INHIBITORY EFFECT OF ETHANOLIC EXTRACT OF CURANGA FEL-TERRAE (PUGUN TANO) LEAVES ON ACETYLCHOLINE MUSCARINIC-3 RECEPTORS INDUCED ON ISOLATED GUINEA PIG TRACHEAL

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ABSTRACT

Objective: The study was aimed to investigate the inhibitory effect of ethanolic extract of Curanga fel-terrae leaves (EECFL) against acetylcholine (ACh)-induced contraction of the ACh-muscarinic-3 receptor.

Materials and Methods: The study of the inhibitory effect of the ethanolic extract on the contraction by ACh concentration series (10−8-10−3 M) was conducted in vitro using isolated guinea pig tracheal organ in the Krebs solution.

Results: Early incubation of tracheal organ with EECFL (0.5, 1, 2 and 4 mg/ml) before contracted by the series of ACh concentration produces the decrease of ACh contraction. The concentration series of ACh sigmoid curve rightward shift without decreasing the maximal contraction. The results of double-reciprocal plot of ACh shows the mean value of the 1/y-intercept of each extract was not different with the control.

Conclusion: The EECFL showed competitive antagonist effect on ACh-muscarinic-3 receptors.

Keywords: Curanga fel-terrae, Pugun tano, Acetylcholine-muscarinic-3 receptors, Competitive antagonist, Ethanolic extract.

INTRODUCTION

Curanga fel-terrae (Lour.) Merr. in Indonesia known as pugun tano is one of the plants of the family Linderniaceae. This plant is found in Asia such as China, India, Indonesia, Philippine, Malaysia, and Myanmar. In Indonesia, the plant can be found in Sumatin, Java, Kaimantan, and Mahaku [1]. Rural communities use the leaves and latex to cure abdominal pain, cough, scabies, bruising, inflammation, and asthma. Previous research suggests that these plants contain glycosides [2,3], flavonoids [4], saponins [5], terpenoids, curangin, and bitter substances [6]. C. fel-terrae has been studied have a pharmacological activity as anthelmintic [7], antidiabetic [8,9], and breast cancer [10-12], diuretic effect [13], and cardioprotective effect [14].

Epidemiology study showed almost 20% people in the world suffer from diseases associated with allergy and respiratory tract irritation induced by allergens and particles in the air [16]. The respiratory diseases such as asthma, chronic bronchitis, and emphysema were the fourth reason of death in Indonesia [17].

Important nervous system associated with human lung is the cholinergic nervous system. Cholinergic or parasympathetic nervous system was largely expressed in the proximal airways and decreases toward the periphery. Acetylcholine (Ach) released by the parasympathetic system plays an important role in controlling the airways and mucus release from submucosal glands, as well as goblet cells in the airway epithelium [18]. In human airway muscarinic receptors overexpressed in M3, M4, and M5 types [18,19]. Excessive activation on the cholinergic nerve can cause of respiratory tract disorders [20].

Indonesia has great biodiversity that potential for the discovery of new drugs. Therefore, it is possible to find a new alternative treatment for asthma from natural resources. Although C. fel-terrae has been used traditionally by the community as a medicine such as for asthma, the scientific data associated with pharmacological activity reports still lack.

Based on this reason, researchers are interested in evaluating the ethanolic extract of C. fel-terrae leaves (EECFL) in the respiratory tract primarily on the contraction induced by ACh on ACh receptors-muscarinic-3.

MATERIALS AND METHODS

Materials

Drugs and chemicals used in this study were ACh, dimethyl sulfoxide (Sigma-Aldrich, USA), and ethanol 96% (Merck). Instrument used in this experiment was organ bath PowerLab (ML0146/50, PanLab, ADInstruments, New Zealand).

Preparation of extract

C. fel-terrae (pugun tano) was collected from Pancur Batu, Deli Serdang District, Sumatera Utara and identified by Indonesian Institute of Sciences. The leaves were washed and dried at 30-35°C, then grinded until dried powder was obtained. The dried powder was percolated using ethanol 96% then the obtained percolate was evaporated and freeze-dried.
**RESULTS**

The effect of EECFL on isolated guinea pig tracheal to the contraction induced by agonist compound could be observed through the changes of isolated tracheal smooth muscle contraction % response with the addition of agonist concentration, ACh (10^{-5}-10^{-3} M) on tracheal organ.

Series concentration of ACh-induced the contraction on guinea-pig isolated tracheal smooth muscle. Response percentage of tracheal smooth muscle increased with the addition of ACh concentration. The maximum ACh concentration for tracheal smooth muscle contraction was 10^{-3} M. The next given concentration (3×10^{-3} M) was not change the percentage of smooth muscle contraction (Fig. 1).

While the end of contraction from each treatment showed 100% contraction. From the Sigmoid graph, it could be concluded that the addition of pugun tano ethanolic extract resulted antagonist effect on ACh-induced contraction.

Inhibition of contraction by the extracts was confirmed by comparing the value of pD_{2} (-log EC_{50}) in Fig. 2 and it showed that the addition of EECFL 1, 2, and 4 mg/ml resulted the decrease of pD_{2} value of ACh (p<0.05). While 0.5 mg/ml EECFL concentration showed not different of pD_{2} value compared to the control. This study shows that EECFL at 1, 2, and 4 mg/ml concentration occur inhibitory effect on tracheal smooth muscle contraction-stimulated by ACh. Increasing concentration of the extract further reduces the potential agonists induce tracheal smooth muscle contraction. These results demonstrate the inhibitory effect of the EECFL is dose dependent manner.

The results of this study indicate that the contraction induced by ACh is significantly inhibited by EECFL at the concentrations of 1, 2, and 4 mg/ml. Tissue incubation with varying concentrations of EECFL before contractions gradually by ACh produces right-shifts contraction curve (Fig. 1) without causing a decrease in maximal contraction (R_{max}) (Table 1). Right-shifts contraction curve indicates that it takes a greater amount of ACh to produce the same effect of contraction due to the obstacles made by the extract. This inhibition does not lowering the ability of ACh to achieve maximum contraction. Both of these results indicate that EECFL occurs the ability to inhibit contraction of ACh.

To find out how the characteristic antagonist of the extracts uses DRP analysis of ACh. DRP provides an easy search antagonism mechanism of natural ingredients such as extracts. DRP results (Table 1) shows the regression equation of the average ACh contraction and ACh+EECFL, y-intercept, and correlation coefficient. The 1/y-intercept average value of each extract was 0.013 (Fig. 3, Table 3). The value is not different from the intercept of ACh (control), it can be concluded that the addition of the EECFL resulting competitive antagonist effect on the contraction induced by ACh.

**DISCUSSION**

ACh administrations have increased tracheal smooth muscle contraction through ACh-M_{3} receptor stimulation [25-27]. ACh-M_{3} receptors expressed in various types of cells and this receptor cellular signal is mediated by ACh. These receptors play an important role in smooth muscle contraction.
controlling the physiological response of the central and peripheral nerve activity [28].

ACh-M3 receptors stimulation could activate phospholipase C enzyme, and enhance the formation of inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). Both of these compounds are the second messenger that plays an important role in increasing Ca2+ intracellular concentration ([Ca2+]i). IP3 play a role in the increased [Ca2+]i through IP3 receptor activation on sarcoplasmic reticulum so that stimulating the release of Ca2+ deposits to the cytosol. DAG activate influx of calcium via opening calcium channels in cell membranes. Increasing levels [Ca2+]i modulates calcium bond with calmodulin which will activating myosin light chain kinase (MLCK). MLCK activation results are cross-linking between actin and myosin. The bond formation between myosin and actin and this may lead to contraction of the smooth muscle [26,29].

Stimulation of ACh-M3 receptor on the smooth muscle of the airways can cause airway disorders such as bronchoconstriction and increased mucus production from the submucosal glands [18,30-32]. It is known that human muscarinic receptor is predominantly expressed in smooth muscle cells, epithelial cells, and fibroblasts [28].

Inhibition of ACh-M receptor induced contraction by extract indicated that the chemical compounds contained in the extracts possess work relaxing effect on smooth muscle muscarinic mediates-receptor such as flavonoid. Galangin, flavonoids have an inhibitory effect on bladder smooth muscle contractility [33]. Flavonoids possess antagonist activity on ACh-M3 receptor [34,35]. Steroidal saponins have bronchial asthma inhibitory via inhibition of mediator release such as prostaglandins, histamine, serotonin and bradykinin [36]. Mahapatra and Pradhan also reported that alkaloids, flavonoids, saponins, tannins, polyphenol antioxidant, and coumarins like biologically active compounds that might be responsible for the anti asthmatic activity [37]. Relevance to that result, C. fel-terrae on this study also contain flavonoids, saponins, steroids, and triterpenes. Further research is needed to determine the chemical compounds of which responsible for the inhibitory effect of ACh-muscarinic-3 receptor.

CONCLUSION

The EECFL showed competitive antagonist effect on ACh-muscarinic-3 receptor.

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