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Analysis of the effectiveness of curcuma longa ethanol extract on isolated guinea pig tracheal smooth muscle in acetylcholine induction

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Abstract
Asthma can be defined as a chronic inflammatory disorder that occurs in the respiratory tract. Airway smooth muscle (ASM) contributes to COPD (Chronic obstructive pulmonary disease). This study aims to analyze the effectiveness of curcuma longa ethanol extract on isolated guinea pig tracheal smooth muscle in acetylcholine induction. This research method is experimental to observe the relationship of ethanol curcuma longa extract (EECL) to the effect of smooth muscle relaxation using male guinea pigs weighing between 300-500 grams with the age of 3-4 months. Experimental animals were divided into two groups, each consisting of 4 animals. that curcuma longa ethanol extract has a relaxing effect on tracheal smooth muscle isolated from experimental mice contracted with acetylcholine. The ethanol extract of curcuma longa has the ability not different from theophylline 2 x 10-4 M in reducing the contraction of the isolated tracheal smooth muscle of cavia porsellus induced by acetylcholine, the strength of acetylcholine without incubation contraction compared with acetylcholine with EECL incubation showed statistically different results ($p < 0.05$). The mechanism of the relaxing effect of curcuma longa on isolated guinea pig smooth muscle is mediated through the inhibition of PDE enzymes.

Keywords: Turmeric, trachea, acetylcholine

Introduction
Asthma can be defined as a chronic inflammatory disorder that occurs in the respiratory tract. Airway smooth muscle (ASM) contributes to COPD (Chronic obstructive pulmonary disease). Asthma asthma treatment consists of drugs that work as inhibitors of airway smooth muscle contractions (bronchodilators) and medicines that prevent and reduce inflammation [1]. The turmeric plant with the scientific name Curcuma longa linn is one of the spice plants and is also a medicinal plant [2]; [3]; [4]. The main compound of turmeric is curcumin [5]. Turmeric has several pharmacological properties, antioxidant properties [6], anti-inflammatory [7], and asthma treatment [8], and its safety level is also excellent (Noorafshan and Esfahani, 2013). According to [9], it is noted that curcumin reduces allergic airway inflammation. In another study in 2014, the use of curcumin can prevent the accumulation of inflammatory cells in the airways, structural changes, and remodeling associated with chronic asthma, such as thickening of smooth muscle, exfoliation of the epithelial layer, and mucus secretion in mice with induced asthma [9]. This study aims to analyze the effectiveness of curcuma longa ethanol extract on isolated guinea pig tracheal smooth muscle in acetylcholine induction.

Research Methods
This research method is experimental to observe the relationship of curcuma longa ethanol extract (EECL) to the relaxation effect of the isolated smooth muscle of guinea pig trachea (tracheal ring chain), which is inserted into an organ bath filled with Kreb's physiological fluid by maintaining a temperature of 35-370C and connected to a macLab recorder. This study was conducted using male guinea pigs. The procedures include collection and preparation of simplisia, screening, making ethanol extract of Curcuma longa (EECL), and testing the contraction of guinea pig trachea using organ bath equipment.
The animals used in this study were eight male guinea pigs (Cavia porcellus), weighing between 300-500 grams and 3-4 months old. The experimental animals were divided into two groups, each consisting of 4 animals. Group 1 was given violin as a positive control group, and Group 2 was given extracts with concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml). Simplisia of turmeric rhizomes then weighed as much as 1 kg. Macerated turmeric powder (1000 grams) with distilled 96% ethanol (7.5 liters) for five days, then remacerated for two days with distilled 96% ethanol (2.5 liters). Filtered, the filtrate is evaporated with a rotary vacuum vaporizer until a thick extract is obtained and put into the refrigerator.
Stages of testing in organ research isolated tracheal cartilage rings with organ bath tools containing Kreb's physiological fluid. This in-vitro study used separate fresh tracheal organs of guinea pigs that had previously been fed for 24 hours. Guinea pigs were sacrificed by dislocating the cervix. Laparotomy was performed for exsanguination (target organ removal) through the abdominal aorta and thoracic cervicotomy for tracheal removal. It starts with creating a pneumothorax by incision with scissors and the diaphragm at the bottom of the rib cage. Then, continue the incision from the bottom of the rib cage to the sternum and two halves. Dissect or remove from the organs above the spine and locate the tracheal organ, which lies directly along the spine. Disconnect all connections to the trachea from the esophagus, lungs, and others. And cut the trachea perpendicular to the spine at the level of the diaphragm. Hold the trachea with forceps and use scissors to dissect the trachea from the spine.
Start the dissection at the lower diaphragm adjacent to the spine and move towards the pharynx. Be sure to take extra

precautions not to pull or tug on the tracheal tissue, which may damage the tissue. Immediately place the trachea into the prepared dissection dish containing Kreb's solution. This allows for wires that can be used to cannulate the aorta and tied to the word, providing stability while in the dissection dish. The black background provides contrast, which aids dissection. Care should be taken using a cannulating wire, as the endothelial cell layer can be removed by excessively rubbing the wire against the vessel's lumen. The trachea is retrieved and then carefully cleaned of fat and connective tissue. When the tissue is relaxed, the trachea is cut in a transverse direction to take 8-9 tracheal rings [10], with 4-5 mm of tracheal tissue cut, there are two cartilage rings, and what is accepted is the tracheal cartilage ring proximal to the lung. If our experiment uses eight tracheal cartilage rings, the tracheal section is 32-40 mm. Using tweezers, the two cut ends of the tracheal rings were tied with thread in each opposite direction.

The data obtained in this study is the contraction or relaxation data of tracheal smooth muscle recorded on the recorder. The data was converted into percentage (%) response to agonist-induced response. Furthermore, a relationship curve between agonist concentration and % response was created (computer program: Lab Chart® 7.0.2). The % response value obtained in testing the relaxation effect of curcuma longa ethanol extract on tracheal smooth muscle was statistically analyzed using the t-test. Still, previously, the Kolmogorov-Smirnov normality test was performed [11].

Results and Discussion

The results of phytochemical screening of the extracts obtained showed the presence of flavonoids, saponins, tannins, glycosides, steroids / triterpenoids. The results of phytochemical screening of turmeric ethanol extract can be seen in the table below:

Table 1. Screening results of ethanol extract of Curcuma longa

No.	Screening	Results
1.	Flavonoid	+
2.	Alkaloid	+
3.	Saponin	+
4.	Tanin	+
5.	Glikosida	+
6.	Steroid/triterpenoid	+

Description (+): present
(-): not present

Testing the relaxing effect of EECL on isolated tracheal smooth muscle was carried out by contracting the tracheal smooth muscle with 2 x 10-4 M acetylcholine, followed by administering a concentration series of EECL 1-8 mg/ml. The relaxation effect of the extract was observed through observation of changes in the % relaxation effect of the section on the tracheal smooth muscle. The administration of the EECL concentration series produced a relaxing impact on contractions induced by 2 x 10-4 M acetylcholine. Table 3. shows the soothing effect of curcuma longa ethanol extract on isolated tracheal smooth muscle. Asthma is one of the COPD diseases besides emphysema and chronic bronchitis. Airway smooth muscle (ASM) is indispensable in airway structure and function. Dysfunction in ASM plays a central role in chronic obstructive pulmonary disease (COPD) pathogenesis. COPD is China's third leading cause of death, with more than 0.9 million deaths in 2013 [12]. Acetylcholine is a choline-ester molecule identified as the most commonly known neurotransmitter [13]; [14]. Turmeric extract can reduce the number of eosinophils in lung tissue. Curcumin, the main compound of the turmeric plant, has beneficial effects on arthritis, allergies, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes, and cancer. This is likely due to its ability to modulate the immune system [15].

Various studies have established its anti-inflammatory effects both *In vitro* and *In vivo* by inhibiting iNOS production and capturing free radicals, inhibiting NF-kappa B activation, activating protein 1 (AP 1), and suppressing proinflammatory cytokine production. Studies have also shown that curcumin decreases the level of iNOS induced by IFN-γ in lung tissue and the expression of cytokines such as IL-2, IL-5, and GM-CSF by acting as an activator of histone deacetylases (HDAC) and inhibiting histamine release from mast cells. It has been demonstrated that curcumin can also restore HDAC activity, thereby restoring corticosteroid function. Curcumin, when added to Dermatophagoides farineae (Der-f)-stimulated lymphocyte cell cultures from allergic asthmatics, inhibited Der-f-induced lymphocyte proliferation and production of IL-2, IL-4, IL-5, and GM-CSF thereby proving that curcumin blocks allergen-triggered inflammatory chemical release in white blood cells taken from asthmatic patients. It also suggests that curcumin may potentially affect the control of allergic diseases through inhibition of cytokine production, eosinophil function, and IgE synthesis [16]; [15]. This study shows that turmeric ethanol extract concentrations (1-8 mg/ml) can relax the smooth muscle of guinea pigs that have contracted due to acetylcholine administration. Based on research, the existence of curcumin or curcuminoid compounds in turmeric is an essential agent to relax smooth muscle.

Conclusion

From the study results, it can be concluded that curcuma longa ethanol extract relaxes isolated tracheal smooth muscle from experimental mice contracted with acetylcholine. The ethanol extract of curcuma longa has the ability not different from theophylline 2 x 10-4 M in reducing the contraction of the isolated tracheal smooth muscle of cavia porsellus induced by acetylcholine, the strength of acetylcholine without incubation contraction compared with acetylcholine with EECL incubation showed statistically different results ($p < 0.05$). The mechanism of the relaxing effect of Curcuma longa on isolated guinea pig smooth muscle is mediated through the inhibition of PDE enzymes.

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