Relaxation Effect of Ethanol Extract Black (EEB) on Plain Muscle Contraction from Insulated Guinea Pig's Trakea by Acethyloline

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Abstract: Indonesia, asthma is a chronic disease that occurs in respiratory tract that make difficulties breathing. This is reflected in the Household Health Survey Study data (SKRT) in various provinces in Indonesia. This study aims to determination of mechanism of relaxation on contraction smooth muscle of guinea pig's trachea.. This research use in vitro model and use organ bath. The parameter measured in this research was contraction and relaxtion of isolated animal trachea. From this reaserch informed that EEBC (0.5-4 mg/ml) concentration effected the trachea smooth mucle and make it contract that induced by acetylcholine 1.43 x 10-4 M ($r=0.982$; $p<0.05$). The concentration of 4mg/ml EEBC, statistically there is no different with atropine sulfate 1 x 10-6 M in decrease the contraction of guinea pig's trachea smooth muscle induced by acetylcholine 1.43 x 10-4 M ($p>0.05$). EEBC has been known have relaxing effect on smooth muscle especially trachea and has the potency to reduce the contraction of trachea contracted with acetylcholine. The action EEBC on isolated trachea was mediated by inhibition of the PDE enzyme.

1 INTRODUCTION

Asthma is a disorder of chronic airway inflammation that causes narrowing of the airways (bronchial hyperactivity), causing recurrent episodic symptoms like wheezing, shortness of breath, chest feeling heavy, and coughing, especially at night or early morning. Asthma is also a serious world health problem that affects all age groups, ranging from children to adults who have many adverse effects both on themselves, family, and society. Based on the results of the Basic Health Research (Riskesdas) in Indonesia in 2013, the asthma prevalence was 4.5% with the highest incidence in women was 4.6% (Runtuwene et al, 2016). At present asthma still shows a high prevalence. Based on data from the World Health Organization (WHO), the number of asthma patients in the world is estimated to reach 300 million people, and it predicted that this number would continue to increase to 400 million sufferers by 2025. This number could be even greater considering asthma is an underdiagnosed disease.

Asthma is a chronic inflammation of the airways with various cell and cellular elements that play a role. Inflammation Constant is associated with hyper-responsive airways which results in repeated episodes of wheezing, chest tightness, shortness of breath and coughing, especially at night or early days. Asthma symptoms vary, multifactorial and in a manner potential associated with bronchial inflammation. Proof that inflammation is a component of asthma Eosinophil, neutrophil, cell degranulation found mast, thickening of the sub-basement membrane, loss of integrity epithelial cells, bronchial lumen obstruction by mucus and Goblet cell hyperplasia at the autopsy performed on asthma patient. Synthetic drugs currently used in asthma pharmacotherapy cannot act at all stages and targets of asthma, but the herbal approach has regained its popularity in traditional treatment systems (proven to provide symptomatic help and help in inhibiting disease progression). Many plants have been documented beneficial for the treatment of various respiratory disorders including asthma. Herbs have shown interesting results in various specific biological activity targets such as bronchodilation, nephroprotective, cardioprotective, anti-inflammatory, anticancer, immunomodulatory and inhibiting mediators such as leukotriene, lipoxygenase, cyclooxygenase, platelet activator, phosphodiesterase and anti-spasmodic cytokines in the treatment of respiratory disorders such as asthma. Ongoing research around the world also provides

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valuable clues about the exact mechanism of action of alternative herbs (Ravindra and Avinash, 2011; Ismail et al, 2020). Asthma can be controlled with drugs that prevent chronic symptoms and relievers for symptoms of asthma that spread. Education and understanding are the keys to controlling asthma effectively, if an asthma attack is not managed properly it can be fatal. Asthma can actually be prevented and treated early by always implementing healthy living behaviors, consuming nutritious food and drinks, exercising and getting enough rest and avoiding things that can potentially cause asthma. For those who have already suffered from Asthma, this disease can be cured through modern medicine or using natural herbal medicine.

Black Intersection is known to have many benefits and pharmacological activities, including bronchodilators, anti-cancer, antidiabetic, antihyperlipidemic, and neutralizing free radicals. Black Intersection is easily found in many tropical regions including Indonesia, Malaysia, and Thailand traditionally used as herbal medicines which are consumed for managing body health (Curcuma aeruginosa Roxb).

Based on the high incidence of asthma that occurs in all age groups and the anti-oxidant and relaxation effects of ethanol extract Black Curcuma (EEBC) are very good, researchers are interested in conducting the benefits of EEBC on tracheal smooth muscle contraction in male guinea pigs induced by acetylcholine in vitro and their role in muscarinic receptors, and their effects in inhibiting the enzyme phosphodiesterase. From several literature studies, research with EEBC is still rarely found.

2 MATERIALS AND METHOD

This research conducted experimentally including the collection of plant material, manufacture of simplicia, characteristics of simplicia, phytochemical screening of simplicia and extraction of the preparation of the test material and the stages of testing the EETH relaxation effect on the contraction of trachea animal induced by an organ organ. In this study there are two variables, namely the independent variable and the dependent variable; independent variables are ethanol extract black methamphetamine (EEBC) with a concentration of 0.5; 1; 1,5; 2; 2.5; 3; 3.5; and 4 mg / ml, while the dependent variable is relaxation of the isolated trachea contracted by acetylcholine (Figure 1). *Sample Preparation and Extraction*: The leaves of *Curcuma aereginosa* (Figure 2) collected from old deli market, Dairi Regency, North Sumatra Province, Indonesia. Simplisia weighed 500 grams put into a container. The first maceration of simplicia was soaked with ethanol as much as 5 L for 6 hours while stirring occasionally, then allowed to stand for 18 hours. The filtrate was separated from the residue. Then the residue was dried in an oven at 500C. After the dry, residue was macerated again with 5 L. ethanol solvent soaked for 6 hours while stirring occasionally, then let stand for 18 hours. The filtrate was separated from the residue and the residue was dried, then maserat evaporated with a rotary evaporator so that a thick extract was obtained.

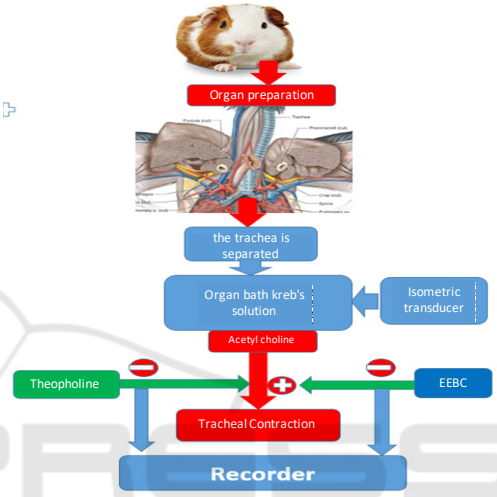

Figure 1. Research conceptual framework

Figure 2. Black Intersection tree (Borobudur Herbal medicine industry).

The animals used in this study were male guinea pigs (Cavia porcellus), weighing between 300-500 grams with ages 3-4 months. This animal was acclimatized for one week with the aim to homogenize its food and life so that it is deemed eligible for research. Before being used in research, animal experiments were fasted for 24 hours so that the tracheal contraction used was not influenced by other substances. The research procedure used first was submitted to the Ethical clearance for research to obtain ethical approval that the procedures performed meet the ethical requirements in experimental animals.

The guinea pig trachea which has been normalize for 45 minutes was contracted by giving acetylcholine solution so as to obtain the maximum submaxative concentration in the organ bath. After obtaining the maximum stable contraction conditions, the graded concentration of EEBC was performed. The response that occurs will be recorded on the order. Testing the mechanism of action through inhibition of muscarinic receptors is done by comparing the contraction pattern of acetylcholine that has been incubated EEBC with the contraction pattern of acetylcholine incubated atropine sulfate and the pattern of acetylcholine contraction without incubation.

The thracea that has been balanced for 45 minutes is incubated for 20 minutes with EEBC in the organ immersion. Then the trachea is contracted by giving acetylcholine chloride solution. Responses to contractions that occur will be recorded. The acetylcholine chloride solution is given until the maximum submaxative concentration is reached. After obtaining a stable maximum contraction condition, EEBC is given with multilevel concentration. The response that occurs will be recorded on the order. The correlation curve of EEBC concentration and % relaxation response compared to % relaxation response due to the administration of EEBC without incubation with theophilin.

The data obtained in this study are % contraction and % relaxation of tracheal smooth muscle on a computer. The relationship graph between the logarithm of concentration and % response is made. EC80 value (concentration of agonist that can produce a response of 80% of the maximum response) receptor agonist, calculated based on the graph of the relationship of concentration to% response. EC80 is calculated based on the equation (1).

$$LogEC_{80}=\left[\frac{80-Y1}{Y2-Y1}\ x\ (X2-X1)\right]+X_1$$

3 RESULTS AND DISCUSSIONS

Based on phytochemical tests on methanol extracts of Black Intersection Rhizome (*Curcuma aeruginosa* Roxb.) With Thin Layer Chromatography (TLC) method to determine the contentthe types of secondary metabolites such as alkaloids, flavonoid, steroid, saponin and tannin. The relaxation effect of the EEBC by increasing the concentration (0,5-4 mg/ml). The administration of the EEBC concentration series produced a relaxing effect on the contractions induced acetylcholine.

Table 1. The % EEBC relaxation data in the tracheal smooth muscle contracted with 1.43 x 10-4 M acetylchoin

No	EEBC (mg/ml)	% Marmot Trachea Relaxation *				Mean	SD	SEM
		1	2	3	4			
1	0,5	6,00	2,94	5,26	9,80	6,00	2,85	1,43
2	1	14,00	5,88	10,53	15,69	11,52	4,33	2,17
3	1,5	20,00	11,76	21,05	29,41	20,56	7,22	3,61
4	2	36,00	20,59	31,58	37,25	31,36	7,58	3,79
5	2,5	54,00	41,18	47,37	41,18	45,93	6,12	3,06
6	3	70,00	67,65	84,21	68,63	72,62	7,79	3,89
7	3,5	92,00	94,12	100,00	94,12	95,06	3,44	1,72
8	4	100,00	100,00	100,00	100,00	100,00	0,00	0

Based on the regression correlation test on the percentage of the effect of relaxation with the EEBC concentration is a positive correlation with a correlation value of 0.970 (the correlation is close to 1) and an R Square value (R2) of 0.940. It can be stated that about 94.0% the percentage of the relaxing effect of extracts on tracheal smooth muscle increases with increasing EEBC concentration. Testing the mechanism of action through inhibition of muscarinic receptors was done by comparing the contraction strength of acetylcholine (1.43 x 10-4 M) which has been incubated with EEBC at a concentration of 3.2 M with the contraction strength of acetylcholine (1.43 x 10-4 M) without incubation and the contraction strength of acetylcholine induced with atropine sulfate (1 x 10-6 M) as shown in Figure 3.

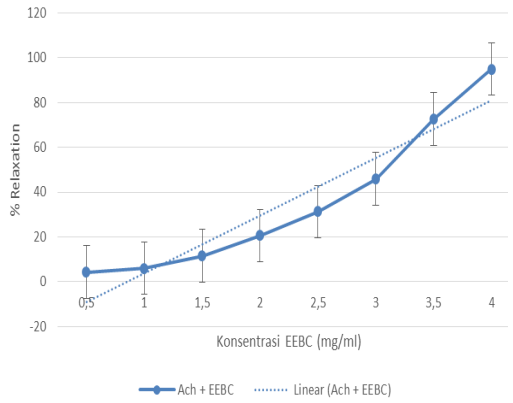

Figure 3. Relaxation effect% EEBC on tracheal guinea pigs contracted using 1.43 x 10-4 M acetylchoin.

The contraction effect of acetylcholine incubated with EEBC compared to acetylcholine without incubation showed statistically significant differences ($p < 0.05$). Statistical results of the comparison of the contraction of acetylcholine (1.43 x 10-4 M) incubated EEBC at a concentration of 3.2 M with acetylcholine (1.43 x 10-4 M) incubated atropine sulfate (1 x 10-6 M) also showed results significantly different ($p < 0.05$). EEBC and atropine sulfate both have a relaxing effect on acetylcholine-induced contractions, but the contraction force incubated with atropine sulfate was smaller, because atropine sulfate used is a pure compound, whereas at EEBC it is not a pure compound but is a plant chemical compound in which it is smaller. There are still some other plant chemical compounds. The results of testing the mechanism of the EEDC's relaxing effect on the tracheal smooth muscle can be seen in Table 2 and Figure 4.

Based on statistical data it can be indicated that EEBC relaxes the guinea pig's smooth muscle by inhibiting muscarinic receptors, this can be proven by looking at the statistical comparison of the results of acetylcholine without incubation and acetylcholine incubated by EEBC which shows significantly different contraction results between the two ($p < 0,05$). EEBC inhibits the increase in contractions by acetylcholine which has been described above that acetylcholine works to stimulate muscarinic receptors which increase smooth muscle contraction. The increase in contractions by acetylcholine decreases because the muscarinic receptors are already largely inhibited by EEBC so that the muscarinic receptors do not all interact with acetylcholine to produce contractions. Inhibiting the release of acetylcholine causes reduced muscarinic receptor activity which mediates smooth muscle contraction [13].

Table 2. % EEBC relaxation data in the tracheal smooth muscle contracted with acetylcholine 1.43 x 10-4 M after incubating theophylline 10-4 M

No	(mg/ml)	% Relaxation *				Mean	SEM
		1	2	3	4		
1	0,5	8,82	6,67	10,53	12,12	9,53	1,17
2	1	20,59	13,33	28,95	21,21	21,02	3,19
3	1,5	32,35	26,67	47,37	36,36	35,69	4,37
4	2	41,18	33,33	52,63	66,67	48,45	7,25
5	2,5	58,82	56,67	76,32	81,82	68,41	6,27
6	3	88,24	90,00	84,21	87,88	87,58	1,22
7	3,5	100,00	100,00	100,00	100,00	100,00	0,00
8	4	100,00	100,00	100,00	100,00	100,00	0,00

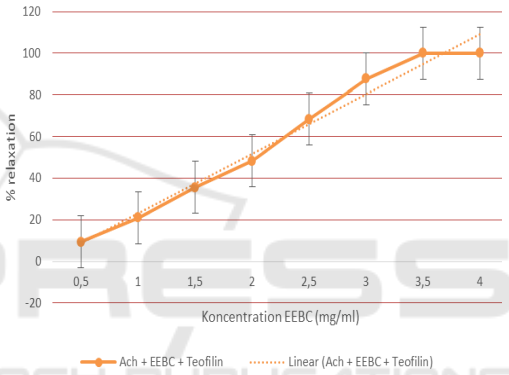

Figure 4. Relaxation effect% EEBC on guinea pigs tracheal contracted using 1.43 x 10-4 M acetylchoin after theophylline incubation.

The initial incubation of the tracheal smooth muscle with theophylline aims to inhibit the activity of phosphodiesterase (PDE). Inhibition of PDE will increase cellular cAMP levels so that it will cause bronchodilation. Testing the mechanism of the EETH relaxation effect on tracheal smooth muscle showed that at initial incubation with theophylline 10-4 M there was no significant difference in the EEBC relaxation effect compared to the relaxing effect on the treatment without theophylline incubation (control) ($p > 0.05$; independent t-test). This result is due to the inhibition of PDE by theophylline causing EEBC no longer relaxes through this mechanism, so the resulting relaxation effect is no different from control. It can be concluded that the relaxing effect of EEBC is related to the inhibitory effect of phosphodiesterase activity.

4 CONCLUSIONS

Black Intersection has a relaxing effect on the isolated tracheal smooth muscle of guinea pigs contracted with acetylcholine, Black Intersection has the ability that is not different from atropine sulfate 1 x 10-6 M in reducing the contraction of isolated guinea pig tracheal smooth muscle induced by acetylcholine, the strength of acetylcholine without incubation contraction compared with acetylcholine with EETH incubation showed statistically significantly different results ($p < 0.05$). This shows that EETH works by inhibiting M-3 receptors. The mechanism of the relaxing effect of EETH on isolated guinea pig smooth muscle is mediated through inhibition of the enzyme PDE.

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