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Research article

Relaxation effect of ethanolic extract of *Picria fel-terrae* (Pugon tanoh) leaves on contraction of isolated rat's ileum contracted by serotonin

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Key words: *Picria fel-terrae,* pugon tanoh, ethanolic extract, serotonin, ondansetron, ileum, rat, relaxation, in vitro.

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Abstract

Objective: This study was aimed to observe relaxation effect of ethanolic extract of *Picria fel-terrae* leaves (EEPFL) on contraction of isolated rat's ileum contracted by serotonin (5-HT). **Methods:** This research was conducted using *in vitro*, the parameter measured is the contraction or relaxation of isolated rat's ileum smooth muscle. The relaxation effect test is done after the rat's ileum is contracted with serotonin, then each of the ileum is given EEPFL and ondansetron cumulative concentrate. **Results:** EEPFL at 0.5 - 4 mg/ml concentration has relaxation effect, EEPFL 3.5 mg/ml has no difference in terms of ability as ondansetron 3 x 10^{-2} M on the ileum smooth muscle contraction induced by serotonin 1.08 x 10^{-6} M. **Conclusion:** EEPFL has relaxation effect on the ileum smooth muscle contraction induced by serotonin.

Introduction

Pugon tanoh (*Picria fel-terrae* Lour.) is a medicinal plant of the Linderniaceae family commonly used by the Karo tribe of Tiga Lingga Village, Kabupaten Dairi, Provinsi Sumatera Utara, as a traditional medicine to treat various diseases [1-2]. Rural communities use the leaves and latex to cure abdominal pain, diarrheae, cough, scabies, bruising, inflammation, and asthma. Previous research suggests that these plants contain glycosides [2-4], flavonoids [2][5], saponins [2][6], tanin [2] dan steroid/ terpenoid [2]. *P. fel-terrae* has been studied have pharmacological activity as anthelmintic [8], antidiabetic [9][10], antibreast cancer [11], diuretic effect [12], cardioprotective effect [13], and inhibitory effect on acetylcholine muscarinic-3 receptors on tracheal [14].

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder characterized by pain or discomfort in the abdomen, bloating and changes in bowel habits. IBS is associated with changes in the pattern of defecation, which can lead to IBS-Diarrhea and IBS-Constipation [15-16]. Diarrhea is still health problem in the world, it can be seen increasing the number of illness from year to year, two billion cases occur every year and as many as 1.9 million children aged <5 years died from diarrhea [17-18]. The prevalence of diarrhea in Indonesia alone was 7% in all age groups, with incidence in infants at 6.7% [19].

Pathophysiological manifestations of diarrhea one of them is increased intestinal motility due to smooth muscle contraction [20-21], so it is needed antispasmodic that can decrease intestinal motility. Treatment of diarrhea in addition to antispasmodic, can also use medicinal plants because the price is cheap, easy to obtain, and also believed to have smaller side effects than modern antispasmodic.

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter found in living organisms of humans, animals, and plants [22-23], and synthesized in serotonergic neurons in the CNS and gastrointestinal enterocromaffin cells, about 80% HT is found in intestinal enterochromaffin cells [24]. Serotonin in GI is involved in the pathophysiology of several diseases such as IBS, diarrhea, and nausea vomiting due to chemotherapy [25]. Therefore serotonin is closely related to the function and physiology of gastrointestinal, as well as gastrointestinal diseases.

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 *P. fel-terrae* have been used traditionally by the community as a medicine such as for diarrheae, the scientific data associated with pharmacological activity reports still lack. Based on this reason, researchers are interested to observe relaxation effect of ethanolic extract of *Picria fel-terrae* leaves (EEPFL) on contraction of isolated rat's ileum contracted by serotonin (5-HT).

Materials and methods

Materials

Drugs and chemicals used in this study were 5-HT (Shanghai Yuanye), dimethyl sulfoxide (Sigma-Aldrich, USA), ethanol 96% (Bratachem), and ondansetron injection (Novell). Instrument used in this experiment

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was tissue bath PowerLab (ML0146/50, PanLab, ADInstruments, New Zealand).

Preparation of extract

P. fel-terrae was collected from Pancur Batu, Deli Serdang District, North of Sumatera, Indonesia and identified by Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia. The 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.

Phytochemical screening of EEPFL

Phytochemical screening carried out on ethanolic extract of *P. fel-terrae* leaves includes examining the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids.

Tissue preparation

Male rat weighting 150-200 g (± 8 weeks) were housed in a room with controlled temperature and lighting and allowed free access to chow and water. The animals were sacrificed by cervix dislocation, then surgically on the abdomen. Ileum was dissected out and the connective tissue was gently removed. Ileum is inserted into tissue bath containing tyrode solution, the temperature of the solution is maintained at 37ºC while iterating with carbogen continuously, both sides ileum was bound with which connected to the transducer MLT0201 (PanLab, ADInstrument) connected with PowerLab T15- 0676 (PanLab, ADInstrument) [26-29].

Experiment of series contractions 5-HT concentrations in smooth muscle ileum

Serotonin testing was performed to measure the maximum extent of rat ileum contraction to obtain Effective concentration 80 (EC80). Rat's ileum was contracted gradually with series concentration of serotonin (1x 10^{-8} – 3 x 10^{-4} M) to the tissue bath as a control concentration-response curve until maximum contraction was achieved [30].

Experiment of relaxation effects of EEPFL on ileum smooth muscle contracted by 5-HT

EEPFL relaxation tests were performed as follows; rat ileum is conditioned with tyrode solution in tissue bath connected to the transducer. Ileum was contracted with EC80 of serotonin in a maximum submaxion of contractions. After obtaining a stable contraction then given cumulative EEPFL concentration (0.5 – 4 mg/ml) [30].

Experiment of relaxation effects of ondansetron on ileum smooth muscle contrainted by 5-HT

Ondansetron relaxation tests were performed as follows; rat ileum is conditioned with tyrode solution in tissue bath connected to the transducer. Ileum was contracted with EC80 of serotonin in a maximum submaxion of contractions. After obtaining a stable contraction then given cumulative ondansetron concentration (1x 10^{-5} – 3 x 10^{-2} mg/ml) [30]. All the experiment conducted using Tyrode buffer with gas flowing O2:CO2 (95%: 5%) [14].

Statistical analysis

The highest contraction induced by 5-HT was considered the maximum response. Log concentration-response curves were constructed. All data are presented as mean ± standard error of the mean and $p<0.05$ were considered significant. The relaxation responses were analyzed using one-way analysis of variance followed by a Tukey *post-hoc* test.

Results and discussion

Phytochemical screening result of EEPFL

Phytochemical screening result showed that ethanolic extract of *P. fel-terrae* leaves positively contains flavonoids, saponins, tanins, glycosides and steroids/triterpenoid.

Series contractions result of 5-HT concentrations in smooth muscle ileum

Experiment of smooth muscle contraction of rat ileum were isolated by addition of serotonin concentrations from 1 x 10^{-8} to 3 x 10^{-4} M to obtain EC80 used to test the relaxation effect of EEPFL. The percentage of maximal contraction of smooth muscle of rat ileum was obtained at serotonin concentration 1 x 10^{-4} M, and submaximal contraction (EC80) at concentration 1.08 x 10^{-6} M (Figure 1).

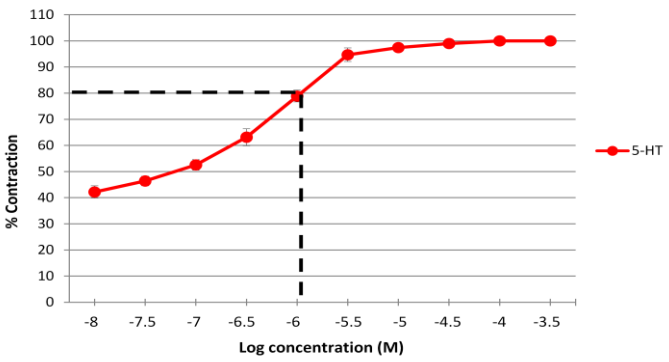


Figure 1. Percentage of smooth muscle contraction of isolated rat ileum contracted with serotonin concentration series.

Serotonin is a monoamine neurotransmitter, about 80% of 5-HT is found in enterocromaffin cells at gut [24]. Serotonin can lead to a number of actions on cells and tissues at intestine, including epithelial secretion, direct or relaxed smooth muscle activation, extrinsic stimulation and intrinsic sensory neurons, and activation of cholinergic neurons resulting in smooth muscle contraction [24]. The serotonin receptor is divided into several subtypes depending on the location of the organ and the function of the organ. In gastrointestinal, serotonin receptor is a 5-HT_3 receptor that regulates gastrointestinal motility and vomiting centers [31].

The 5-HT_3 receptor is a non-selective ionic channel because it can be passed by Na^+, K^+ and Ca^{++} cations [32], 5-HT_3 receptors mediate rapid depolarization responses in both pre-synaptic and post-synaptic neurons. Opening of Ca^{++} channels causes increase intracellular Ca^{++} levels up to 100 mM, which can lead to smooth muscle contractions. 5-HT_3 receptor activation by 5-HT, will stimulate the cholinergic nerve to release achetylcholine (Ach) [31, 33-34].

Ach is a cholinergic agonist that stimulates or enhances cholinergic nerve activity, ach will interact with M3 receptors in the cells of cholinergic nerve effector organs, for example, parietal cells of the stomach, heart muscle, and smooth muscle of the gastrointestinal tract. In the ileum, ach will interact with the M_3 receptor that triggers an increase in smooth muscle motility [35].

Relaxation effects result of EEPFL versus Ondansetron on ileum smooth muscle contracted by 5-HT

EEPFL relaxation tests on isolated ileum muscle were performed by contracting the smooth muscle of ileum with serotonin 1.08 x 10^{-6} M, followed by concentration series of EEPFL 0.5 - 4 mg/ml. Relaxation effects were observed by increased percentage of relaxation on ileum.

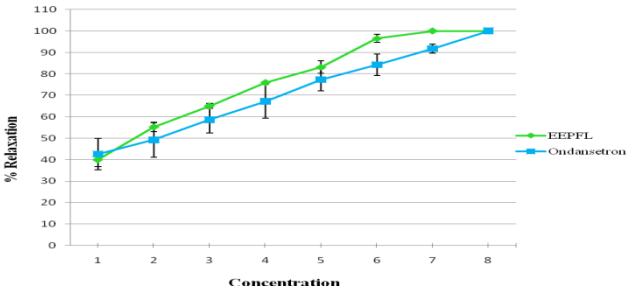


Figure 2. Effect of EEPFL (1 = 0.5; 2 = 1; 3 = 1.5; 4 = 2; 5 = 2.5; 6 = 3; 7 = 3.5; 8 = 4 mg/ml) and ondansetron (1 = 1 x 10^{-5}; 2 = 3 x 10^{-5}; 3 = 1 x 10^{-4}; 4 = 3 x 10^{-4}; 5 = 1 x 10^{-3}; 6 = 3 x 10^{-3}; 7 = 1 x 10^{-2}; 8 = 3 x 10^{-2} mg/ml) on serotonin-induced contractile response in isolated rat ileum. Data presented as mean ± standard error of mean from n=4, *p<0.05.

In figure 2 EEPFL has a greater relaxation effect than ondansetron, but the required smaller dose of ondansetron to relax the ileum smooth muscle is contracted with serotonin, so it can be stated that the ondansetron relaxation effect is greater than extract. EEPFL at a concentration of 3.5 mg/ml has achieved a relaxation effect of 100%, while ondansetron achieves maximum effect at concentrations of 3 x 10^{-2} M.

Statistical analysis at both of ondansetron and extract shows that ondansetron concentration 3 x 10^{-2} mg / ml did not different significantly with EEPFL concentration of 3.5 mg/ml (Table 1).

When serotonin concentrations increase, then 5-HT_3 receptors will be activated. 5-HT_3 receptors work in two ways, first acting as ion channels permeable to Na^+, K^+, and Ca^{++}. The activation of 5-HT_3 receptors in this way leads to rapid depolarization due to Ca^{++} channel opening, thus increasing intracellular Ca^{++} levels [31-33]. The second way is through activation of the cholinergic nerve, if acetylcholine is released, binds to the M_3 receptor. The M_3 receptor is a receptor coupled with G protein, activating this receptor through a series of biochemical processes involving phospolipase C (PLC). Actived PLC will increase the hydrolysis of phosphoinositol 4,5-bisphosphate to inositol triposfat (IP_3) and diasilgliserol. IP_3 will then occupied IP_3 receptors in the calcium store in the sarcoplasmic reticulum, thus opening the calcium canal. The calcium released into the cytosol increases intracellular calcium levels. Intracellular calcium is then bound to calmodulin, thus forming a calcium-calmodulin complex which will then activate myosin light chain kinase (MLCK). MLCK then phosphorylates myosin light chains and activates the ATPase myosin necessary to encourage crosslinking between the myosin-muscles resulting in smooth muscle contraction [36-39].

EEPFL is thought to work by affecting the process of intracellular Ca^{++} uptake into the calcium store in sarcoplasmic reticulum and affecting Ca^{++} efflux so that the receptors can not be occupied by serotonin, which in turn inhibits the formation of the actin-myosin cross linking to smooth muscle relaxation. The effects of medicinal plants relaxation are thought to be due to flavonoids, reported that traditional flavonoid-rich plants and phenol groups can be used as vasorelaxation. EEPFL screening contains flavonoids, glycosides, saponins, tannins and steroids / triterpenoids, the possibility of ileum smooth muscle relaxation effects is due to flavonoids [40-41]. Huang, et al., (1999), shown that *P. fel-terrae* contains apigenin 7-*O*-β-glucuronide, luteolin 7-*O*-β-glucuronide dan apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl) glucuronide.

Table 1. Statistical results EEPFL vs Ondansetron in various concentrations

Relaxation_percentage							
Tukey HSD[a]							
Object	N	Subset for aplha=0.05					
		1	2	3	4	5	6
EEPFL 0.5	4	40.017500					
Ondansetron 1 x 10^{-5}	4	42.637500					
Ondansetron 3 x 10^{-5}	4	49.252500	49.252500				
EEPFL 1	4	55.185000	55.185000	55.185000			
Ondansetron 1 x 10^{-4}	4	58.662500	58.662500	58.662500			
EEPFL 1.5	4		65.095000	65.095000	65.095000		
Ondansetron 3 x 10^{-4}	4		67.195000	67.195000	67.195000		
EEPFL 2	4			75.947500	75.947500	75.947500	
Ondansetron 1 x 10^{-3}	4			77.350000	77.350000	77.350000	
EEPFL 2.5	4				83.15000	83.15000	83.15000
Ondansetron 3 x 10^{-3}	4				84.302500	84.302500	84.302500
Ondansetron 1 x 10^{-2}	4					91.790000	91.790000
EEPFL 3	4					96.537500	96.537500
EEPFL 3.5	4						100.000000
EEPFL 4	4						100.000000
Ondansetron 3 x 10^{-2}	4						100.000000
Sig.		0.196	0.246	0.052	0.162	0.098	0.339

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Ondansetron is a potent and selective 5-HT_3 receptor antagonist, that is why ondansetron is used to treat chemotherapy-induced nausea and vomiting, as chemotherapy releases 5-HT_3 to the small intestine which then stimulates the vomiting reflex by activating the afferent vagal fibers via the 5-HT_3 receptor, so the reflex is inhibited. Activation of afferent vagal fibers can also lead to the release of 5-HT_3 in the postrema area which can also stimulate the emesis through a central mechanism. Ondansetron effect in the treatment of chemotherapy-induced nausea and vomiting is due to the 5-HT_3 receptor in the central nervous system and the peripheral nerve system inhibited by ondansetron [42].

Conclusion

Ethanolic extract of *Picria fel-terrae* leaves has relaxation effect on the ileum smooth muscle contraction induced by serotonin.

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