

## **RELAXATION EFFECT OF EXTRACT ETHANOL CURCUMA LONGA ON ISOLATED GUINEA PIG TRACHEA**

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### **Abstract**

**Aims:** Asthma is a chronic obstructive pulmonary disease which is a world health problem that is not only infected in developed countries but also in developing countries. According to the National Asthma Education and Prevention Program (NAEPP), asthma can be defined as a chronic inflammatory disorder that occurs in the airways, which involves inflammatory cells. curcumin reduces allergic airway inflammation in mice through inhibition of a specific pathway aimed at investigating the anti-inflammatory effect of curcumin in acute allergic asthma and its underlying mechanisms in mice.

**Study design:** This study is experimental study.

**Methodology:** The experimental animals were divided into 2 groups, each group consisting of 4 animals. Group 1 gave theophylline 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, 7 mg / ml and 8 mg / ml). These animals are acclimatized for 1 (one) week with the aim of homogenizing their food and life with the same conditions so that they are considered eligible for research. **Results:** The ethanol extract of curcuma longa has no different ability from atropine sulfate  $1 \times 10^{-6}$  M in reducing the smooth muscle contraction of isolated guinea pig tracheal induced by acetylcholine, the strength of acetylcholine without incubation contraction compared to acetylcholine with EETH incubation showed statistically different results ( $p < 0.05$ ).

**Conclusion:** The mechanism of the relaxing effect of curcuma longa on isolated mouse smooth muscle is mediated through inhibition of the PDE enzyme.

*Keywords: [Asthma, Curcuma Longa, PDE]*

### **1. INTRODUCTION**

Asthma is a chronic obstructive pulmonary disease a public health problem worldwide not only contracted in developed countries but also in developing countries. According to the National Asthma Education and Prevention Program (NAEPP), asthma can be defined as a chronic inflammatory disorder that occurs in the respiratory tract, which involve inflammatory cells. Respiratory tract inflammation found in asthma patients is believed to be the underlying disorder that is the function of airway obstruction that restricts air flow.

In susceptible individuals, this inflammation causes repeated episodes of wheezing, shortness of breath, a feeling of tightness in the chest, and coughing, especially at night or early morning (Kelly and Sorkness, 2009). According to data from a 2012 Global Initiative for Asthma (GINA) report, it is stated that the estimated number of people with asthma worldwide is three hundred million people, with the number of deaths that continues to increase to 180,000 people per year. WHO data also shows similar data that the prevalence of asthma has continued to increase in the last thirty years, especially in developed countries. Nearly half of all asthma patients have been hospitalized and made visits to the emergency department every year (Rengganis, 2008). A variety of cellular mediators and pathways contribute to the pathogenesis of COPD generating a large number of airway and pulmonary dysfunctions. Smoking is one of the main causes, while exposure to air pollution such as cooking biomass, heating, exhaust gases is also a trigger factor, besides environmental changes, genetic disorders, abnormal lung development, and premature aging also contribute to the development of COPD (Yan et al. al, 2018).

As for the treatment of asthma, airway smooth muscle relaxation produced by the inhibitory effect of phosphodiesterase activity. Phosphodiesterase also works by phosphorylating contractile proteins and reducing  $Ca^{2+}$  levels (Husori, 2011). Basically the treatment of asthma consists of two categories: drugs that work as inhibitors of smooth muscle contraction airways (bronchodilators) and -obat drugs that prevent and reduce inflammation. Bronchodilator drugs such as adrenergic agonists, xanthine derivatives and anti-cholinergics; whereas drugs that prevent and reduce inflammation are glucocorticoids, leukotriene inhibitors and mast cell-stabilizing agent (McFadden, 2001).

Indonesia is a country that has considerable biological wealth that can be developed especially for traditional medicine. Of the many varieties of plants in Indonesia that can be developed into traditional medicine, one of them is the turmeric plant (*Curcuma longa* Linn.). The genus *Curcuma* (family Zingiberaceae) consists of more than 100 species and about 40 to 50 species distributed in the Malesian region, Indo-China, Taiwan, Thailand, Malesia, to the Pacific and northern parts of Australia, widely used as food and traditional medicine. Indonesia is home to many species of *Curcuma*. The various *Curcuma* species that are frequently used are *C. longa* (turmeric), *C. xanthorrhiza*, *C. heyneana*, *C. aeruginosa*, *C. mango*, and *C. zedoaria*. Turmeric is the plant most often used in traditional medicine in Indonesia (Setiadi, 2016). Scientific studies have demonstrated beneficial pharmacological effects of curcumin. Curcumin is a bright yellow spice, derived from the rhizome of *Curcuma longa* Linn. It has been shown that curcumin is a highly pleiotropic molecule that can modulate the intracellular signaling pathways that control cell growth, inflammation, and apoptosis. Curcumin may be a potential candidate for prevention and / or treatment of several diseases because of its antioxidant, anti-inflammatory activity and excellent safety profile (Noorafshan and Esfahani, 2013).

## **2. MATERIAL AND METHODS**

### **2.1 Materials**

Surgical instruments, laboratory glassware, amplifiers (Adinstrument), blenders, drying cabinets, micro-volume pipettes (Socorex, Switzerland), mortars and stamps, analytical balances, rough balances, bath organ volume 40 ml (Panlab), ovens electricity, recorder (Adinstrument), rotary evaporator (Haake D), animal scale, transducer (Adinstrument), vortex, carbogen tube (ordered through an "Aneka Gas" Medan agent). Oxygen regulator, stainless steel wire, clamp pliers. ethanol 96%, distilled water, carbogen gas containing 95% oxygen and 5% carbon dioxide (Various gases, Medan), Krebs's solution (containing sodium chloride, potassium chloride, calcium chloride, magnesium sulfate, sodium bicarbonate,

potassium dihydrophosphate and glucose), dimethyl sulfoxide (DMSO), acetylcholine chloride, and theophylline.

The animals used in this study were 8 male rats (*Cavia porcellus*), body weight between 300-500 grams and 3-4 months of age. The animals were housed under standard laboratory conditions (12 hour light / dark cycle at 21 ° C), with free access to food and water (Albuquerque et al., 2016).

## **2.2 Ethics Commission Approval**

This study has received ethical clearance from the Ethics Commission of health and science commission, Universitas Prima Indonesia, Medan, Indonesia.

## **2.3 Extraction of *Curcuma longa***

Simplicia turmeric rhizome, then weighed as much as 1 kg. It is macerated turmeric powder (1000 grams) with 96% ethanol distilled (7.5 liters) for 5 days, then remacerated for 2 days with distilled 96% ethanol (2.5 liters). It was filtered and the filtrate was evaporated with rotary vacuum evaporator until a thick extract was obtained and put in a refrigerator.

## **2.3 Experimental Design**

The experimental animals were divided into 2 groups, each group consisting of 4 animals. Group 1 gave theophylline 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, 7 mg / ml and 8 mg / ml). These animals are acclimatized for 1 (one) week with the aim of homogenizing their food and life with the same conditions so that they are considered eligible for research. Before being used at the research stage, experimental animals were fasted for 24 hours so that the contraction of the trachea used was not affected by other substances.

## **2.4 Statistical Analysis**

The data obtained in this study were tracheal smooth muscle contraction or relaxation data recorded on the recorder. The data is converted into percentage (%) responses to agonist-induced responses. Next, a curve of the relationship between agonist concentration and % response was made (computer program: LabChart® 7.0.2). The % response value obtained in testing the relaxation effect of the ethanol extract of *curcuma longa* on tracheal smooth muscle was statistically analyzed using the t-test, but previously the Kolmogorov-Smirnov normality test was carried out (Husori et al., 2012).

## **3. RESULTS AND DISCUSSION**

**The results of testing the mechanism of action on the relaxing effect of Ethanol Extract *Curcuma Longa* (EECL) on tracheal smooth muscle through inhibition of muscarinic receptors (acetylcholine).**

Testing EECL relaxing effect on isolated tracheal smooth muscle is done by contracting the tracheal smooth muscle by acetylcholine  $2 \times 10^{-4}$  M, followed by administration of a concentration series EECL 1-8 mg / ml. The relaxing effect of the extract was observed by observing the change in the % effect of the extract relaxation on tracheal plain muscle. Giving series EECL concentration produces a relaxing effect on the contraction induced by acetylcholine  $2 \times 10^{-4}$  M. Table 1 shows the effect of the ethanol extract of *Curcuma longa* relaxation of smooth muscle isolated trachea.

**Table 1. The relaxing effect of % EECL on rat trachea constructed using  $2 \times 10^{-4}$  M acetylcholin.**

No	EECL (mg/ml)	% Trachea Relaxation				Mean $\pm$ SD
		1	2	3	4	
1	1	7,21	2,54	5,33	10,7	8,247 $\pm$ 5,9
2	2	13,51	5,12	10,21	13,87	10,677 $\pm$ 4,1
3	3	21,34	10,23	23,19	28,79	20,887 $\pm$ 3,08
4	4	37,91	19,18	30,91	38,61	31,652 $\pm$ 2,98
5	5	53,12	40,41	48,23	43,71	46,367 $\pm$ 4,67
6	6	71,44	65,31	82,61	67,46	71,705 $\pm$ 4,65
7	7	91,45	94,67	100,00	95,98	95,525 $\pm$ 2,78
8	8	100,00	100,00	100,00	100,00	100,00

**The results of the contraction strength value of the EECL relaxation effect on tracheal smooth muscle in rats through inhibition of muscarinic receptors (acetylcholine) and incubation of theophylline (phosphodiesterase inhibitor)**

Testing the relaxation effect of EECL on tracheal smooth muscle contracted with  $2 \times 10^{-4}$  M acetylcholine was observed by testing that begins with tracheal incubation with a phosphodiesterase inhibitor, theophylline  $10^{-4}$  M, for 20 minutes. The resulting relaxation effect was then compared with the relaxation effect of EECL performed without incubation with theophylline (testing in the previous procedure). This procedure has been a simple test to study the possible role of phosphodiesterase inhibition in the relaxing effect of a substance. The results of testing the relaxation effect of EEDC on tracheal smooth muscle can be seen in Table 2.

**Table 2. % EECL relaxation in rat trachea constructed with  $2 \times 10^{-4}$  M acetylcholine after  $10^{-4}$  M theophylline incubation**

No	(mg/ml)	% relaxation effect*				Mean $\pm$ SD
		1	2	3	4	
1	1	8,82	6,67	10,53	12,12	9,53 $\pm$ 1,45

2	2	20,59	13,33	28,95	21,21	21,02 ± 1,98
3	3	32,35	26,67	47,37	36,36	35,69 ± 4,14
4	4	41,18	33,33	52,63	66,67	48,45 ± 2,90
5	5	58,82	56,67	76,32	81,82	68,41 ± 2,09
6	6	88,24	90,00	84,21	87,88	87,58 ± 1,56
7	7	100,00	100,00	100,00	100,00	100,00
8	8	100,00	100,00	100,00	100,00	100,00

Bronchial asthma, a chronic bronchial inflammatory disorder, is characterized by bronchoconstriction, increased mucus production, and a hyper airway response. Increased inflammatory infiltrate, strong mucus and exudate obstruction and enlargement of bronchial smooth muscle especially in medium sized bronchi cause the resulting symptoms of wheezing, coughing and dyspnea. Bronchodilators and inhaled / systemic corticosteroids are usually used as first-line treatment for symptom management but their use is limited because of long-term side effects. Prophylactic therapy is important in asthma but does not prevent recurrent episodes.

Over the years, there is growing evidence that curcumin, the phytochemical present in *Curcuma longa* (turmeric), has a wide spectrum of therapeutic properties including modulation of inflammation and oxidative stress. Various studies have clearly established anti-inflammatory effects both in vitro and in vivo by inhibiting iNOS production and scavenging free radicals, inhibiting NF-kappaB activation and activating protein 1 (AP 1) and suppressing proinflammatory cytokine production. Studies have also shown that curcumin decreases the levels of iNOS induced by IFN- $\gamma$  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 shown that curcumin can also restore HDAC activity, thereby restoring corticosteroid function.

Kobayashi T et al., Demonstrated that curcumin when added to Dermatophagoidesfarineae (Der-f) stimulated lymphocyte cell cultures of allergic asthmatics inhibits Der-f-induced lymphocyte proliferation and production of IL-2, IL-4, IL-5 and GM-CSF thus proved that curcumin blocks the release of allergen-induced inflammatory chemicals in white blood cells taken from asthma patients. It also suggests that curcumin may have a potential effect on allergic disease control through inhibition of cytokine production, eosinophil function and IgE synthesis.

In this study, it can be seen that the ethanol extract of turmeric in concentration (1-8 mg / ml) has the ability to relax the smooth muscle of rats experiencing contractions due to acetylcholine administration. Based on research, the presence of curcumin or curcuminoid compounds in turmeric is a very important agent for relaxing smooth muscle.

#### **4. CONCLUSION**

The ethanolic extract of *curcuma longa* has a relaxing effect on tracheal smooth muscle isolated from the experimental rat contracted with acetylcholine.

## COMPETING INTERESTS DISCLAIMER:

**AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.**

## REFERENCES

- Albuquerque, A.A.S., M.T.M. Carvalho., P.M. Evora., T.R. de Nadai., A.C. Celotto., P.R.B. Evora. 2016. In vitro reactivity (“organ chamber”) of guinea pig tracheal rings methodology considerations. *Ann Transl. Med.* 4(11): 216.
- American Lung Association (ALA). (2012). Trends in Asthma Morbidity and Mortality. Washington D.C: American Lung Association Epidemiology and Statistics Unit Research and Health Education Division. Hal. 2.
- Amri, M.H., G. Gabella. 1988. Structure of the Guinea Pig Trachea at Rest and in Contraction. *Anat Embryol.* 178(5): 389-397.
- Anas, Y. 2012. Percobaan dengan Alat Organ Terisolasi. Yance Anas Community. <https://yanceanascommunity.blogspot.com/2012/03/percobaan-dengan-alat-organ-terisolasi.html>
- Anto, R.J., G. Kuttan., K.D.V. Babu., K.N. Rajsekharan. 1996. Antiinflammatory activity of natural and synthetic curcuminoids. *Amala Research Bulletin.* 16(1996): 73-77.
- Anto, R.J., G. Kuttan., K.D.V. Babu., K.N. Rajsekharan. 1994. Antiinflammatory activity of natural and synthetic curcuminoids. *Amala Research Bulletin.* 14(1994): 60-65.
- Azis., Tanaka, K., Kadota, S., & Tezuka, Y. (2011). Sesquiterpenes from the rhizomes of *Curcuma heyneana*. *Journal of natural products*, 76(2), 223-229.
- Baluk, P., G. Gabella. 1989. Innervation of the guinea pig trachea: A quantitative morphological study of intrinsic neuron and extrinsic nerves. *Journal of Comparative Neurology.* 285(1).
- Barnes, P.J. (1993). Muscarinic Receptor Subtypes in Airways. *European Respiratory Journal.* 6(1). Hal. 328-331
- Barnes, P.J. (1995). Cyclic Nucleotides and Phosphodiesterases and Airway Function. *European Respiratory Journal.* 8(1). Hal. 457-462.
- Biology dictionary. Smooth Muscle. <https://biologydictionary.net/smooth-muscle/>
- Cahyono, B., Huda, M. D. K., & Limantara, L. (2011). Pengaruh proses pengeringan rimpang temulawak (*Curcuma xanthorrhiza*) terhadap kandungan dan komposisi kurkuminoid. *Reaktor*, 13(3), 165-171.

- Cahyono, I.D. 2009. Neurotransmitter Dalam Fisiologi Saraf Otonom. *Jurnal Anestesiologi Indonesia*. 1(1): 42-55.
- Depkes RI. (1979). Farmakope Indonesia. Edisi III. Jakarta: Departemen Kesehatan RI.
- Depkes RI. (1995). Farmakope Indonesia. Edisi IV. Jakarta: Departemen Kesehatan RI
- Deter, M., C. Siegers., P. Muhl., W. Hansel. 1999. Choleric effect of Curcuminoids on an acute cyclosporine induced cholestasis in rats. *Planta Medica*. 65(7): 610-613.
- Ditjen POM. (1995). *Materia Medika Indonesia*. Jilid VI. Jakarta: Departemen Kesehatan RI. Hal. 300- 306, 321, 325, 333-337.
- Dono, N.D. 2013. Turmeric (*Curcuma longa* Linn.) Supplementation as an Alternative to Antibiotics in Poultry Diets. *Wartazoa*. 23(1): 41-49.
- Fahrum. (2018). Efek Relaksasi Ekstrak Etanol Daun Biwa (*Eryobotrya Japonica* (Thunb.) Lindl.) Terhadap Otot Polos Terisolasi Trakea Tikus dan Pengaruhnya pada Fosfodiesterase.
- Fawcett, D.W. (2002). *Buku Ajar Histologi*. Jakarta: EGC. Hal. 257.
- Hajare, R., Darvhekar, V.M., Shewale, A., dan Patil, V. (2010). Evaluation of Antihistaminic Activity of Piper betel Leaf in Guinea pig. *African Journal of Pharmacy and Pharmacology*. 5(2). Hal. 113-117.