

RELAXATION EFFECT OF ETHANOLIC EXTRACT OF *AVERRHOA BILIMBI* L. LEAVES ON ILEUM SMOOTH MUSCLE CONTRACTION OF *IN VITRO* ISOLATED RAT (*RATTUS NORVEGICUS*)

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Received: 07 March 2018, Revised and Accepted: 25 March 2018

ABSTRACT

Objective: The study was aimed to investigate the relaxation effect of ethanolic extract of *Averrhoa bilimbi* L. leaves (EEABL) and ondansetron against 5-HT-induced contraction of the 5-HT₃ receptor.

Methods: The study of the relaxation effect of the cumulative concentration EEABL leaves (0.5–4 mg/mL) and ondansetron (10^{-5} – 3×10^{-2}) after the contracted with 5-HT (EC₈₀: 1.158×10^{-6} M) was conducted *in vitro* using isolated rat's ileum organ in the Tyrode solution.

Results: The EEABL and ondansetron decreased ileum contraction after induced by 5-HT. EEABL has no difference in terms of ability as ondansetron in reducing the ileum smooth muscle contraction induced by 5-HT ($p > 0.05$).

Conclusion: The EEABL has relaxation effect on smooth muscle of rat's isolated ileum which has induced by 5-HT.

Keywords: *Averrhoa bilimbi* L., Serotonin (5-HT), 5-HT₃ receptor, Ethanolic extract.

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INTRODUCTION

Averrhoa bilimbi Linn. is found in Asia [1]. Although a native of Malaysia and Indonesia, it is a widely cultivated tree in southern India, particularly in Mangalore and Udupi [2]. *A. bilimbi* leaves have been widely used as traditional medicine to treat a cough, itches, boils, and diarrhea. *A. bilimbi* studied have a pharmacological antibacterial activities [3,4], antidiabetic activity [1,5], antioxidant activities [6], apoptogenic effects [2], and antihyperlipidemic activity [1].

Diarrhea is still a health problem in the world, the increase of morbidity every year, 2 billion cases occur every year and as many as 1.9 million children under 5 years died from diarrhea [7,8]. Serotonin (5-HT) is an important signaling molecule in the gut targeting enterocytes, smooth muscles, and enteric neurons. In physiological studies of gut smooth muscle, 5-HT can make the bowel contract, depending on the experimental conditions [9]. 5-HT increases in diarrhea and decreases in constipation [10].

Indonesia has great biodiversity that potential for the discovery of new drugs. Therefore, it is possible to find a new alternative treatment for diarrhea from natural resources. Although *A. bilimbi* has been used traditionally by the community as a medicine such as for diarrhea, the scientific data associated with pharmacological activity reports were still lack.

In the current study, the relaxation effect of ethanolic extract of *A. bilimbi* L. leaves (EEABL) on ileum smooth muscle was evaluated.

MATERIALS AND METHODS

Materials

Drugs and chemicals used in this study were serotonin, ondansetron, dimethyl sulfoxide (Sigma-Aldrich, USA), and ethanol 96% (Merck, Indonesia). Instrument used in this experiment was organ bath PowerLab (ML0146/50, PanLab, AD Instruments, New Zealand).

Preliminary phytochemical screening

A. bilimbi L. was collected from Medan, North Sumatera, Indonesia, and identified by Indonesian Institute of Sciences, Research Center for

Biology (No:1151/IPH.1.01/If.07/IV/2017). Phytochemical screening was carried out on *A. bilimbi* L. leaves powder to determine the constituents, which include alkaloid, flavonoids, glycosides, saponins, tannins, triterpenoids, and steroids [11,12].

Preparation of extract

A. bilimbi L. leaves were washed and dried at 30–35°C, then grinded until dried powder was obtained. The dried powder was percolated using ethanol 96%, then the obtained percolate was evaporated.

Tissue preparation

Male rat weighing 200–300 g (2–3 months) was housed in a room with controlled temperature and lighting and allowed free access to chow and water. The experimental protocol of this study was evaluated by Animal Research Ethics Committees, Universitas Sumatera Utara (No: 246/TGL/KEPK FK USU-RSUP HAM/2017). The animals were sacrificed by cervix dislocation. The intestine was dissected out and the mesenteric coating was gently removed. Subsequently, the ileum was cut with a length of 1–5 cm with which connected to the transducer MLT0201 (PanLab, AD Instrument) connected with PowerLab T15-0676 (PanLab, AD Instrument) [13].

The contraction of ileum smooth muscle induced by 5-HT

Serotonin testing was performed to measure the maximum extent of rat ileum contraction to obtain EC₈₀. Following 60-min equilibration, the ileum smooth muscle strips were contracted with series concentration (10^{-8} – 10^{-4} M) of 5-HT (agonists). After the contraction reached a maximum, the tissue strips were washed by fresh Tyrode solution in the same condition for 60 min (with replacement of the Tyrode solution every 15 min) [13].

The relaxation effect of EEABL and ondansetron of ileum smooth muscle induced by 5-HT

After equilibration, rat ileum was contracted gradually with single concentration of 5-HT (EC₈₀) to the tissue bath as a control concentration-response curve until maximum contraction was achieved. The ability of EEABL and ondansetron to challenge 5-HT-induced ileum contraction was tested using cumulative addition of EEABL (0.5–4 mg/mL) and ondansetron (10^{-5} – 3×10^{-2}). All the experiment conducted using Tyrode buffer with gas flowing O₂:CO₂ (95%:5%) [14].

Statistical analysis

Log EC₈₀ and relaxation data were analyzed using SPSS 22 version (Table 1). The contractile responses to cumulative 5-HT, EEABL, and ondansetron were analyzed using one-way analysis of variance followed by a Tukey *post hoc* test. All data are presented as mean ± standard error of the mean and p<0.05 was considered statistically significant.

RESULTS

Phytochemical screening

Table 1 shows the Screening result of *A. bilimbi* L. Leave powder.

Effects on the contraction of ileum induced by 5-HT

Series concentration of 5-HT-induced the contraction on rat isolated ileum smooth muscle. Response percentage of ileum smooth muscle increased with the addition of 5-HT concentration. The maximum 5-HT concentration for ileum smooth muscle contraction was 3×10^{-4} M. The next given 80 effective concentration or EC₈₀ (1.15×10^{-6} M) was not change the percentage of smooth muscle contraction (Fig. 1).

The relaxation effect of EEABL and ondansetron of ileum smooth muscle induced by 5-HT

The effect of EEABL on isolated rat ileum to the contraction induced by agonist compound could be observed through the changes of isolated ileum smooth muscle contraction % response with the addition of EEABL (0.5–4 mg/mL) and ondansetron (10^{-5} – 3×10^{-2}) on ileum organ.

Addition a series of concentrations of EEABL and ondansetron resulted in the relaxation effect on the contraction of smooth muscle ileum mice induced by 5-HT (1.158×10^{-6} M) (Fig. 2). The relaxation effect produced by ondansetron was compared with the relaxation effect of EEABL in the rat ileum that was contracted with 5-HT. Ondansetron concentration 1×10^{-5} has a relaxation effect of 27.3750 ± 1.8630 showed no statistically significant difference with the concentration of EEABL 0.5 mg/mL (33.3775 ± 1.35640) (p>0.05). Ondansetron concentration 3×10^{-2} mg/mL showed maximum relaxation result of $97.59 \pm 1.40\%$ and showed no statistically significant difference (p>0.05) with EEABL concentration 3.5 mg/mL ($100 \pm 0.00\%$) (Tables 2 and 3). EEABL has no statistically significant difference in terms of ability as ondansetron in reducing the ileum smooth muscle contraction induced by 5-HT (p>0.05).

Table 1: Screening results of *A. bilimbi* L. leave powder

Screening	<i>A. bilimbi</i> L. leaves powder
Alkaloids	-
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Triterpenoid/steroids	+

A. bilimbi: *Averrhoa bilimbi*

Table 2: The relaxation effect of EEABL after the contracted with 5-HT (EC₈₀: 1.158×10^{-6} M)

EEABL concentration (mg/ML)	Relaxation (%)	SEM
0.5	33.3775	1.3567
1	47.1500	2.0065
1.5	55.7704	2.9706
2	70.3100	2.5961
2.5	82.2600	1.6811
3	88.8825	0.3765
3.5	100.0000	0.0000
4	100.0000	0.0000

EEABL: Ethanolic extract of *Averrhoa bilimbi* L. leaves, *A. bilimbi*: *Averrhoa bilimbi*

DISCUSSION

5-HT administrations have increased ileum smooth muscle contraction through 5-HT₃ receptor stimulation. 5-HT₃ receptors expressed in various types of cells and this receptor cellular signal is mediated by 5-HT [15,16]. These receptors play an important role in controlling the physiological response of the central and peripheral nerve activity [17].

5-HT is ligand-gated ion channel receptor, 5-HT₃ receptors are not selective and can be passed by Na⁺, K⁺, and Ca²⁺ [18]. Activated 5-HT₃ receptor by 5-HT stimulates the cholinergic nerve and release acetylcholine [9]. Acetylcholine binds to the ACh-M₃ receptor, activation of this ACh-M₃ receptor through G protein, then stimulates phospholipase C inducing the formation of two intracellular messengers, IP₃ and diacylglycerol (DAG) [13,19]. Both of these compounds are the second messenger that plays an important role in increasing Ca²⁺ intracellular concentration ([Ca²⁺]). IP₃ play a role in the increased [Ca²⁺] through IP₃ receptor activation on sarcoplasmic reticulum so that stimulating the release of Ca²⁺ deposits to the cytosol [20,21]. DAG activates influx of calcium through opening calcium channels in cell membranes. The contraction of smooth muscle is triggered when phosphorylation of myosin light chain (MLC) occurs by MLC kinase. This kinase is activated by binding to the complex of intracellular Ca²⁺-calmodulin [22,23].

In the study, we found that EEABL inhibited the contraction of ileum smooth muscle induced by 5-HT in Tyrode solution. Relaxation effect of EEABL compared with ondansetron (5-HT₃ antagonist). The finding indicates that EEABL tends to inhibit the release of intracellular Ca²⁺ from intracellular Ca²⁺ store and affect efflux Ca²⁺ possess. Extract indicated contained glycosides, flavonoids, saponins, tannins, and triterpenoid/steroids. The relaxation effect of EEABL indicated that the chemical compounds contained in the extracts possess inhibit the release Ca²⁺. Traditional plants contained flavonoids have a vasorelaxant effect [24]. Several compounds have been reported possess relaxation

Table 3: The relaxation effect of ondansetron after the contracted with 5-HT (EC₈₀: 1.158×10^{-6} M)

Ondansetron concentration (mg/ML)	Relaxation (%)	SEM
1×10^{-5}	27.3750	1.8630
3×10^{-5}	33.1800	2.1133
1×10^{-4}	45.1525	2.3073
3×10^{-4}	56.7975	3.0240
1×10^{-3}	67.2550	2.5957
3×10^{-3}	72.1325	0.9724
1×10^{-2}	85.0275	2.5189
3×10^{-2}	97.5950	1.4046

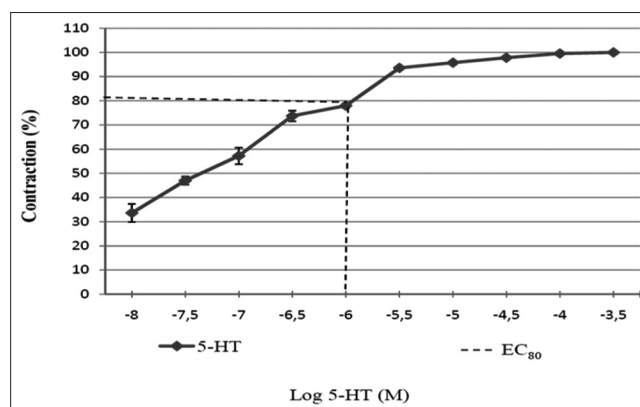


Fig. 1: Concentration-response curves to 5-HT (10^{-8} M– 3×10^{-4} M). Data presented as mean ± standard error of mean from n=3

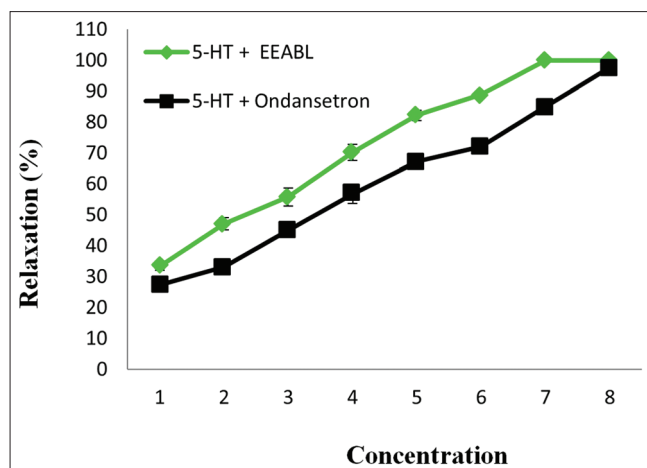


Fig. 2: The relaxation effect of ondansetron concentration compared with ethanolic extract of *Averrhoa bilimbi* L. concentration on smooth muscle of rat's ileum isolated induced by 5-HT (EC80: 1.158×10^{-6} M). Data presented as mean \pm standard error of mean from n=5

effect on smooth muscle muscarinic-mediated receptor such as flavonoid and flavonoids have an inhibitory effect on bladder smooth muscle contractility [20,25].

CONCLUSION

The EEABL showed relaxation effect on smooth muscle of rat's ileum isolated induced by 5-HT.

ACKNOWLEDGMENTS

The authors would like to thank the head of the pharmacology and phytochemical laboratory (Faculty of Pharmacy, Universitas Sumatera Utara) for helpful support in this project research.

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