured together to form a tracheal chain.

Tissue was then suspended in a 40 mL organ bath (organ bath PowerLab, ML0146/50 PanLab, AD Instrument, New Zealand) containing Krebs solution of the following composition: NaCl 5.9 g, NaHCO₃ 2.1 g, MgSO₄ 0.11 g, KH₂PO₄ 0.16 g, KCl 10.35 g, and glucose 2.0 g. The Krebs solution was maintained at 37°C and gassed with 95% O₂ and 5% CO₂. Tissue was allowed to equilibrate for at least 45 min [12].

Protocols

Effective concentration (EC₅₀) acetylcholine testing on tracheal smooth muscle

EC₅₀ of ACh to contract tracheal smooth muscle was determined. Then, this concentration would be used to contract the muscle before the extract. Measurements of contraction were performed on a graded

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Keywords: Eriobotrya japonica, Biwa, ACh-muscarinic-3 receptor, Theophylline, Aspirin.
basis by giving a series of acetylcholine (ACh) concentrations of $10^{-3} \times 10^{-2}$ M. Tracheal marmots which had been forty-brated for 45 min (with alternation of Kreb's solution every 15 min for 3 times) were given ACh chloride solution with the concentrations of $10^{-2}-3 \times 10^{-3}$ M (smooth tracheal muscle of marmots shows maximum contraction response). The smooth muscle contraction of tracheal marmots that occurred was recorded on the recorder. In this experiment, the effect of eight cumulative concentrations from the extract was examined; the effect of the extract pre-incubated with theophylline and aspirin was also measured to investigate the possible mechanism. A decrease in tone was considered as an inhibitory effect and expressed as positive percentage change in proportion to maximum contraction obtained due to contractile agents. The study was approved by the Animal Research Ethic Committee, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

**Statistical analysis**

The data of the inhibitory effect of different experiments were expressed as mean ± SEM. The graph patterns of effect of ethanol extract of *Eriobotrya japonica* leaves (EEEJL) in the absence and presence of theophylline and aspirin were compared using independent t-test. Significance was accepted at $p<0.05$.

**RESULTS**

**Phytochemical screening**

The phytochemical analysis conducted on *E. japonica* extract revealed the presence of flavonoids, tannins, glycosides, saponins, and steroids/triterpenoids (Table 1). These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the relaxant effect of the plant extract used in this study.

**Effect of ethanol extract of *E. japonica* on isolated tracheal strips**

Series concentration of ACh ($10^{-8}-3 \times 10^{-4}$ M) was applied on isolated tracheal strips to obtain EC$_{50}$, this concentration will be used to induce contraction. Fig. 1 showed that percentage contraction of tracheal smooth muscle increased with the addition of ACh concentration. The maximum ACh concentration for tracheal smooth muscle contraction was $3 \times 10^{-4}$ M. The next given concentration did not change the percentage of smooth muscle contraction. EC$_{50}$ of ACh was $1.25 \times 10^{-4}$ M.

It can be seen that EEEJL (1-8 mg/mL) relaxed the isolated tracheal strips pre-incubated with ACh (Fig. 2). EEEJL 1 mg/mL relaxed the isolated organ as much as 59%. The following cumulative concentrations of EEEJL showed significant increase reaching 80, 98, and 116% at concentration of 2, 3, and 4 mg/mL, respectively. The next cumulative concentrations at 5-8 mg/mL did not show significant increases. EEEJL was effective to relax the isolated organ with EC$_{50}$ 1.36 mg/mL.

To investigate the possible mechanism of EEEJL on the inhibition of PDE, the relaxation effect of EEEJL pre-incubated with theophylline was tested. Fig. 3 shows that no significant difference between EEEJL and EEEJL pre-incubated with theophylline ($p>0.05$).

Furthermore, to explore the possible mechanism of EEEJL on COX, the relaxation effect of EEEJL pre-incubated with aspirin was carried out. Fig. 4 shows that no significant difference between EEEJL and EEEJL pre-incubated with aspirin ($p>0.05$).

**DISCUSSION**

In the present research, EEEJL induced relaxation precontracted by AChs on tracheal strips. This effect may be due to the role of its chemical content. The phytochemical screening showed that this extract contains varied kinds of compounds, namely: flavonoids, tannins, glycosides, saponins, and steroids/triterpenoids. These chemical constituents, which are also present in many other plants, relaxed bronchus through their inhibition on ACh and histamine [13]. To assess the extract mechanism as a bronchodilator, specifically its possibility in the inhibition of PDE and COX, EEEJL was pre-incubated with theophylline, a PDE inhibitor, and aspirin, a COX inhibitor, before being contracted. The results showed that theophylline and aspirin did not modify the pattern of the relaxation graphs, it can be seen that the EEEJL graph pre-incubated with theophylline and aspirin did not have significant difference compared to that of without theophylline and aspirin. These results suggested that bronchodilator effects of this extract were related to the inhibition of PDE and this extract avoided the damage of prostaglandin E2 (PGE2). Theophylline acts as a bronchodilator through its mechanism in the inhibition of PDE [14], whereas, aspirin could cause bronchoconstriction by its inhibitory effect on COX, which leading to the decrease of PGE2, a lung protector [15]. These inhibitions

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**Table 1: The phytochemical components of *E. japonica* based on the preliminary ethanol crude leaf extract screening**

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Presence</th>
</tr>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>+: Present, -: Absent</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1:** Effect of acetylcholine ($10^{-3}-3.10^{-4}$ M) on isolated tracheal strips (n=4)

**Fig. 2:** Effect of ethanol extract of *Eriobotrya japonica* leaves (1-8 mg/mL) on isolated tracheal strips pre-incubated with acetylcholine
may be contributed by the presence of flavonoids, tannins, glycosides, saponins, and steroids/triterpenoids in the extract.

CONCLUSION

The results suggest that EEEJL induces relaxation in isolated tracheal strips through the inhibition of PDE and the protective effect on PGE2.

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CONFLICT OF INTEREST

The authors have no conflict of interest in this research.

REFERENCES