Pak. j. life soc. Sci. (2024), 22(2): 6850-6860 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences

www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.2.00517

RESEARCH ARTICLE

Eleutherin palmifolia Bulb Extract (EPBE) Reduce Diarrhea Symtomps and Increases Histopathology of Duodenum of Male Mice (Mus musculus)

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ARTICLE INFO

Received: Aug 11, 2024

Accepted: Oct 3, 2024

Keywords

Flavonoid

Tannin

Herbal medicines

Traditional medicine

TCM

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ABSTRACT

Dayak onion (Eleutherine palmifolia) is a plant endemic to Kalimantan that is traditionally used by the Dayak people as herbal medicine. The content of tannin and flavonoids in Dayak onion has the potential for use as antidiarrheal therapy. This study aims to evaluate the effectiveness of Dayak onion extract on antidiarrheal activity and the improvement of duodenal histopathology in male mice (Mus musculus) induced by Oleum Richini. The antidiarrheal test was performed using a diarrhea protection model induced by Oleum Richini. The mice were divided into six groups: Non treatment group which was given distilled water without induction,a negative control group (KN(-)) which was given Na-CMC and induced with Oleum Richini, a positive control group (KP(+)) which was given loperamide suspension and induced with Oleum Richini, and three treatment groups which were given Dayak onion extract at doses of 250 mg/kg BW, 500 mg/kg BW, and 750 mg/kg BW, each induced with Oleum Richini. Diarrhea parameters, including defecation frequency and stool consistency, were statistically analyzed using ANOVA and Kruskal-Wallis tests.After treatment showed that the dose of 250 mg/kg BW was effective but not as effective as Loperamide, while the doses of 500 mg/kg BW and 750 mg/kg BW showed effectiveness equivalent to KP(+). Histopathological analysis showed that a dose of 500 mg/kg BW can improve inflammation in the duodenum of mice.Dayak onion extract at a dose of 500 mg/kg BW is effective as an antidiarrheal and is able to improve duodenal histopathology in male mice induced by Oleum Richini.

INTRODUCTION

Diarrhea is a clinical condition characterized by an increase in the frequency and volume of fecal elimination, which is usually liquid or even watery, more than three times per day. The term “diarrhea” is derived from the Greek, “diarroi,” meaning “to flow continuously,” and describes an abnormal condition with frequent fecal output (Wulan *et al*.,2022). The diagnosis of acute diarrhea, particularly those caused by bacterial infections, requires a systematic and thorough evaluation approach. The diagnosis process involves collecting the patient’s medical history, including history of illness, environmental factors, history of medication use, especially antibiotics, and travel history. A careful physical examination and supporting examinations are also required to establish an accurate diagnosis and determine appropriate therapy (Lukman *et al.,*2022).Diarrheal disease is an endemic condition that has the potential to cause extraordinary events (KLB) and is one of the leading causes of death in Kalimantan, especially in the under-five group. In 2020, the total incidence of diarrhea reached 3,252,277 cases across all age groups, with a special incidence in children under five as many as 1,140,503 cases (Purnamiasih, *et al.*,2022). Data from the 2018 Basic Health Research (Riskesdas)

showed a diarrhea prevalence of 8% for all age groups, 12.3% for toddlers, and 10.6% for infants. In addition, data from the Sample Registration System in 2018 identified diarrhea as one of the leading causes of death in neonates with a contribution of 7% and in infants aged 28 days with a contribution of 6% (Qisti, *et al*.,2021).

Treatment of diarrhea is done by taking chemical drugs such as Loperamide. The treatment can cause side effects such as nausea, vomiting, abdominal pain, and skin rashes. The presence of side effects causes people to prefer medicinal plants as an alternative treatment. Some previous studies explain that some medicinal plants are effective in treating diarrhea due to the content of tannin compounds, phenols, saponins, essential oils, alkaloids, and flavonoids such as guava leaves (Moila, *et al*.,2013). Another medicinal plant that is still not utilized by the community is papaya fruit seeds. The utilization of papaya fruit seeds is still low, even though papaya fruit seeds also contain active compounds such as tannins, phenols, saponins, and alkaloids (Winarno, *et al*.,2003).Indonesian people have long recognized and used medicinal plants as an effort to overcome health problems. Knowledge of medicinal plants is based on experience and skills that have been passed down from generation to generation (Permana,*et al* 2009). Many herbal medicines have been widely accepted in almost all countries in the world. According to the World Health Organization (WHO), countries in Africa, Asia, and Latin America use herbal medicine as a complement to the primary treatment they receive. The advantage of herbal medicine lies in its natural ingredients so that side effects can be minimized (Hidayah *et al*., 2015). Dayak onion (*Eleutherine palmifolia*) is a plant of Kalimantan that is commonly used by people in the Kalimantan Central to be a potion or traditional medicine (Narko *et al*., 2017).

Dayak onion bulbs (*Eleutherine palmifolia*) contain flavonoid secondary metabolite compounds (Hidayah *et al*., 2015). In addition, dayak onion bulbs (*Eleutherine palmifolia*) also contain secondary metabolite compounds of the naphthoquinone group and its derivatives such as elecanacin, eleutherin, eleutherol, eleutherinol, eleutherinon, eleuthoside B, and eletherinoide A (Narko *et al*., 2017).

According to Muti'ah, Dayak onion is one of the feed additives that contains very complete active compounds. These compounds include flavonoids, alkaloids, steroids, glycosides, phenolics, saponins, and tannins. Flavonoids are one of the secondary metabolites. Their presence in the leaves is influenced by the photosynthesis process, so young leaves do not contain too many flavonoids (Kesumasari, *et al*., 2018). Some flavonoid functions for plants include growth regulation, photosynthesis regulation, antimicrobial properties, and antiviral properties (Kurniawan, *et al*., 2017). The mechanism of flavonoid compounds as antidiarrheals inhibits intestinal motility, thereby reducing fluid and electrolyte secretion and prolonging intestinal transit time (Inayathulla, *et al*., 2010).

Tannins are astringent substances (adstringensia) that function to shrink the intestinal mucous membrane and pores. This inhibits the secretion of fluids and electrolytes and is thought to block the absorption of germs and toxins while reducing excessive fluid expenditure. These astringent properties can alleviate diarrhea by shrinking the intestinal mucous membrane and depositing proteins on the intestinal surface, making the intestines more resistant to irritation or stimulation from chemical compounds that cause diarrhea, bacterial toxins, and induction of diarrhea by *Oleum ricini* (Tjay *et al*,. 2007). As a chelator, tannins have a spasmolytic effect that can constrict the intestines, reducing intestinal peristalsis (Pratiwi *et al* .,2015). Another effect of tannins as antibacterials causes diarrhea by inhibiting bacterial growth through changes in the permeability of the cytoplasmic membrane (Kayaputri, *et al*., 2014).

Additionally, according to research conducted by Subramaniam, Dayak onions have antimicrobial activity effects on several bacterial pathogens such as Shigella sp, Klebsiella, Lactobacillus, Streptococcus sp, Salmonella sp, Pseudomonas sp, and Bacillus sp, which are mostly pathogens that cause diarrhea. This makes Dayak onion a great potential as an antimicrobial.

The number of studies on the utilization of Dayak onion bulbs (*Eleutherine palmifolia*) is still lacking, inspiring researchers in this study. Therefore, the researchers aim to prove that the ethanol extract of Dayak onion bulbs (*Eleutherine palmifolia*) Merr has antidiarrheal properties in mice (*Mus musculus*) induced by *oleum ricini* through an examination of intestinal histopathology in male mice (*Mus musculus*).

Oleum ricini derives its name from castor oil, which is a substitute for castoreum, a basic perfume ingredient. The plant (*Ricinus communis* L.) belongs to the *Euphorbiaceae* family. Castor oil can be used for medical applications, such as overcoming constipation, but in this case, it is used as a diarrhea inducer. Castor oil contains *ricinoleic acid* (90%), *linoleic acid* (4%), *oleic acid* (3%), *stearic acid* (1%), and *linolenic acid* less than 1% (Venkatesh *et all.,* 2023).

Based on the explanation of the previous research, this study aims to evaluate the effectiveness of Dayak onion extract on antidiarrheal activity and improvement of duodenal histopathology in male mice (*Mus musculus*) induced by *oleum ricini*.

Figure 1. Dayak onion samples *(Eleutherine palmifolia (L.) Merr.)*

METHODS:

Male white mice (*Mus musculus*) weighing 12-30 mg, acclimatized for 1 week in order to adjust to the environment. The test animals were selected as many as 30 mice and divided into 6 groups,M1 Aquadest netral group, M2 NACMC as negative control group,M3 Loperamide as positive control group, and the treatment group using dayak onion extract M4 dose 250mg/KgBW,M5 dose 500mg/KgBW,M6 dose 750mg/KgBW oral induction was carried out for 24 hours treatment and histological testing and data processing will be carried out.

RESULT:

The results showed that the dose of 250 mg/kg BW was effective but not as effective as Loperamide, while the doses of 500 mg/kg BW and 750 mg/kg BW showed effectiveness equivalent to KP(+). Histopathological analysis showed that a dose of 500 mg/kg BW can improve inflammation in the duodenum of mice

CONCLUSION

The conclusion of this study is that Dayak onion extract at a dose of 500 mg/kg BW is effective as an antidiarrheal and is able to improve duodenal histopathology in male mice induced by Oleum Richini.

Introduction

Diarrhea is a common health problem that affects various age groups worldwide. This condition is characterized by increased frequency of bowel movements, often accompanied by loose or liquid stools. Diarrhea can be caused by a variety of factors, including infections, viruses, bacteria, or parasites, as well as factors such as contaminated food, allergic reactions, stress, and certain medical problems. (Hartati, et al., 2018) It can affect a person's quality of life, interfere with daily activities, and in some cases, can lead to dehydration and even death. (WHO., 2021). According to data from the World Health Organization (WHO), there are 2 billion cases of diarrhea in adults worldwide each year. In the United States, the incidence of diarrhea cases reaches 200 million to 300 million cases per year. Approximately 900,000 cases of diarrhea require hospitalization. Worldwide, approximately 2.5 million cases of death due to diarrhea per year. In the United States, diarrhea-related mortality is high in the elderly. One study of national mortality data reported more than 28,000 deaths from diarrhea in 9 years, 51% of deaths occurred in the elderly. In addition, diarrhea is still a cause of death in children worldwide, despite advances in management.

Opiate group as antidiarrhea in this group includes codeine phosphate, loperamide HCl, and a combination of diphenoxylate and atropine sulfate. The use of codeine is 15-60 mg 3 times a day, loperamide 2-4 mg / 3-4 times a day. The effects of this group of drugs include inhibition of propulsion, increased fluid absorption, so that it can improve stool consistency and reduce the frequency of

diarrhea. If given correctly, it is quite safe and can reduce the frequency of defecation by up to 80%. This drug is not recommended for acute diarrhea with symptoms of fever and dysentery syndrome.(Stevani *et all.*, 2016).

Oleum ricini or castor oil is a triglyceride containing the active component ricinoleic acid (Patel, et al., 2017) The mechanism of action of oleum ricini in the small intestine is hydrolyzed by lipase into glycerol and its active substance, ricinoleic acid, which mainly works in the small intestine to stimulate fluid and electrolyte secretion and stimulate intestinal peristalsis (Suliska, et all., 2019). Ricinoleic acid is a component that provides laxative properties to ricinus oil. It is used as a laxative to overcome constipation problems (Amalia, et al., 2012) Ricinoleic acid stimulates intestinal contractions and promotes bowel movements. (Suliska, 2019). Castor oil is a triglyceride that is effective as a laxative. In the small intestine, this oil undergoes hydrolysis and produces ricinolate acid which stimulates the intestinal mucosa, thus accelerating peristaltic motion and causing rapid expulsion of intestinal contents (Ita, 2010). The dose of castor oil is 2 to 3 tablespoons (15 - 30 ml), given on an empty stomach. The effect occurs 1 to 6 hours after administration, in the form of loose bowel movements (Stevani et all., 2016).

Dayak onion (Eleutherine palmifolia (L.) Merr.) is a plant in the Kalimantan forest that is commonly used by the people of the interior of Central Kalimantan as a herbal medicine or traditional medicine. In general, the parts of the plant used are the bulbs and leaves. The experimental animals used were Male white mice (*Mus musculus*) weighing 12-30 mg, acclimatized for 1 week in order to adjust to the environment. The test animals were selected as many as 30 mice and divided into 6 groups,M1 Aquadest netral group, M2 NACMC as negative control group,M3 Loperamide as positive control group, and the treatment group using dayak onion extract M4 dose 250mg/KgBW,M5 dose 500mg/KgBW,M6 dose 750mg/KgBW. Samples of experimental animals in the form of white mice will be selected and those that have a minimum weight of 25 grams, animals that have been selected will be acclimatized first at least 7 days before treatment is given, the goal is that the test animals are not stressed. The mice used are male mice to avoid the influence of the reproductive cycle or pregnancy.

MATERIAL AND METHODS

Place and preparation of dayak onion samples

E. palmifolia plants were purchased from and identified by CV Indonegri (Malang, Indonesia) with specimen No. #23032192892. Simplicia of *E. palmifolia* bulbs were oven-dried and ground into a fine powder using a grinder. Dried ground powder of *E. palmifolia* was extracted by maceration with 95% ethanol and incubated for 24 h. After filtration, the filtrate was collected and the sediment was re-extracted five times. The macerate was filtered using a vacuum pump and evaporated using a rotary evaporator at 42°C to obtain a viscous extract. The ethanol extract was then analyzed using thin-layer chromatography (TLC CAMAG Linomat 5 S/N 210989; Camag, Muttenz, Switzerland) to determine the content of the active compound andrographolide (Cat No.365645; Sigma-Aldrich, St. Louis, MO, USA).

Phytochemical screening of dayak onion samples (Eleutherine palmifolia (L.) Merr.)

This secondary metabolite test was conducted in the phytochemistry laboratory, Faculty of Pharmacy, Hasanuddin University. The test material used in this study was the Dayak onion bulbs Eleutherine palmifolia (Mill.) Urb) obtained in Samarinda City, East Kalimantan. Fresh Dayak onion bulbs were processed into simplicia through a series of processes, namely wet sorting, washing, slicing, drying, and grinding. From 4 kg of fresh Dayak onion bulbs that had been cleaned, 1.8 kg of simplicia powder was produced. Furthermore, the Dayak onion powder was dried using an oven at a temperature of 50-60°C for 2 days.Dayak onion powder was macerated with 1000 mL of 96% ethanol solvent for 3 x 24 hours while stirring occasionally. The macerate results obtained were then separated and evaporated using a rotary vacuum evaporator at a temperature of 50°C to obtain a liquid extract. The liquid extract was then solidified on a hot plate at a temperature of 50°C until thick.The Ethanol extract obtained was then tested to determine the group of secondary metabolite compounds contained in the Dayak onion Ethanol extract. The test was carried out with Iron (III) chloride (FeCl3) and Magnesium (Mg) powder reagents. From the results of the phytochemical test, the results were obtained in the following table 1.

Acclimatization and Preparation of Mice Model *(Mus musculus)*

A total of 30 male mice were divided into six groups, each group consisting of 5 mice. The selected male mice (Mus musculus) were 3-4 months old and had an average weight of 25 grams. The mice were placed in cages with a base of rice husks and covered with wire. The feed given was in the form of pellets, and drinking water was given ad libitum; M1 Aquadest netral group, M2 NACMC as negative control group,M3 Loperamide as positive control group, and the treatment group using dayak onion extract M4 dose 250mg/KgBW,M5 dose 500mg/KgBW,M6 dose 750mg/KgBW. Sonde was needed when inserting the extract into the stomach of the mice. This treatment was carried out for 24 days. On the next day, a necropsy was performed, and the duodenum organs were taken to make histopathology preparations.

Duodenum histopathology

After taking the stomach from the mice, experts from the pathology department of FK UNHAS interpreted it.

RESULTS

Phytochemical screening of dayak onion samples *(Eleutherine palmifolia (L.) Merr.)*

In the phytochemical screening process of secondary metabolites in Dayak onion extract *(Eleutherine palmifolia (L.) Merr.),* the results can be found in Table 1

No	Secondary Metabolites	Result	Picture	Information
1	Flavonoid	Yellow Brownish		+
2	Tanin	Blackish Green		+

Figure 2. Secondary metabolite content of dayak onion *(Eleutherine palmifolia (L.) Merr.)*

Information: (+) Positive for Containing Secondary Metabolite Compounds

Table 1 Effect of Dayak onion on diarrhea frequency after treatment

Based on Table 1 of *Tukey's Post Hoc* test reveals significant differences between negative control groups, including extract (EPBE 250 mg/bw, EPBE 500 mg/bw, EPBE 750 mg/bw), Loperamide, and normal control groups. However, these groups do not differ significantly from normal control groups.

Table 1 Effect of Dayak onion on diarrhea frequency after treatment

Treatment	Mean ± SD[$]	p-value[#]
Na- CMC1%	5.60 ± 0.548	0.013
Loperamide	4.40 ± 0.548*	
EPBE 250 mg/bw	5.20 ± 0.837*	
EPBE 500 mg/bw	5.00 ± 1.000*	
EPBE 750 mg/bw	4.00 ± 0.707**	
Aquadest	5.40 ± 0.548*	

Source: Primary Data

Notes: [#]ANOVA test,[$] Tukey's Post hoc test, significant if ($p<0.05$), (*) significance level against negative control. (**) significance level greater than (*) against negative control.

Negative control there is a significant difference to the extract group, (EPBE 250 mg/bw, EPBE 500 mg/bw, EPBE 750 mg/bw), Loperamide and normal control group. While the Loperamide treatment group and the EPBE extract treatment group (250 mg/bw and 500 mg/bw) and the normal group

against EPBE 750 mg/bw extract there was a significant difference. However, the Loperamide treatment group, EPBE extract treatment group (250 mg/bw and 500 mg/bw) and the normal group were not significantly different.

2. Effects of Dayak Onion on Mucus and Stool Weight

Table 2 Effects of Dayak Onion on Mucus and Stool Weight after Treatment

Treatment	Mean ± SD	
	Mucus Weight (g)$	Soft Stool Weight (g)#
Na- CMC1%	0.122 ± 0.044	0.300 ± 0.023
Loperamide	0.059 ± 0.032**	0.150 ± 0.065
EPBE 250 mg/bw	0.116 ± 0.042*	0.188 ± 0.081
EPBE 500 mg/bw	0.026 ± 0.016***	0.260 ± 0.014
EPBE 750 mg/bw	0.026 ± 0.004***	0.260 ± 0.015
Aquadest	0.094 ± 0.0195*	0.142 ± 0.016

Source: Primary Data

Description: $Tukey post hoc test,# Kruscal-wallis test. (*) significance level against negative control. (**) more significant than (*) and so on.

Based on Table 2 on the Mucus Weight variable, it shows that the negative control has a significant difference to the extract treatment groups (EPBE 250 mg/bw, EPBE 500 mg/bw, EPBE 750 mg/bw) and Loperamide positive control and normal control.However, it is not significant to the EPBE 250 mg/bw extract group. Loperamide variable was significantly better than EPBE 250 mg/bw but significantly not better than EPBE 250 mg/bw, EPBE 500 mg/bw, EPBE 750 mg/bw had no significant difference.

In the mushy stool weight variable, it showed that the negative control did not have a significant difference against all variable groups of EPBE extract (250 mg, 500 mg, and 750 mg) and positive control and normal control ($p<0.05$). However, it tended to be better than the negative control although not significant.

3. Effects of Dayak Onion on Histopathology of the duodenum

The group of treated mice that experienced diarrhea had a histopathological picture of the duodenum given the treatment different from the group of mice without treatment which can be seen based on the results of histopathological observations of duodenal organs (Figure 3.1).

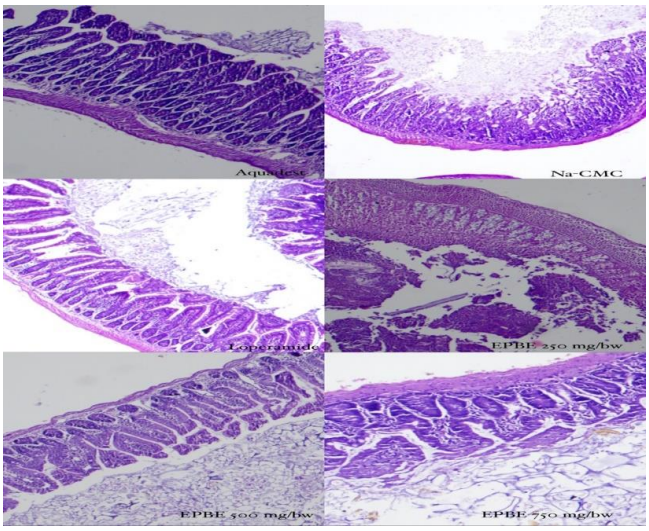

Figure 3.1 Histopathological features of duodenal organs of treated mice at 100x magnification with HE staining, with the description of Non-Treatment (Aquadest), Control (KN-), Positive Control (KP+); Control Dayak Onion with a dose of 250 mg (EPBE 250 mg).

Based on the results of histopathological examination of duodenal organs of treated mice, it can be seen in Figure 3.1 shows that in the normal group (without treatment only aquadests) no abnormalities were found. In the negative control treatment (CMC), abnormalities of surface epithelial erosion and epithelial ulceration were found. In the positive control Loperamide still found abnormalities Desquamation of the epithelium. In the administration of 250 mg/bw dayak onions, surface epithelial erosion and epithelial ulceration were found, at 500 mg/bw dayak onions, epithelial desquamation was found, while at 750 mg/bw dayak onions, surface epithelial erosion abnormalities were found in the duodenum of mice.

This table shows that the negative control showed differences in EPBE (500 mg/bw and 750 mg/bw), Loperamide, and normal control, but was not significant for EPBE 250 mg/bw, EPBE 500 mg/bw, EPBE 750 mg/bw, Loperamide, and normal control.

Table 3 Duodenal Histopathology of Mice

Treatment	Mean Rank	Mean ± SD
Na- CMC1%	14.33	2.333 ± 0.577
Loperamide	6.17	0.333 ± 0.577**
EPBE 250 mg/bw	15.67	2.667 ± 0.577
EPBE 500 mg/bw	6.17	0.333 ± 0.577**
EPBE 750 mg/bw	10.17	1.333 ± 1.155*
Aquadest	4.50	0.000 ± 0.000***

Source: Primary Data

Note: (*) indicates significant level (p<0.05)

Based on Table 3, the histopathology score variable shows that the negative control is significantly different from EPBE extract (500 mg/bw and 750 mg/bw), Loperamide, and normal control. However, it is not significantly different from EPBE 250 mg/bw extract. While EPBE extract 500 mg/bw against EPBE 750 mg/bw is significant and not significant against Loperamide. Normal control was significant against all groups.

DISCUSSION

Based on figure 2, before the intervention was carried out on all experimental groups, the initial stage was phytochemical screening with the aim of determining the secondary metabolite compounds contained in the dayak onion extract to be used. From the results of the phytochemical screening test in figure 2, it was obtained that the extract contained tannin and flavonoids. This is because the tannin compound has strong astringent properties. When interacting with the intestinal mucosa, tannin will precipitate protein to form a strong protective layer. This layer plays a dual role: first, as a shield that protects the intestinal mucosa from irritation and inflammation which are often the cause of diarrhea. Second, this layer also functions as a barrier that reduces fluid secretion from mucosal cells. This, the volume of stool is reduced and the frequency of bowel movements becomes less frequent, alleviating diarrhea symptoms (Adrianto *et al*., 2017; De Jesus *et al*., 2012).

Based on some of the results obtained, it is found that in Table 1, shows that there is a significant difference between the negative control and all variable groups with a value of p = 0.13. Negative group with Mean ± SD value (5.60 ± 0.548) and EPBE extract treatment group (250 mg/bw, 500 mg/bw, 750 mg/bw) with Mean ± SD value respectively (5.20 ± 0.837, 5.00 ± 1.000, 4.00 ± 0.707), loperamide with Mean ± SD value (4.00 ± 0.707), and normal control group with Mean ± SD value (5.40 ± 0.548).

The negative control, which is a group that is only induced with *Oleum Ricini* + CMC without any additional treatment, shows significant differences to all treatment groups, namely dayak onion extract at doses of 250 mg/bw, 500 mg/bw, and 750 mg/bw, as well as loperamide. This explains that EPBE extract at all doses tested showed statistically different effects compared to the negative control group.

This significant difference explains that EPBE extract has the potential to reduce diarrhea symptoms caused by *Oleum ricini*. Based on the previous explanation above, that Dayak onions contain secondary

metabolite compounds tannins and flavonoids, also because the flavonoids in Dayak onion extract can inhibit the secretion of lysosomal enzymes and capillary permeability. This reduces the release of histamine and other inflammatory factors, and reduces inflammation in intestinal tissue. This, dayak onion extract can reduce inflammation, which is the main cause of diarrhea symptoms. This delay is related to the ability of tannins to slow down bowel movements and reduce excess fluid secretion. In addition, diarrhea is also usually caused by bacteria, diarrhea can also be caused by bacteria including *Escherichia coli, Salmonella,* and *Shigella sp*.(Narko *et al.,*2017 *)*

The normal control group, which was not induced with *Oleum Ricini*, showed significantly different results from the negative control. This indicates that the normal control group did not experience diarrhea because it was not treated and the negative control group was induced with oleum ricini. This explains that *Oleum ricini*, or castor oil, causes diarrhea in mice through several main mechanisms. The active component in this oil, *ricinoleic acid*, stimulates prostaglandin receptors in the intestinal wall, which increases intestinal motility by accelerating smooth muscle contractions. This results in faster movement of feces through the digestive tract. In addition, *ricinoleic acid* increases fluid secretion from intestinal cells and has an osmotic effect by drawing water into the intestinal lumen, which increases fecal moisture. *Oleum Ricini* can also interfere with intestinal epithelial function by altering ion transport and damaging the protective layer of the intestine, reducing fluid absorption, and affecting secretion. The combination of these effects leads to an increase in defecation frequency and a decrease in stool consistency, which overall causes diarrhea in mice.(Irwinmwiduwa *et al*.,2023)

Meanwhile, the Loperamide treatment group and the EPBE extract treatment group (250 mg/bw and 500 mg/bw) and the normal group to 750 mg/bw EPBE extract there were significant differences. This is because, in the 750 mg EPBE extract group, Dayak onion extract at high doses shows better effectiveness as antidiarrheal. High doses of 750 mg EPBE extract may contain greater concentrations of active compounds such as flavonoids and tannins possessed by Dayak onions that have high potential in reducing diarrhea symptoms. Such compounds work by inhibiting the secretion of intestinal fluid and reducing gastrointestinal motility, providing a strong therapeutic effect.

The research conducted by Efendi 2015 which shows that dayak onion extract can reduce diarrhea symptoms in mice induced by *oleum ricini*. In this study, a dose of 750 mg/bw showed higher effectiveness in overcoming diarrhea symptoms (Efendi *et al*.,2015).

The loperamide treatment group, dayak onion extract at doses of 250 mg/bw and 500 mg/bw, and the normal control group did not show significant differences. However, when compared between the three treatment groups, the group receiving loperamide showed higher effectiveness compared to the group receiving dayak onion extract at a dose of 500 mg/bw. In addition, the dayak onion extract group at a dose of 500 mg/bw also showed better effectiveness than the group receiving the extract at a dose of 250 mg/bw. Dayak onion extract at doses of 250 mg/bw and 500 mg/bw showed antidiarrheal effects, but the level of effectiveness varied. Administration of the 250 mg/bw dose may not have been sufficient to achieve a significant therapeutic effect, while the 500 mg/bw dose, although more effective, has not yet reached a level of effectiveness equivalent to loperamide.

Loperamide is an effective antidiarrheal agent, working by the mechanism of binding to μ (mu) opioid receptors located in the intestinal wall. Activation of these receptors reduces intestinal motility and fluid secretion.(Nazir *et al.,*2021) By binding to μ receptors, loperamide also decreases the secretion of fluid and mucus from the digestive glands. This reduces the volume of fluid entering the intestinal lumen, thereby helping to reduce the frequency of stool((Irwinmwiduwa *et al*.,2023).

Based on Table 2**,** the variable weight of mucus, shows that the negative control has a significant difference from the treatment group (EPBE 250, 500 and 750 mg/bw) and Loperamide positive control and normal control. This is because *Oleum Ricini*, which is known as a diarrhea inducer, significantly affects mucus weight in mice. The increase in mucus weight in the negative control group explains that Oleum Ricini causes an increase in mucus secretion in response to the induced diarrhea condition.

While in the variable group Loperamide was significantly better than EPBE 250 mg/bw but significantly not better than EPBE 500 mg/bw and 750 mg/bw, while EPBE 500 mg/bw and 750 mg/bw were not significant. This is because Loperamide, which is a clinically proven antidiarrheal

drug, works by binding to μ (mu) opioid receptors in the intestinal wall, which inhibits intestinal motility and reduces fluid and mucus secretion in the gastrointestinal tract. This mechanism of action effectively reduces stool frequency and mucus volume, making it more efficient in addressing mucus secretion compared to the low dose of EPBE extract (250 mg/bw) which may not have reached the optimal concentration of active compounds for the same effect. Conversely, EPBE extract at a dose of 250 mg/bw may not have reached the right dose, thus unable to provide comparable effects to loperamide(Irwinmwiduwa *et al*.,2023).

In contrast, the dayak onion extract group at doses of 500 mg/bw and 750 mg/bw showed higher effectiveness in reducing mucus secretion compared to loperamide. This is influenced by higher doses of EPBE extract which may contain a greater concentration of active compounds, which can increase the reduction of mucus secretion.

Increasing the dose of dayak onion extract from 500 mg/bw to 750 mg/bw did not show a significant increase in effect as the 500 mg/bw dose may have reached the optimal level of therapeutic effectiveness for reducing mucus secretion. In this case, the 500 mg/bw dose appears to be close to the maximum level of effectiveness, so increasing the dose does not provide any additional benefit. As a result, there was no statistically significant difference between the 500 mg/bw and 750 mg/bw doses in the EPBE extract group, indicating that the 500 mg/bw dose was sufficient to effectively reduce mucus secretion.

In the Soft Stool Weight variable, it showed that the negative control did not have a significant difference against all EPBE variable groups (250, 500, and 750 mg/bw) and the positive control and normal control ($p<0.05$). However, it tends to be better than the negative control although not significant. This is because tannins in dayak onions can help increase water absorption in the intestine. With increased water absorption, the consistency of feces will become denser and the weight of mushy feces will decrease (Efendi *et al*.,2015).

In addition, the active substances in Dayak onion can reduce intestinal motility, which means that bowel movements become slower. Thus, food will stay longer in the large intestine, providing more time for absorption of water and nutrients, which will result in denser stools and reduce the frequency of diarrhea. Although the results obtained in the study were not significant, there was a reduction in the weight of mushy feces after being treated with Dayak onion (Efendi *et al*.,2015).

In Table 3 and Figure Histopathology Duodenum Mice showed that the administration of dayak onion 500, and 750 mg/bw was able to improve the duodenum of mice significantly compared to abnormalities in the negative control although not yet able to improve as in the duodenum of normal group mice. However, the administration of dayak onion 250 mg/bw, has not been able to improve the duodenum of mice. Where as the administration of 500 mg/bw dayak onions has the same effect as the Loperamide control in improving the duodenum of mice and better than the administration of 750 mg/bw dayak onions. This can be caused by high doses that can have side effects.

This is explained in the findings of research conducted by Lestari 2019 which showed that a dose of 750 mg/bw of dayak onion extract can improve inflammation in the duodenum of mice, but can also damage duodenal histopathology. This suggests that higher doses can have significant side effects on intestinal tissue.(Lestari *et al*.,2019)

The effectiveness of the 750 mg/bw dose in overcoming diarrhea may be accompanied by side effects, such as damage to the histopathology of the duodenum of mice. This effect may occur because high doses of dayak onion extract can cause irritation or oxidative stress to intestinal tissues, affecting the integrity of the mucosal layer and increasing cellular damage. High doses may trigger an inflammatory response or local toxicity that damages the histological structure of the intestine (Thedsawad *et al*.,2019).

CONCLUSION

Based on the results of the study, it can be concluded that 500 mg/bw dayak onion extract (*Eleutherine palmifolia)* is effective as antidiarrheal and able to improve the histopathology of *the Duodenum of* Male Mice (*Mus Musculus*) Induced by *Oleum Richini.*

ACKNOWLEDGMENT

The author would like to thank the Hasanuddin University Postgraduate School, parents, and the Ministry of Education and Culture's superior scholarships that have supported the course of this research, friends, and all those involved in helping this research. This research was supported by CV. Indonesia Green Innovation, Malang, East Java.

AUTHOR CONTRIBUTIONS

SKN was in charge of collecting data and had the role of the main researcher, S was in charge of revising and improving the data collection process. AK was in charge of assisting the final process of the research.

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