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Research Article

IN VITRO ANTICONVULSANT EFFECT OF ETHYL ACETATE FRACTION OF TITANUS LEAF *(LEEA AEQUATA* L.) ON ISOLATED COLON

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ABSTRACT

Objective: The excessive activity of the parasympathetic nervous system in the intestinal smooth muscle is important role in the increased intestinal motility, so antispasmodic medications are needed that can decrease intestinal motility such as atropine. Treatment may also use medicinal plants that are cheaper and easy to obtain, and also believed to have smaller side effects than modern antispasmodic drugs. This study aimed to determine the anticonvulsant or relaxation effects of the ethyl acetate fraction of titanus leaf (EAFTL) against contracted guinea pig colon.

Methods: The parameters measured are smooth muscle relaxation. Before testing, guinea pig colon was equilibrated for 45 min to obtain a stable condition in Tyrode's solution with a temperature of 37°C aerated with carbogen gas (O_2:CO_2) with a ratio of 95%:5%. The relaxing effect of the colon was tested after inducing by acetylcholine chloride; then, each colon was given cumulative concentration of EAFTL and atropine sulfate. The concentration of acetylcholine chloride required to increase the contraction of the guinea pig colon was 1.76×10^{-4} Mol. The cumulative concentration of EAFTL given was 0.5–4 mg/Ml and cumulative concentration of atropine sulfate given was 6.95×10^{-6}–2.08×10^{-2} mg/Ml.

Results: The EAFTL has a relaxing effect. Statistical analysis of EAFTL at a concentration of 4 mg/Ml (100.000±1.7417) in reducing the smooth muscle of colon contraction induced by acetylcholine chloride 1.76×10^{-4} Mol ($p>0.005$), has not statistically significant compared to that of atropine sulfate 6.95×10^{-3}mg/Ml (105.7292±0.8161).

Conclusion: EAFTL has relaxing effect on the smooth muscle of the colon.

Keywords: Titanus leaf, Colon, Guinea pig, Relaxation, *In vitro*.

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INTRODUCTION

Tetanus is one of the potentially fatal causes, characterized by increased spasms of skeletal muscle, one of the manifestations of seizures is the continuous contraction of the muscles in the body or the entire body. Tetanus is caused by the release of toxin compounds by bacterial spores of *Clostridium tetani*.[11]

The mechanism of tetanus is by inhibiting transmitter in the synapse so that excitatory transmitters will predominate on the synapse, the high excitatory transmitter in this synapse that will increase contraction continuously to cause seizures. The balance between transmitter inhibitory and excitatory transmitter on synapse transmission is essential to maintain the normal functioning of the nervous system. The existence of an imbalance between transmitter inhibitory and excitatory transmitter in the synapse will cause problems in the body. Examples of inhibitory transmitters are GABA, glycine, and nitric oxide, and excitatory transmitters are acetylcholine, histamine, norepinephrine, and serotonin.[13]

The excessive activity of the parasympathetic nervous system in the intestinal smooth muscle is an important role in the increased intestinal motility [6], so antispasmodic medications are needed that can decrease intestinal motility such as atropine. In addition to using antispasmodic drugs, treatment may also use medicinal plants that are cheaper and easy to obtain, and also believed to have smaller side effects than modern antispasmodic drugs.

Titanus leaf (*Leea aequata* L.) is a plant that is used as a traditional medicine. Its barks and roots are used as astringent, anthelmintic, indigestion, jaundice, chronic fever, and malaria. Its leaves and twigs are used as an antiseptic and treat wounds [3]. The content of titanus leaf is like flavonoids are known to have biological and pharmacological activity tested *in vitro* as antiallergic, anti-inflammatory, antioxidant, antibacterial, anticancer, and antidiarrheal [1,2,4]. While based on study, the ethanol extract titanus leaf has a relaxation effect on the contraction of the isolated guinea pig ileum (smooth muscle) was induced by acetylcholine.

MATERIALS AND METHODS

Research tool

The tools used in this study include laboratory glassware, analytical balance (Boeco Germany), animal weights (Presica Geniweigher), a set of organ preparation equipment (Germany), vortex (Boeco Germany), stirrer (Dell), four set organ bath volume 50.0 ml (ML0146/50, Panlab magnet (Bel-Art Products), isometric transducers (MLT0201, Panlab, ADInstruments, Spain), computers (ADInstruments, Spain), microvolume pipette (Socorex, Switzerland) heating and magnetic stirrer (Velp Scientifica, Europe), thermostat (ML0146/50, Panlab, ADInstruments, Spain), PowerLab 15T (T15-0676 series, ADInstruments, Australia), and Quad Bridge Amplifier (serial 224-0448, ADInstruments, Australia).

Materials research

The samples used in this study were titanus leaf (*L. aequata* L.), the chemicals used were Tyrode solution (consisting of NaCl, KCl, MgCl2, NaH2PO4, CaCl2, NaHCO3, and D-Glucose) (Merck), carbogen gas containing 95% oxygen and 5% carbon dioxide (Tri Gases, Medan, Indonesia), acetylcholine HCl (Sigma, Switzerland), atropine sulfate (Sigma, USA), dimethyl sulfoxide (Merck), and aquadest. This study uses guinea pigs as animal's experiments and the use of animals has been approved by the issuance of Letter of Recommendation of Health Research Ethical Approval from Animal Research Ethics Committees of FMIPA USU with the number: 498/KEPH-FMIPA/2017 [12].

Methods

Contraction of smooth muscle (colon) induced by acetylcholine

The muscarinic agonists are performed to measure the maximum limit that can be demonstrated to guinea pig colon contractions to obtain

effective concentration (EC80) by acetylcholine. Measurements are performed gradually with cumulative administration of acetylcholine to obtain concentrations in the organ bath 10–8 to 10–3 Mol. It has been prepared for 45 min with tyrode solution every 15 min.

The relaxation effect of ethyl acetate fraction of titanus leaf (EAFTL) on colon guinea pig by acetylcholine induction

The isolated colon of guinea pig is contracted by administering 35.2 µl of a 2×10^{-1} Mol acetylcholine solution, equivalent with concentration of acetylcholine 1.76×10^{-4} Mol in organ bath. The activity of acetylcholine to increase the contraction of guinea pig colon was done by EAFTL in organ bath is to decrease the contraction. So that it can be obtained the concentration of stratum, i.e., 0.5–4 mg/Ml EAFTL, has been prepared for 45 min (with Tyrode solution every 15 min).

The relaxation effect of atropine sulfate on colon guinea pig by acetylcholine induction

The isolated colon of the guinea pig is conditioned by a Tyrode solution in the organ bath connected to the isometric transducers. Colon is contracted with 35.2 µl of acetylcholine (1.76×10^{-4} Mol) in the organ bath. After the contraction obtaining stable then performed the concentration of atropine sulfate.

RESULTS AND DISCUSSION

The result of contraction smooth muscle (colon isolated from guinea pig) induced by acetylcholine

Contractions triggered by acetylcholine chloride can be observed by observing a change in the smooth muscle (colon isolated) contraction response to the addition of a series acetylcholine chloride concentrations (10^{-8}–10^{-3} Mol). The percentage of colon contraction obtained at the stratified with acetylcholine concentration (EC80).

Acetylcholine is a cholinergic agonist which means a drug that promotes cholinergic nerve activity. Acetylcholine will interact with the muscarinic acetylcholine receptor on the cells of the effector organ of the cholinergic nerve such as parietal cells of the stomach, heart muscle, and smooth muscle of the gastrointestinal tract. In the ileum, acetylcholine will interact with muscarinic receptors which will lead to an increase in smooth muscle motility [7]. The results obtained in accordance with the theory obtained (Table 1 and Fig. 1), with the increased concentration of acetylcholine, the intestinal motility will increase.

For more details of Table 1 can be shown in Fig. 1

The result of relaxation effect of EAFTL on isolated colon guinea pig contraction by acetylcholine induction

The relaxation effect of EAFTL against isolated colon (smooth muscle) from guinea pig was performed by contracting the smooth muscle of colon by acetylcholine 1.76×10^{-4} Mol, followed by series concentration of 0.5–4 mg/Ml EAFTL (Table 2 and Fig. 2).

For more details of Table 2 can be shown in Fig. 2.

The result of relaxation effect of atropine sulfate on isolated colon guinea pig contraction by acetylcholine induction

Testing of atropine sulfate relaxation effect on isolated small intestinal muscle was done by contracting the smooth muscle of colon with acetylcholine 1.76×10^{-4} Mol, followed by series of atropine sulfate concentration 6.95×10^{-6}–2.08×10^{-2} mg/Ml (Table 3 and Fig. 3).

For more details of Table 3 can be shown in Fig. 3.

Comparison of % relaxation of atropine sulfate and EAFTL on contraction of colon by induced acetylcholine

The correlation of percentage of relaxation effect with the concentration of titanus leaf ethyl acetate and the atropine sulfate is not different ($p>0.05$). The % relaxation comparison graph of EAFTL and atropine sulfate can be shown in Fig. 4.

The comparison of the relaxation effect between atropine sulfate at concentrations of 6.95×10^{-3}mg/Ml (105.7292±0.8161) with EAFTL in the administration of 4 mg/Ml (100,000±1.7417) for the contraction of the colon induced by acetylcholine showed that the percentage difference relaxation between the two did not significant different ($p>0.05$). In Fig. 5, it is indicated that EAFTL concentrations of

Table 1: Data of % contraction by series concentrations of acetylcholine on smooth muscle of the colon

Concentration of acetylcholine (Mol)	% Contraction of colon*			Mean	Error Standard
	1	2	3		
1×10^{-8}	32.1428	28.5714	28.1250	29.6131	1.2714
3×10^{-8}	25.0000	45.2380	28.1250	32.7877	6.2900
1×10^{-7}	28.5714	45.2380	34.3750	36.0615	4.8844
3×10^{-7}	28.5714	38.0952	37.5000	34.7222	3.0801
1×10^{-6}	28.5714	42.8571	37.5000	36.3095	4.1665
3×10^{-6}	35.7142	47.6190	37.5000	40.2777	3.7065
1×10^{-5}	39.2857	59.5238	40.6250	46.4782	6.5340
3×10^{-5}	46.4285	59.5238	50.0000	51.9841	3.9082
1×10^{-4}	67.8571	71.4285	78.1250	72.4702	3.0094
3×10^{-4}	92.8571	90.4761	81.2500	88.1944	3.5395
1×10^{-3}	100.0000	100.0000	100.0000	100.0000	0

*% relaxation is calculated from the maximum contraction point achieved by the administration of acetylcholine

Table 2: Data on the relaxation effect of EAFTL on colon contraction by induction of acetylcholine 1.76×10^{-4} Mol

Dose of EAFTL (mg/Ml)	% Relaxation of colon						Mean	Error Standard
	1	2	3	4	5	6		
0.5	38.4616	37.2882	50.9804	59.0909	53.9479	50.8197	48.4314	3.5589
1.0	40.6594	37.2882	70.5883	80.3031	59.2105	60.6557	58.1175	6.8180
1.5	42.8572	32.2034	76.4706	86.3637	64.4737	65.5738	61.3237	8.3151
2.0	46.1539	47.4577	90.1961	91.6667	75.0000	73.7705	70.7075	8.1468
2.5	49.4506	55.9323	96.0785	93.9394	81.5789	77.0492	75.6715	7.8843
3.0	57.1429	77.9662	100.000	95.4546	82.8947	78.6885	82.0245	6.1951
3.5	58.2418	98.3051	100.000	96.9697	86.8421	85.2459	87.6008	6.3892
4.0	91.2088	100.000	100.000	96.9697	97.3684	90.1639	95.9518	1.7497

*% relaxation is calculated from the maximum contraction point achieved by the administration of acetylcholine

Table 3: Data on the effects of atropine sulfate relaxation on intestinal contractions by acetylcholine induction 1.76×10^{-4} Mol

Concentration of atropine sulfate (mg/Ml)	% Relaxation of colon						Mean	Error standard
	1	2	3	4	5	6		
6.95×10^{-6}	39.7261	37.8379	42.7084	39.7261	24.1379	63.6364	41.2955	5.2048
2.08×10^{-5}	61.6439	56.7568	66.6667	61.6439	39.6552	78.1819	60.7581	5.1695
6.95×10^{-5}	63.0137	91.8919	81.2500	63.0137	75.8621	80.0000	75.8386	4.5953
2.08×10^{-4}	78.0822	94.5946	82.2917	78.0822	87.9311	83.6364	84.1030	2.5812
6.95×10^{-4}	83.5617	97.2973	87.5000	83.5617	94.8276	89.0909	89.3065	2.3361
2.08×10^{-3}	93.1507	100.000	88.5417	93.1507	98.2759	100.000	95.5198	1.8948
6.95×10^{-3}	94.5206	100.000	90.6250	94.5206	98.8276	100.000	96.4156	1.5519
2.08×10^{-2}	110.958	105.405	120.833	110.958	100.000	105.454	108.935	2.9113

*% relaxation is calculated from the maximum contraction point achieved by the administration of acetylcholine

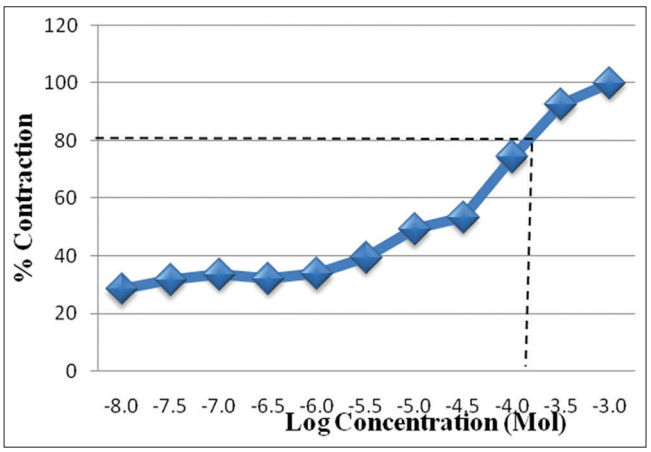

Fig. 1: Graphic % contraction of smooth muscle (colon isolated) by giving series of acetylcholine concentration, n=3

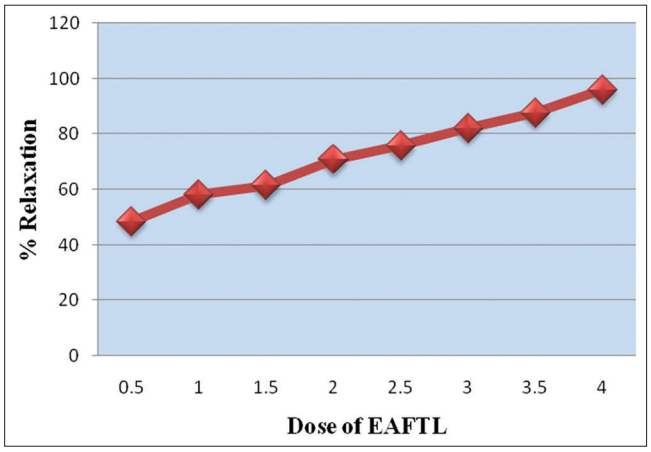

Fig. 2: Graph % relaxation after the series concentration of EAFTL on smooth muscle of colon contracted with acetylcholine1.76×10^{-4} Mol, n=6

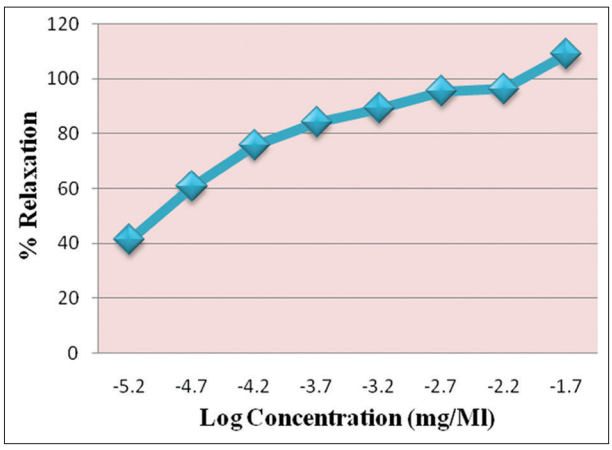

Fig. 3: Graph % relaxation after the series concentration of atropine sulfate on smooth muscle of colon contracted with acetylcholine 1.76×10^{-4} Mol, n=6

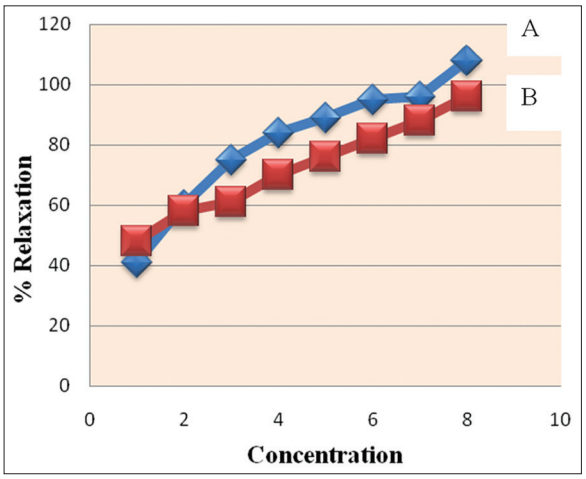

Fig. 4: Graph % relaxation after series concentration (A) atropine sulfate (1=6.95×10^{-6}; 2=2.08×10^{-5}; 3=6.95×10^{-5}; 4=2.08×10^{-4}; 5=6.95×10^{-4}; 6=2.08×10^{-3}; 7=6.95×10^{-3}; 8=2.08×10^{-2}mg/Ml) and (B) EAFTL (1=0.5; 2=1;3=1.5; 4=2; 5=2.5; 6=3; 7=3.5; 8=4 mg/Ml) in the colon contracted with acetylcholine 1.76×10^{-4} Mol, n=6

4 mg/ml have a capability not much different from the 6.95×10^{-3} mg/Ml concentration of atropine sulfate in reducing the contraction induced by acetylcholine 1.76×10^{-4} Mol.

The existence of a relaxation effect of the EAFTL is probably due to secondary metabolites. Secondary metabolites of *L. aequata* are alkaloids, glycosides, steroids/terpenoids, flavonoids, and tannins [1,5,10]. According to Raihan *et al*., 2011[9], that *Leea indica* that has the same family has a strong sedative effect on mice and according to Rahman, *et al*., 2012, [8] *L. indica* has a secondary metabolite similar to *L. aequata*. Therefore, further research is needed on the effect of selective secondary metabolites on the relaxation of smooth muscle of the colon.

CONCLUSION

Based on the study of the relaxation effect of EAFTL (*L. aequata* L.) to the contraction of colon (smooth muscle of guinea pig) *in vitro*, it can be concluded:

The EAFTL (*L. aequata* L.) has a relaxant effect on the colon contraction induced by acetylcholine ($p>0.05$).

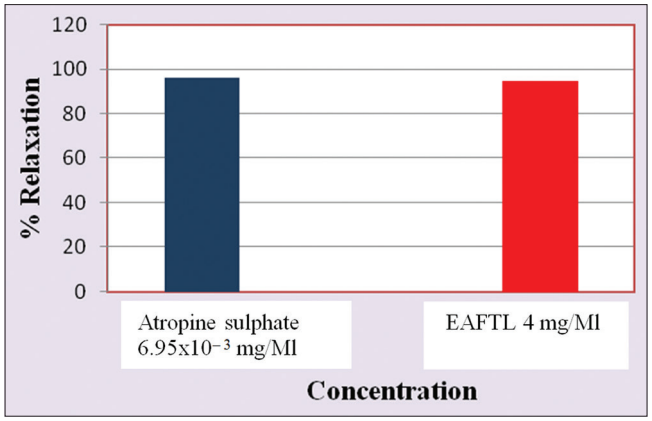

Fig. 5: The % relaxation value of EAFTL concentration 4 mg\Ml and atropine sulfate 6.95×10^{-3} mg/Ml after contracted with acetylcholine 1.76×10^{-4} Mol (n=6)

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