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# Effects of Curcuma Longa Ethanol Extract on Isolated Guinea Pigthile Smooth Muscle in Acetylcholine Induction

**Zheng Yuebin[1], Florenly[2], Liena[3], Fioni[4]**
[1,2,3,4]Departement of Medicine, Faculty of Medicine, Universitas Prima Indonesia
Email: ly@unprimdn.ac.id

***Abstract:***

*Epidemiological studies show that nearly 20% of the world's population suffers from diseases related to allergies and asthma. The main compound of turmeric is curcumin has several pharmacological properties, antioxidant properties, anti-inflammatory, asthma treatment. The study aimed to determine the effects of ethanol extract Curcuma longa on the smooth muscle of the isolated guinea pigtic trachea in acetylcholine induction. This research method was experimental to observe the relationship of Curcuma longa ethanol extract (EECL) to the relaxing effects of isolated smooth muscle trachea marmot (tracheal ring chain) inserted into a bath organ filled with Kreb's physiological fluid by maintaining a temperature of 35-370C and associated with a Matlab recorder. Samples used by male guinea pigs and ethanol extract Curcuma longa (EECL). The results of the study that ethanol extract Curcuma longa has a relaxing effect on the smooth muscles of the trachea isolated from the experimental rats contracted with acetylcholine. Ethanol extract Curcuma longa has the ability not dising from theophylline 2 x 10-4 M in reducing smooth muscle contraction of insulated Cavia porcelain trachea induced by acetylcholine, acetylcholine strength without incubation contraction compared to acetylcholine with EECL incubation showed statistically different results (p <0.05). The mechanism of Curcuma longa relaxation effect on isolated guinea pig smooth muscle is mediated through inhibition of the enzyme PDE.*

***Keywords:***
*turmeric; trachea; acetylcholine*

## I. Introduction

Epidemiological studies show that nearly 20% of the world's population suffers from diseases related to allergies and asthma (WHO, 2003). Asthma is a chronic inflammatory disease of the lungs characterized by reversible airway obstruction, hyperresponsive airways, and airway inflammation (Holgate 2012); (Kudo, Ishigatsubo, and Aoki 2013). Asthma can be defined as a chronic inflammatory disorder that occurs in the respiratory tract. Smooth muscles of the airway (ASM; Airway smooth muscle) contributes to COPD (Chronic obstructive pulmonary disease). ASM dysfunction contributes to modulating airway response to stimuli, remodeling, and fibrosis, as well as affecting lung adherence (Yan et al. 2018); (Lam, Lamanna, and Bourke 2019); (Putrawan and Ngurah Rai 2008). As for the treatment of asthma, relaxation of smooth muscles of the respiratory tract is produced by the inhibition of the effects of phosphodiesterase activity (Gultom 2018). Basically asthma treatment consists of two categories, namely drugs that work as inhibitors of contraction of the smooth muscles of the airways (bronchodilators) and drugs that prevent and reduce inflammation (Tri Yus Priyantoro, Sudjari, and Karyono 2004).

Turmeric plant with scientific name Curcuma longa Linn is one of the spice plants and is also a medicinal plant (Ariani 2017); (Sabale, Modi, and Sabale 2013); (Hartati 2013). The main compound of turmeric is curcumin (Nabofa et al. 2018). Turmeric has several pharmacological

properties, antioxidant properties (Wanninger et al. 2015), anti-inflammatory (Park et al. 2020), asthma treatment (Manarin et al. 2019), the level of security is also very good (Noorafshan dan Esfahani, 2013). According to (Ningrum, Suprihati, and Santosa 2016), noting that curcumin reduces allergic airway inflammation. In other studies, curcumin use may prevent the accumulation of inflammatory cells in the airways, structural changes, and remodeling associated with chronic asthma, such as thickening of smooth muscles, exfoliation of epithelial layers, and mucus secretion in mice with asthma induced asthma (Ningrum, Suprihati, and Santosa 2016). The study aimed to find out the effect of ethanol extract Curcuma longa on the smooth muscle of the insulated guinea pigtic trachea in the induction of acetylcholine.

## II. Review of Literature

Asthma is one of the COPD diseases in addition to emphysema and chronic bronchitis. The smooth muscle of the airway (ASM) [Airway smooth muscle] plays an indispensable role in the structure and function of the airway. Dysfunction in ASM plays a central role in the pathogenesis of chronic obstructive pulmonary disease (COPD=COPD). COPD is the third leading cause of death in China with more than 0.9 million deaths in 2013 (Yan et al. 2018). Acetylcholine is the most commonly known ester-choline molecule identified as the most commonly known neurotransmitter (nerve stimulus delivery chemical) (Gosens and Gross 2018); (Kistemaker and Gosens 2015). Turmeric extract can decrease the amount 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 (Jagetia and Aggarwal 2007).

## III. Research Methods

This research method was experimental to observe the relationship of curcuma longa ethanol extract (EECL) to the relaxing effects of isolated smooth muscle trachea marmot (tracheal ring chain) inserted into a bath organ filled with Kreb's physiological fluid by maintaining a temperature of 35-370C and associated with a macLab rekorder. The study was conducted using male guinea pigs. Procedures include collection and manufacture of simplisia, screening, making ethanol extract curcuma longa (EECL), and testing of guinea pigtic contractions using bath organs.

### 3.1 Tool

The tools used in this study are surgical tools, laboratory glass tools, amplifiers (Adinstruments), blenders, dryer cabinets, micro volume pipettes (Socorex, Switzerland), mortar and stamfer, analytical balance sheet, rough balance, organ bath volume of 40 Ml (Panlab), electric oven, recorder (Adinstrument), rotary evaporator (Haake D), animal scales, transducer (Adinstrument), vortex, carbogen tube (ordered through medan 'Aneka Gas" agent). Oxygen regulator, stailess steel wire, brace pliers.

### 3.2 Material

The sample used in the study was curcuma longa (purchased from one of The Petisah Medan's traditional markets). The chemicals used are 96% ethanol, distilled water, carbogen gas contains 95% oxygen and 5% carbon dioxide (Various gases, Fields), Kreb's solution (containing sodium chloride potassium chloride, calcium chloride, magnesium sulfate, sodium bicarbonate, potassium dihydrophosphate and glucose), dimethyl sulfoxide (DMSO), acetylcholine chloride, and theophylline.

### 3.3 Animal Experiment

The animals used in the study were 8 male marmotes (Cavia porcellus), weighing between 300-500grams by the age of 3-4 months. The animals were divided into 2 groups, each group consisting of 4 tails. Group 1 administered theoflin as a positive control group and group 2 gave extracts with concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml).

### 3.4 Making Ethanol Extract Curcuma Longa

Simplisia rhizome turmeric, then weighed as much as 1 kg. Dimaserasi turmeric powder (1000 grams) with ethanol 96% distillation (7.5 liters) for 5 days, then in remassion for 2 days with ethanol 96% distillation results (2.5 liters). Filtered and filtratenya evaporated with a rotary vacuum vaporist until a thick extract is obtained and put in the refrigerator.

### 3.5 Phytochemical Screening of Curcuma Longa Ethanol Extract (EECL)

Screening of phytochemical extracts is carried out in the Phytochemical Laboratory of the Faculty of Pharmacy, University of North Sumatra with examination of alkaloid group compounds (Depkes RI, 1995), flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids (Buckley 1966).

### 3.6 Krebs Solution Making

The physiological solution used is Kreb's solution. To make 1liter of Kreb's weighed solution (Hajare et al. 2011).

**Table 1.** Content in the Manufacture Of Kreb's Solution

| | |
|---|---|
| NaCl | 5,9 gram |
| KCl | 0,25 gram |
| $CaCl_2$ | 0,28 gram |
| $MgSO_4$ | 0,11 gram |
| $NaHCO_3$ | 2,1 gram |
| $KH_2PO_4$ | 0,16 gram |
| Glucose | 2,0 gram |

NaHCO3 (2.1 grams) and glucose (2.0 grams) are dissolved with some distilled water until dissolved. Other materials (NaCl, KCl, MgSO, KH2PO4, CaCl2) are dissolved separately with distilled water until dissolved, then added a solution of sodium bicarbonate and glucose. After all the ingredients are mixed, the solution is coerated with carbogen (O2 95%, CO2 5%) so that no deposition of calcium salts is characterized by turbidity. Next the solution is set at a temperature of 370C and Ph 7.4.

### 3.7 Manufacture of Ethanol Extract Solution Curcuma Longa

A total of 1600 mg of ethanol extract curcuma longa (EECL) is dissolved with 1 ml of DMSO (Dimethyl sulfoxide), then suffocate with a solution of krebs up to 5 ml. Obtained extract concentration of 320 mg / ml. DMSO is an inert, non-toxic solvent, and can dissolve almost any compound and is a semipolar solvent, but can still mix with krebs media (Velasco et al. 2003); (Bertoluzza et al. 1979). The limit for the use of the number of DMSO solvents added to the bath organ (40ml) is 200 µl or 1% v/v, and the dilution of EECL uses the formula: V1M1=V2M2, where V1 (Volume-1) M1 (Concentration-1) and V2 (Volume-2) M2 (Concentration-2) (Husori, 2011).

- Picked 125 µl EECL into organ bath volume 40 Ml so that the concentration of extract in organ bath obtained 1 mg / Ml.

- Picked 125 µl EECL into the bath organ volume of 40 Ml so that the concentration of extract in the bath organ is obtained 2 mg / Ml.
- Picked 125 µl EECL into organ bath volume 40 Ml so that the concentration of extract in organ bath obtained 3 mg / Ml.
- Picked 125 µl EECL into the bath organ volume of 40 Ml so that the concentration of extract in the bath organ is obtained 4 mg / Ml.
- Picked 125 µl EECL into the bath organ volume of 40 Ml so that the concentration of extract in the bath organ is obtained 5 mg / Ml.
- Picked 125 µl EECL into the bath organ volume of 40 Ml so that the concentration of extract in the bath organ is obtained 6 mg / Ml.

**3.8 Manufacture of Acetylcholine Solution**

The study used acetylcholine as a musarinic receptor agonist. These compounds can cause isolated smooth muscle contractions in the trachea. The parent solution is made by dissolving acetylcholine into aqua destilata so that a concentration of 2x10-1 M is obtained. Manufacturing of acetylcholine chloride concentration series:

- Acetylcholine chloride $2x10^{-2}$ M
  Picked 500 Ml solution acetylcholine chloride concentration 2x10-1 M, put in a test tube, add 4500 Ml aquadest. Vortex for 1 minute.
- Acetylcholine chloride $2x10^{-3}$ M
  Picked 500 Ml solution acetylcholine chloride concentration 2x10-2 M, put in a test tube, add 4500 Ml aquadest. Vortex for 1 minute.
- Acetylcholine chloride $2x10^{-4}$ M
  Picked 500 Ml solution acetylcholine chloride concentration 2x10-3 M, put in a test tube, add 4500 Ml aquadest. Vortex for 1 minute.
- Acetylcholine chloride $2x10^{-5}$ M
- Picked 500 Ml solution acetylcholine chloride concentration 2x10-4 M, put in a test tube, add 4500 Ml aquadest. Vortex for 1 minute.
- Acetylcholine chloride $2x10^{-6}$ M
  Picked 500 Ml solution acetylcholine chloride concentration 2x10-5 M, put in a test tube, add 4500 Ml aquadest. Vortex for 1 minute.

**3.9 Teofiilin Solution Setup**

Similarly to making a concentration of acetylcholine-chloride, the standard solution of theophylline concentration 2x10-1, dissolved into consecutive, 2x10-2, 2x10-3, 2x10-4, 2x10-5, 2x10-6.

**3.10 Testing Stages**

The testing stage in the study of isolated organs of tracheal cartilage rings with bath organ tools containing Kreb's physiological fluids. In this in-vitro study, fresh tracheal organs were used separate guinea pigs that had previously been satisfied for 24 hours. Guinea pigs are sacrificed by dislocation of the spinal cord (cervix). Laparatomy is performed for exsanguination (retrieval of target organs) through the abdominal aorta and thoracic cervicotomy for the tracheal retrieval process. It starts with the manufacture of pneumothorax by making an incision with scissors along with 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 parts. Dissect or set aside from the organs that are above the spine and find the tracheal organ, which is located directly along the spine. Disconnect all connections to the trachea; from the esophagus,

lungs, etc. And cut the trachea perpendicular to the spine as high as the diaphragm. Hold the trachea with forceps and use scissors to dissect the trachea from the spine.

Begin dissection in the lower diaphragm area adjacent to the spine and move toward the pharing. Be sure to take extra precautions not to attract or pull the tracheal tissue, which can damage the tissue. Immediately place the trachea into a prepared dissection plate containing Kreb's solution. This allows for the use of wires that can be used to simulate the aorta and be fastened to the disc, providing stability while on the dissection disc. The black background provides contrast that helps with surgery. Treatment should be taken using cannulating wire because the endothelial cell layer can be removed by excessive rubbing of the wire against the lumen of the vessel. The trachea is taken and then carefully cleansed of fat and connective tissue. When the tissue is relaxed, cut the trachea in a transverse direction to take 8-9 trachea rings (Hock 2015), With the cutting of tracheal tissue along 4-5 mm there are 2 rings of cartilage, and taken is a ring of proximal tracheal cartilage to the lungs. And when our experiment uses 8 rings of tracheal cartilage, then the tracheal cutting is 32-40 mm. Using tweezers, both ends of the tracheal ring pieces are tied with thread in each opposite direction.

### 3.11 Preparation of Ligature or Wire Assembly

To measure the smooth muscle preparation response, start setting ligatures or wire assemblies (stainless steel clips) attached to a fixed stand at the bottom of an organ soak containing Krebs solution, while others are attached to strength transducers to measure the mechanical "isometric" response (i.e., contraction or relaxation) of smooth muscle. The lower thread of the trachea is tied to the tissue retaining rod and the top thread of the trachea is connected to the transducer. It is then inserted into a bath organ filled with 40 Ml of Kreb's solution, with a solution temperature of 370C and is co-matched with carbogen continuously. The isolated tissue is isolated for 45 minutes until it is in stable condition (Hock 2015).

### 3.12 Placement of Tracheal Network in Bath

Note that in this system, the bath tissue itself does not move. At this time, fill the tissue bath with a warmed and aerated PSS (Physiological Salt solution) and let the solution reach temperature. With silk seams, tie one of the hooks on the tissue preparation to the peg on the stainless steel rod. Place this end into the bath organ. Connect the rod to the ring mount and place the other end on a staining plate filled with PSS to keep the network immersed in the buffer. Place the stems and tissues in the tissue bathroom and make sure the wipes are fully immersed in the PSS and the stems are safe. Tie another stitch to a style transducer and be sure to relax the seams between the tissues and the style transducer.

### 3.13 Preparation of Kreb's Liquid Making

Ideally, cut tissue from the animal immediately before use and insert it directly into the PSS (Kreb's solution) to keep the tissue durable. Some networks can be stored in a pss one night at 4°C, but proper control must be exercised to validate network functions after long storage.

### 3.14 Kreb's Fluid Filling into the Bath Organ

The bath organ is filled with 10 mL of Krebs solution (the same composition used for the aorta) is maintained at 37 °C and inflated with a cariogenic mixture (95% O2 and 5% CO2). One of the handles is anchored to fixed support, and the other is connected to a transducer (Grass force-displacement transducer FT03, Grass Instrument CO, Quincy, USA) for isometric force measurements. The transducer is then coupled to an 8-channel recorder (Gould, Cleveland, USA), thus allowing simultaneous recording of isometric tension.

### 3.15 Set Passive Voltage

Initial experiments to determine the optimal strain voltage that reaches this length should be performed for each type of tissue to be examined. Thracea marmot has an optimal passive strain voltage of 4 g. Use micrometers/racks and pinions to increase tension by up to 2 g and wait for the tissue to reach a plateau. Once the plateau is reached, raise the tension by another 2grams and wait until the network reaches the plateau. In the tracheal ring, once the initial tension of 2g has been established and a plateau is reached, the tissue will then relax to 1.6g. The second 2 g application will take the network to 3.6 g, from which the network will relax again and the tension will decrease. So, after adding 4 g of total voltage, the software must show approximately 3.2 g of voltage. Repeat this step for the second tracheal ring and bathroom tissue.

### 3.16 Turn on the Heat of Water Gas

The temperature is usually kept at 37 °C, but this can vary depending on the body temperature of the animal from which the tissue is from, or other experimental needs. Allowing a sufficient period of equilibrium at 37°C allows muscles to produce ATP, reactivate atp-dependent systems and restore the potential of resting systems (Hock 2015). Heat the tissue handling system to 37 °C by turning on the recirculating hot water handler. Critical Steps: Each component of the system is water jacketed, ensuring that they are connected sequentially to each other. The direction of the flow is very important, ensuring that water flows to each component at the lowest spiked joint and exits at the highest spiked joints (Lam, Lamanna, and Bourke 2019).

### 3.17 Turn on Data and Software Acquisition

Turn on the data acquisition system. Power on a force transducer at least 15 minutes before the experiment to balance the temperature. Most force transducers use strain gauges that are sensitive to temperature variations and show thermal deviations initially after power is applied. Launch data acquisition software and ensure connection with the data acquisition system. Please follow the factory instructions to enable data recording. Make sure the power transducer is calibrated before the network is placed in a network container and before data recording begins; follow the manufacturer's instructions for calibration.

### 3.18 Gas Cylinder Preparation

Several solutions are used, which differ in the composition and concentration of salts and buffer systems used. Solutions containing bicarbonate require CO2 gas to regulate pH. Make sure the oxygen tube contains carbogens. Install the oxygen tube regulator. Connect the tissue bath system to a medical-grade gas cylinder of 95% O2/5% CO2. Check for gas leaks and then put pressure on the system.

### 3.19 Effective Concentration (EC50) Acetylcholine Testing on Isolated Smooth Muscle Trachea Guinea Pigs (Cavia porcellus)

Testing of muskarinic agonists was conducted to measure the limits of the maxitive. ang can be indicated against the contraction of the guinea pigs, to get the price of EC50. Contraction measurements are carried out in stages with the provision of a series of acetylcholine concentrations ing from 2x10-5 to x10-1 M. Thracea guinea pigs that have been crucified for 45 minutes (with the change of Kreb's solution every 15 minutes three times) are given a solution of acetylcholine chloride with concentrations in bath organs 2x10-5 M to 2x10-1 M (smooth muscle of hammeren trachea shows maximum contraction response). The response of smooth muscle contraction of guinea pigs that occur will be recorded on the recordder.

### 3.20 Testing the Relaxing Effect of Curcuma Longa Ethanol Extract on the Isolated Smooth Muscle of the Marmot Trachea (Cavia porcellus)

Three relaxation mechanisms: (I) guanosine 3,5-cyclic monophosphate (cGMP)/NO-dependent; (II) depending on 80denosine 3, 5-cyclic monophosphate (cAMP)/PGI2; and (III) depending on hyperpolarization (Hock 2015). Guinea pigs that have been crucified for 45 minutes (with the change of Kreb's solution every 15 minutes three times) are contracted with the administration of acetylcholine-clorida starting consecutively from the lowest dose $2x10^{-5}$M, $2x10^{-4}$M, until ec-50 is reached in the bath organ (until $2x10^{-1}$M). The response that occurs will be recorded on the record. After obtaining a stable maximum contraction condition (EC-50), the fluid in the bath organ is replaced every 15 minutes to 3 x replacement, then given in a graded extract of ethanol curcuma longa (EECL) as much as 125 µL to 1000 µL so that the concentration in the bath organ 1 mg / mL, and tested with the lowest dose of 1 mg/ mL, 2 mg / mL, 3 mg/mL until a perfect relaxation level is reached (up to 6 mg/mL). The response that occurs will be recorded on the recordder.

### 3.21 Testing of the Theophylline Relaxing Effect on the Isolated Smooth Muscle of the Marmot Trachea (Cavia porcellus)

Guinea pigs that have been crucified for 45 minutes (with the change of Kreb's solution every 15 minutes three times) are contracted with the provision of acetylcholine-clorida until EC-50 is reached in the bath organ. The contraction with acetylcholine-clorida starts again from the administration of acetylcholine-clorida liquid the lowest dose of 2x10-5.M, and so on until it reaches EC-50, the response will be recorded on the recorder. After obtaining a stable maximum contraction condition (EC-50) then given in a theophylline level starting from successive concentrations $2x10^{-5}$M, $2x10^{-4}$M, until achieving a perfect concentration of relaxation (up to 2x10-1). The response that occurs will be recorded on the recordder.

### 3.22 Testing of the Mechanism of the Relaxing Effect of Curcuma Longa Ethanol Extract on the Isolated Smooth Muscle of the Trachea through Inhibition of the Enzyme Phosphodiesterase by Theophylline

Guinea pigs that have been crucified for 45 minutes (with the change of Kreb's solution every 15 minutes three times). Inc incredated for 20 minutes with the theophylline until the isolated trachea reaches perfect relaxation, do it again for 45 minutes (with a change of Kreb's solution every 15 minutes three times). Then do the induction of acetylcholine-chloride until it reaches the IC-50 concentration, do again the equilibrium for 45 minutes (with the change of Kreb's solution every 15 minutes three times). Then do the testing with EECL starting from the concentration of 1 mg / mL until achieving a perfect relaxation concentration (up to 8 mg / mL). The response that occurs will be recorded on the recordder.

### 3.23 Comparison Curve

After the EC-50 test and perfect relaxation as many as 4 repetitions. Followed by observations of the EECL concentration relationship curve and % relaxation response. Then compared to the % relaxation response due to the administration of EECL that is not initially incestinated with theophylline.

### 3.24 Data Analysis

The data obtained in this study is data on contraction or relaxation of the smooth muscle of the trachea recorded on the recorder. The data is converted into a percentage (%) of responses to agonist-induced responses. Next, a curve of relationships is made between the

concentration of agonists to % of responses (computer program: LabChart® 7.0.2). The % value of the response obtained in testing the relaxing effect of curcuma longa ethanol extract on the smooth muscle of the trachea was statistically analyzed using the t-test, but previously performed the Kolmogorov-Smirnov normality test first (Husori 2012).

## IV. Discussion

### 4.1 Phytochemical Screening of Ethanol Extract Curcuma Longa (EECL)

The results of phytochemical screening extract 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 2.** Screening Results of Ethanol Extract Curcuma Longa

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

Information (+): exist
(-): none

### 4.2 Results of Testing Mechanisms of Action on the Relaxing Effects of Curcuma Longa Ethanol Extract (EECL) on the Smooth Muscle of the Trachea through Inhibition Of Musarinic Receptors (Acetylcholine)

Testing of the relaxing effect of EECL on isolated tracheal smooth muscle is done by contracting the smooth muscle of the trachea with acetylcholine 2 x $10^{-4}$ M, continued with the administration of series concentration of EECL 1-8 mg / ml. The relaxation effect of the extract was observed through observation of the %change in the extract's relaxation effect on the plain otos of the trachea. Serial administration of EECL concentrations produces a relaxing effect on acetylcholine-induced contractions 2 x $10^{-4}$ M. Table 3. Demonstrates the relaxing effect of curcuma longa ethanol extract on isolated tracheal smooth muscle.

**Table 3.** Relaxation Effect % Extract of Ethanol Curcuma Longa (EECL), in Contracted Guinea Pighea Using 2x10-4 M Acetylchoin.

| No | EECL (mg/ml) | % Relaxation of the Thrace Marmut | | | | Average |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | |
| 1 | 1 | 7,21 | 2,54 | 5,33 | 10,7 | 8,247 |
| 2 | 2 | 13,51 | 5,12 | 10,21 | 13,87 | 10,677 |
| 3 | 3 | 21,34 | 10,23 | 23,19 | 28,79 | 20,887 |
| 4 | 4 | 37,91 | 19,18 | 30,91 | 38,61 | 31,652 |
| 5 | 5 | 53,12 | 40,41 | 48,23 | 43,71 | 46,367 |
| 6 | 6 | 71,44 | 65,31 | 82,61 | 67,46 | 71,705 |
| 7 | 7 | 91,45 | 94,67 | 100,00 | 95,98 | 95,525 |
| 8 | 8 | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 |

**4.3 Results in the Contraction Strength Value of the EECL Relaxation Effect on Smooth Muscle of the Guinea Pigtics through Inhibition of Musarinic Receptors (Acetylcholine) and Theophylline Incubation (Phosphodiesterase Inhibitors)**

Testing of the mechanism of EECL relaxation effect on the smooth muscle of the trachea contracted with acetylcholine 2 x 10-4 M was observed with testing beginning with the incubation of the trachea with a phosphodiesterase inhibitor i.e. theophylline 10-4 M, for 20 minutes. The resulting relaxation effect was then compared to the EECL relaxation effect performed without incubation with theophylline (testing on previous procedures). This procedure has become a simple test to study the possible role of phosphodiesterase inhibition in the relaxing effects of a substance. Results from testing of the mechanism of EECL relaxation effect on the smooth muscle of the trachea can be seen in Table 4.

**Table 4.** Relaxation Effect % Ethanol Extract Curcuma Longa (EECL), in Contracted Guinea Pigs Using 2x10-4 M Acetylchoin and Incubation Theophylline (Phosphodiesterase Inhibitor)

| No | % Relaxing effect * | | | | Average |
|---|---|---|---|---|---|
| | (mg/ml) | 1 | 2 | 3 | 4 | |
| 1 | 1 | 8,82 | 6,67 | 10,53 | 12,12 | 9,53 |
| 2 | 2 | 20,59 | 13,33 | 28,95 | 21,21 | 21,02 |
| 3 | 3 | 32,35 | 26,67 | 47,37 | 36,36 | 35,69 |
| 4 | 4 | 41,18 | 33,33 | 52,63 | 66,67 | 48,45 |
| 5 | 5 | 58,82 | 56,67 | 76,32 | 81,82 | 68,41 |
| 6 | 6 | 88,24 | 90,00 | 84,21 | 87,88 | 87,58 |
| 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 infiltration, strong exudate and mucus blockages and enlargement of the bronchial smooth muscles 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 treatments for symptom management but their use is limited due to long-term side effects. Prophylactic therapy is important in asthma but does not prevent recurrent episodes.

Over the years, more and more evidence has found that curcumin, a phytochemical present in Curcuma longa (Turmeric), has a broad 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 catching free radicals, inhibiting NF-kappaB activation and activating protein 1 (AP 1) and suppressing the production of proinflammatory cytokines. Studies have also shown that curcumin lowers IFN-γ-induced iNOS levels in lung tissue and cytokine expressions 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 proven that curcumin can also restore HDAC activity, thereby restoring corticosteroid function. Curcumin when added to the stimulated cell culture of Dermatophagoides farineae (Der-f) from allergic asthmatics inhibits the proliferation of Der-f-induced lymphocytes and the production of IL-2, IL-4, IL-5 and GM-CSF thereby proving 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

potential effects on allergy disease control through inhibition of cytokine production, eosinophil function and IgE synthesis (Khairinal 2012); (Jagetia and Aggarwal 2007). In this study it can be seen that turmeric ethanol extract concentration (1-8 mg / ml) has the ability to relax the smooth muscle of guinea pigs that contract due to the administration of acetilcholine. Based on research the presence of curcumin or curcuminoid compounds in turmeric becomes a very important agent to relax smooth muscle.

## V. Conclusion

From the research has, it can be concluded that ethanol extract Curcuma longa has a relaxing effect on the smooth muscle of the trachea isolated from the experimental rats contracted with acetylcholine. Ethanol extract Curcuma longa has the ability not dising from theophylline 2 x 10-4 M in reducing smooth muscle contraction of insulated Cavia porcelain trachea induced by acetylcholine, acetylcholine strength without incubation contraction compared to acetylcholine with EECL incubation showed statistically different results ($p < 0.05$). The mechanism of Curcuma longa relaxation effect on isolated guinea pig smooth muscle is mediated through inhibition of the enzyme PDE.

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