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

by Marianne Anne4
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MARIANNE*, URIP HARAHAP, EMIL SALIM, DADANG IRFAN HUSORI, FAHRUMSYAH JALI RAMBE, 
NONI KRISTIANI

Department of Pharmacology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia. 
Email: marianne80@usu.ac.id

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ABSTRACT

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

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

Results: The contractile response evoked by ACh (125 x 10^-7 M) was decreased by EEJL (effective concentration, EC₅₀ = 1.36 mg/mL) and has no significant difference of relaxant effect to that of EEJL pre-incubated with theophylline and aspirin (p>0.05).

Conclusion: The EEJL 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.

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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 unrecorded 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. Ficus eliolobifera (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, nicotine neutralize pain reliever, and to smooth the skin. FE has several other biological activities such as anti-diabetes [4], neuroprotective [5], antioxidant [6], and anti-inflammatory effects [7]. In the previous studies, FE was demonstrated to exert a noticeable effect in ovalbumin-induced asthma [8] and relieve the symptoms of chronic bronchitis rats [9]. The phytochemical studies on FE showed the presence of various terpenes, flavonoids, sesquiterpenes, and tannins [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, Medan, Sidikalang, North Sumatera, and dried at room temperature in the absence of sunlight. The plant was identified by botanists in the Herbarium Medanense, Universitas Sumatera Utara; the specimen number of the plant is 1445/MBDA/2017.

The ethanol extract was prepared as follows. 10 g of the chopped, dried plant was macerated with 300 mL ethanol for 40 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 trachea were removed. Each trachea was cut into 10 rings (each containing 2-3 cartilaginous rings). All the rings were then cut open opposite the tracheal musculature and saturated 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 159 g, NaHCO₃ 2.1 g, MgSO₄ 0.11 g, KCl 0.16 g, K₂HPO₄ 0.35 g, sodium lactate 2.5 g. These compounds were dissolved in 1 mL of distilled water. 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
basis by giving a series of acetylcholine (ACh) concentrations of 10⁻⁶, 3 × 10⁻⁸ M. Tracheal marmots which had been forty-bracted for 45 min (with alternation of Krebs's solution every 15 min for 3 times) were given ACh chloride solution with the concentrations of 10⁻⁴, 3 × 10⁻⁵ 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 percent change in proportion to maximum contraction obtained due to contractile agents. The study was approved by the Animal Research Ethics 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 Eroboteza japonica leaves (EEJL) 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 screening 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 (10⁻⁶ to 3 × 10⁻⁵ M) was applied on isolated tracheal strips to obtain EC₅₀, 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 × 10⁻⁵ M. The next given concentration did not change the percentage of smooth muscle contraction. EC₅₀ of ACh was 1.25 × 10⁻⁵ M.

It can be seen that EEJL (1-8 mg/mL) relaxed the isolated tracheal strips pre-incubated with ACh (Fig. 2). EEJL 1 mg/mL relaxed the isolated organ as much as 59%. The following cumulative concentrations of EEJL showed significant increase reaching 80, 90, 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. EEJL was effective to relax the isolated organ with EC₃₀, 1.36 mg/mL.

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

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

DISCUSSION

In the present research, EEJL induced relaxation precontracted by ACh 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, EEJL 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 EEJL graph pre-incubated with theophylline and aspirin did not have significant difference compared to that of without theophylline and aspirin. These results suggest 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 protectors [15]. These inhibitions

![Fig. 1: Effect of acetylcholine (10⁻⁷ to 3×10⁻⁵ M) on isolated tracheal strips (n=4)](image)

![Fig. 2: Effect of ethanol extract of Eroboteza japonica leaves (1-8 mg/mL) on isolated tracheal strips pre-incubated with acetylcholine)](image)

| Phytochemical compounds | Presence |
|-------------------------|---------|
| Alkaloid                 | -       |
| Flavonoids              | +       |
| Tannins                 | +       |
| Glycosides              | +       |
| Saponins                | +       |
| Steroids/triterpenoids  | +       |
| - Present, - Absent     |         |
CONCLUSION
The results suggest that EEEJL induces relaxation in isolated tracheal strips through the inhibition of PDE and the protective effect on FGEJ.

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CONFLICT OF INTEREST
The authors have no conflict of interest in this research.

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