THE TEST OF *Padina australis*-BASED TABLET AS AN ANTI-DIARRHEAL MEDICINE ON *Escherichia coli* INDUCED MALE SPRAGUE DAWLEY RATS

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Abstract

Diarrhea is a process of discharging feces from the body (defecation) in which the feces is half liquid or liquid-shaped and more watery than normal. One of its cause is the *Escherichia coli* bacteria. Diarrhea is often treated using chemical medicine like Loperamid, but because of its many side effects, people start using herbal medicine instead. *Padina australis* is a species of seaweed that can be used as a medicine, specifically to reduce the growth of *Escherichia coli*, which is a diarrhea-caused bacterium. This research aims to determine the effect of *Padina australis*-based tablet and its optimal dosage as an anti-diarrheal medicine. This research use tablets made from *Padina australis* extract and 30 male Sprague Dawley rat with age 2-3 months and weight ± 250 gr as the test subject. The method used in this research includes environmental adaptation on the test subject, producing *Padina australis* extract tablet suspension, testing tablet suspension on various concentrations, macroscopic and microscopic examination of test subject's intestines using intestinal transit method, and histopathology examination of test subject's organ. The result indicates that 3.78 mg/200 gr weights is the most optimal dosage to restore the condition of test subject's intestine. Microscopic examination shows no tissue damage on the test subject's digestive organs. Therefore, we conclude that *Padina australis* extract tablet has an anti-diarrheal effect on male Sprague Dawley rat with 3.78 mg/200 gr weights as the most optimal dosage.

Keyword: anti-diarrhea, male rat digestive organs, *Padina australis* extract tablet.

INTRODUCTION

Diarrhea is a process of discharging feces from the body (defecation) in which the feces is half liquid or liquid-shaped and more watery than normal (more than 200 gr or 200 ml/24 hour) (Zein, 2004). Diarrhea occurred because of irritation on the intestine caused by virus infection or by bacteria. Irritation caused by microbe also affects muscle tissue and increase intestinal motility (Corwin, 2009).

Diarrhea is often treated using chemical medicine like *Loperamide*, but its usage can cause some side effects such as nausea, vomiting, abdominal pain, and skin rash. Those side effects have caused people to choose herbal medicine as an alternative medication. For example, by using medicine made from seaweed. *Padina australis* is one example of Indonesian seaweed that can be used as a medicine. It can reduce the growth of *Escherichia coli*, a diarrhea-caused bacterium (Triastinurmiatiningsih dan Haryani, 2008).

Hanura et al. (2006) has made a study about the effectivity test of *Padina australis*-based tablet as an antibacterial against *Escherichia coli*. They conclude that to reduce the growth of *E. coli* bacteria, the most effective formula for *Padina australis*-based tablet consists of 30% *Padina australis* extract, 60% amprotab as an excipient (tablet filler), 5% Avicel PH-102, 1% talc, and 1% magnesium stearate. In spite of the result of their research, there is still a necessity to do research about the effect of *Padina australis*-based tablet and its effective dosage as an anti-diarrheal medicine on *E. coli* induced male rat.

RESEARCH METHOD

Research Tools

50 ml beaker glass (Pyrex), 25 ml laboratory flask (Pyrex), digital scale (Tanita KD-160), hotplate stirrer (Wisdom MSH-20D), basin-shaped cage for rats (Krisbow), binocular microscope (Yazumi xsz 107BN), camera (Nikon Coolpix), surgery tools, feeding tube (catheter), spuit (One Med 3 cc).

Research Material

100 250 mg *P.australis* extract tablet, Loperamide HCl (Lodia®), 20 Sprague Dawley male rat, food for lab rat (BR-512), purified water (aquades), 0.5% CMC-Na, 10% neutral buffered formalin.

Producing Test Material

The trial for anti-diarrhea effect includes producing 0.5% CMC-Na, medicine for comparison (Loperamide HCl), and *Padina australis* tablet.

a. Producing 0.5% CMC-Na
   Put 0.5 gr CMC-Na into 20 ml hot purified water, then close and set it aside for 30 minutes until a transparent mass is gotten. After that, crush and dilute it with purified water until its volume reach 100 ml (Anief, 1999). Norit is used in this research as a marker to calibrate intestinal transit method. The utilized norit is not activated and is inert.
b. Producing Loperamide HCl tablet
   Loperamid HCl (Lodia®) contain 2 mg active compound. Weigh tablets, then crush and take 108 mg of its powder. Put the powder into a mortar and crush it while being added by 0.5% CMC-Na suspension bit by bit until homogen. Afterwards, add 0.5% CMC-Na until its volume reach 25 ml. Normal dosage of Loperamide HCl for adult is 2-8 mg per day and the maximum dosage is 16 mg per day. Dosage that is utilized for testing in this research is 2 mg conversed into 0.0504 mg/200 gr body weight for each male rat.
c. Producing *Padina australis* tablet suspension
   Weigh tablet powder according to each concentration (157.5 mg; 315 mg; and 630 mg) Put it into a mortar, add a little 0.5% CMC-Na with weight/volume (W/V) ratio, and stir until homogen. Add 0.5% CMC-Na W/V suspension until its volume reach 25 ml.

Preparing Test Animals

This research utilized ± 250 gr weight and 2-3 months old Sprague Dawley male rat as test animals. The test animals were divided into 5 groups with 4 rats in each group. Each of them was induced orally by 10^9 cfu/ml *E. coli* bacterial culture with 1 ml dosage per day for 7 days. After 7 days and they started appear to having a diarrhea, each group were not given any food for 18 hours before we started giving them some treatments as below:

Table 1. Treatment to test animals according to dosage

Group of Test Animals	Treatment
Group 1 (K-)	Rats without any treatment (diarrhea)
Group 2 (K+)	Rats given 0.0504 mg/200 gr body weight dose of Loperamide HCL tablet
Group 3 (P1)	Rats given 1.89 mg/kg body weight dose of *P. australis* extract tablet
Group 4 (P2)	Rats given 3.78 mg/kg body weight dose of *P. australis* extract tablet
Group 5 (P3)	Rats given 7.56 mg/kg body weight dose of *P. australis* extract tablet

Macroscopic Observation of Test Animals' Intestinal Organ

Intestinal transit method was utilized in macroscopic observation of test animals' intestinal organ. *P. australis* and Loperamide HCl tablet were given to test animals in the beginning of this research. An hour later, all test animals were given 1 ml norit suspension orally. An hour after receiving norit suspension, all test animals were dissected and their intestines were taken out carefully. The length of intestine that was passed by norit marker was measured starting from the pylorus until the blackened end. The entire length of the test animal's intestine starting from pylorus until rectum was also measured. Afterwards, the percentage of the trajectory passed by the norit marker to the entire length of the intestine was calculated (Inayathulla et al., 2010).

$$\text{Norit trajectory ratio} = \frac{\text{The length of intestine passed by norit marker}}{\text{The entire length of the intestine}} \text{ x } 100\%$$

Histopathology Observation of Test Animals' Intestinal Organ

Samples for histophatology preparation were prepared by utilizing HE stain. Samples of small intestine were fixated inside NBF (Neutral Buffered Formalin) solution for 24 hours. Afterwards, the samples were dehydrated inside graded consentrations of alcohol for 2 hours in each grade. Then, the samples were cleared inside graded concentrations of xylol for 40 minutes in each grade, infiltrated, and embedded. Afterwards, the samples inside paraffin block were cut into thickness around 4-5 μm. Each sample was then deparaffinizated for 1 minute and colored by Hematoxylin-Eosin. Afterwards, the samples were mounted on top of microscopic slide by using entellan as adhesive.

Note: Approval letter from Ethic Comittee is being processed.

RESULTS

1. **Macroscopic Observation of Test Animals' Intestinal Organ**

   Macroscopic observation on each group of test animals' intestinal organ –with group 1 is *E. coli* induced-diarrhea rats without any further treatment; group 2 is diarrhea rats which were given 0.0504 mg/200 gr body weight dose of Loperamide HCl tablet and 1 ml norit marker; group 3, 4, and 5 are diarrhea rats which were given 1.89 mg, 3.78 mg, and 7.56 mg respectively, per 200 mg body weight dose of *P. australis* extract tablet and 1 ml norit marker– showed comparable result of the percentage of norit marker trajectory inside their intestinal organ. Detailed result is shown in table 2.

2. **Histopathology Observation of Test Animals' Intestinal Organ**

   Histopathology observation on test animals' intestinal organ that has been given various treatments was conducted on their duodenum, jejunum, and ileum. Detailed result is shown in picture 1, picture 2, and picture 3. Picture 1 (duodenum tissue sample) shows that all treatments didn't result in any damage on each duodenum tissue structure, as seen on every normal tissue structures and those that were given treatments (F). Picture 2 (jejunum tissue sample) shows differences between each tissue structure that were given various treatments according to their respective groups. In group with 3.78 mg/200 gr body weight dose (D), mucosa structure appears to be intact just like a normal structure (F). Picture 3 (ileum tissue sample) shows differences between the results of various treatments. Group with 3.78 mg/200 gr body weight dose (D) and 7.56 mg/200 gr body weight dose (E) show no damage on tissues as shown in their mucosa structures which appear like a normal one (F).

DISCUSSION

1. **Macroscopic Observation of Test Animals' Intestinal Organ**

   The negative control treatment (K-) resulted in 90.38% norit marker trajectory which showed a condition of diarrhea. In this research, *E. coli* bacteria were utilized to induce diarrhea. *E. coli* will affect fluid secretion in digestive system and cause atrophy and necrosis on the intestine, resulting in diarrhea (Suwito, 2010). *E. coli* body will attach itself on small mucosa cell and bring damage on epithelium by means of microcolony establishment which is shown through localized attachment (Savkovic et al., 2005). Furthermore, 10^5-10^{10} dose of *E. coli* can results in around 5 days of diarrhea (Janda and Abbot, 2006).

   In the positive control treatment (K+), the administration of 0.054 mg/200 gr body weight dose of Loperamide HCL to *E.coli*-induced diarrhea rats result in decrease of norit marker trajectory percentage (61.15%). Loperamide was utilized as a comparison because it can restore cell in hypersecretion to normal resorption condition. It can also increase small intestine transit time and body's ability to absorb water, sodium, and chloride when

there is some electrolite disruption (Purwaningdyah et al., 2015). Loperamide has effect of constipation by means of slowing digestive tract's motility and intestinal flow rate to colon. Loperamide also normalize the balance between fluid absorption and secretion on intestinal mucous membrane (Anas et al., 2016). Normal dosage of Loperamide for adult is 2-8 mg per day with 16 mg per day as maximum dosage (Dewoto, 2007). The dosage utilized in this research was 2 mg, which is converted into 0.054 mg/200 gr body weight for adult rats.

The treatment with administration of 1.89 mg, 3.78 mg, and 7.56 mg per 200 gr body weight of *P. australis* extract tablet to *E.coli*-induced diarrhea rats results in decrease of norit marker trajectory percentage. 1.89 mg/200 gr body weight dose has 71.12%, 3.78 mg/200 gr body weight dose has 57.94%, and 7.56 mg/200 gr body weight dose has 48.82%, as shown in Table 1. 7.56 mg/200 gr body weight dose of *P. australis* extract tablet suspension has the lowest norit marker percentage of all. This results show that 7.56 mg/200 gr body weight dose of *P. australis* extract tablet suspension has the strongest anti-diarrheal effect compared to other doses that were tested.

Another test by utilizing Duncan's method indicates that 1.89 mg, 3.78 mg, and 7.56 mg per 200 gr body weight doses can be utilized as an anti-diarrheal medicine because of the decrease of norit marker percentage inside *E.coli*-induced diarrhea rats' intestine that they bring about. *P. australis* extract tablet can be utilized as an anti-diarrheal medicine because it contains secondary metabolite such as: triterpenoid/steroid (Shaha et al., 2012), tannin and saponin, and flavonoid (Anas et al., 2012). As an anti-diarrheal, steroid can increase water and electrolite absorption inside intestine and makes them back to normal (Anas et al., 2012). Tannin can decrease diarrhea intensity by means of shrinking intestinal mucous membrane and pores to decrease fluid and electrolite secretion (Tjay and Rahardja, 2007). Furthermore, adstringent trait that tannin has will makes intestine more resistant to bacterial toxin and chemical stimulation that could cause diarrhea. Flavonoid stops *E.coli*-induced diarrhea by means of obstructing intestinal motility to decrease fluid and electrolite secretion (Di Carlo, 1993).

2. Histopathology Observation of Test Animals' Intestinal Organ

Picture 1 shows no damage on duodenum tissue structure on every given treatment, as shown by comparing every tissue structure with their respective treatment to the normal one (F). This indicates that all treatment have a nearly same effect to duodenum. This is happened because duodenal lumen is rich with bile salt and pancreas secretion can obstruct the microorganism's growth (Jonqueira and Carneiro, 2007). Furthermore, duodenum's fast peristaltic movement can prevent patogen from staying in it (Lu, 2001).

Picture 2 shows some differences on jejunum tissue structures between various treatments. In group of treatment with 3.78 mg/200 gr body weights dose (D), mucosa composition appear to be intact like in normal tissue (F). In negative control treatment group (A) and 1.89 mg/200 gr body weights dose treatment group (C), there are some erosion in the lamina propria inside mucous membrane. In positive control group (B) and 7.56 mg/200 gr weights dose treatment group (E), a lymphoid follicular proliferation is occured. This indicates that the administration of 3.78 mg/200 gr body weights dose of *P. australis* tablet can reduce tissue damage in jejunum that is caused by *E. coli* infection. The damage in jejunum happened because jejunum has longer length and slower peristaltic movement than duodenum, so it receives longer exposure from *E. coli* bacteria. Furthermore, jejunum has slower regeneration speed of villi epithelial cell than duodenum, so *E. coli* that stick there is eliminated at a slower rate (Rajha et al., 2009).

Picture 3 shows differences on ileum tissue structures between various treatments. In 3.78 mg/200 gr body weights dose (D) and 7.56 mg/200 gr body weights dose (E) treatment groups, there are no apparent tissue damage. It is indicated by their mucosa

compositions which appear like a normal one (F). This indicates influences from tannin and saponin compounds in *P. australis* that can decrease bacteria growth (Hanura et al., 2016). In negative control group (A), there is some erosion in the lamina propria inside mucous membrane. In 1.89 mg/200 gr body weights dose treatment group (C), there appear to be extensive infiltration of lymphocytes imflammatory cells. In positive control group (B), lymphoid follicular proliferation seems to be occured with details shown in appendix 3. The occurence of lymphoid follicular is part of body's immune system and has an important role in adaptive and natural immune response. Lymphoid follicular enlargement is body's immune response as a sign of our body having some tissue damage. It is also an immune response triggered by humoral response and disease, including virus and bacterial infection (Miranda, 2013). This indicates treatment in positive control group and the administration of 1.89 mg/200 gr body weights dose of *P. australis* tablet are less effective to counter *E. coli* agression to the ileum so as causing damage to ileum tissue.

The above results indicate that administrating 3.78 mg/200 gr body weights dose of *P. australis* tablet is effective to restore the condition of small intestine's mucous membrane. Furthermore, group without any treatment and left out to having diarrhea experiences continous tissue damage.

CONCLUSION

According to the results of this research, it can be concluded:

1. *Padina australis* extract tablet has an anti-diarrheal effect on *Sprague Dawley* white male rat.
2. Dosage of 3.78 mg/200 gr body weights is the most effective dosage to be utilized as an anti-diarrheal.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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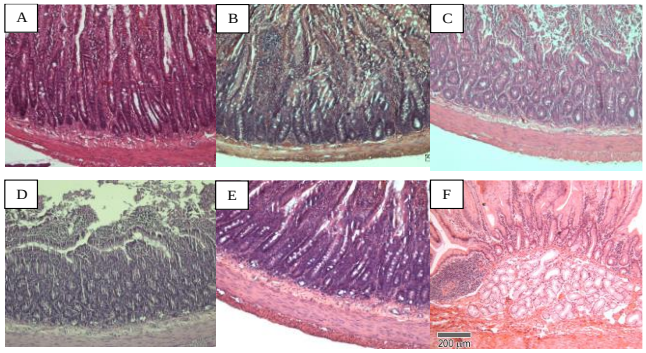
Table 2. Percentage of norit marker trajectory inside rat's intestine

Ulangan Hewan Uji	K +	K-	*Padina australis* tablet (mg/200 gr body weights)		
			1.89	3.78	7.56
1	84.40%	59.20%	60.30%	47.25%	43.10%
2	94.31%	53.54%	75.40%	68.35%	49.62%
3	94.01%	60.47%	86.13%	69.90%	53.17%
4	88.83%	71.40%	62.66%	46.27%	49.41%
Average value	90.38% ^{c}	61.15% ^{ab}	71.12% ^{b}	57.94% ^{ab}	48.82 ^{a}

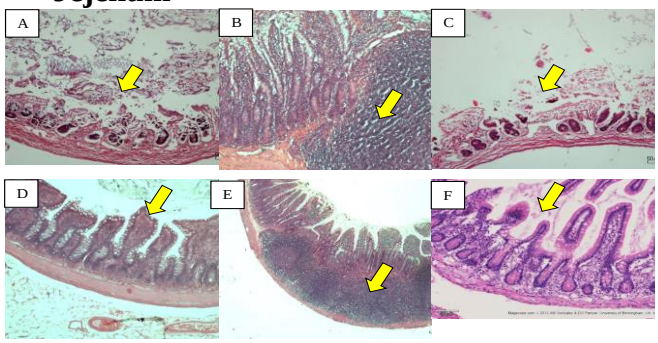
Note: Average values followed by different superscript letter inside same column/line show a real difference influence ($p<0.05$)

Duodenum



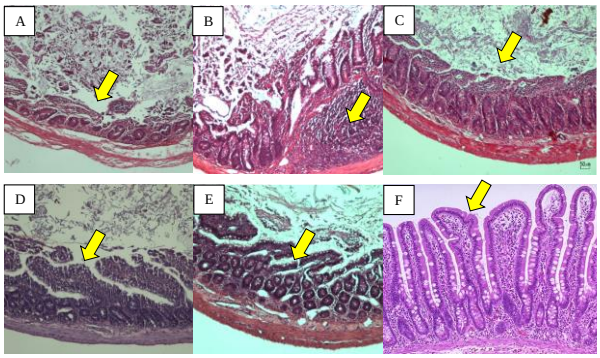
Picture 1. Transverse slices of rat's duodenum with HE stain and 100x magnification. Negative control (A), postive control (B), 1.89 mg/200 gr body weights dose (C), 3.78 mg/200 gr body weights dose (D), 7.56 mg/200 gr body weights dose (E), and normal tissue (F).

Jejenum



Picture 2. Transverse slices of rat's jejunum with HE stain and 100x magnification. Negative control (A), postive control (B), 1.89 mg/200 gr body weights dose (C), 3.78 mg/200 gr body weights dose (D), 7.56 mg/200 gr body weights dose (E), and normal tissue (F).

Ileum



Picture 3. Transverse slices of rat's ileum with HE stain and 100x magnification. Negative control (A), postive control (B), 1.89 mg/200 gr body weights dose (C), 3.78 mg/200 gr body weights dose (D), 7.56 mg/200 gr body weights dose (E), and normal tissue (F).