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Efficacy of Shallots Infusion (*Allium Cepa L.*) as an Antidiarrhea in Mice (*Mus Musculus*)

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ABSTRACT

Shallots have been used for generations as traditional medicine, one of which is used in the treatment of diarrhea. This study determined the antidiarrheal activity of the shallot bulbs using intestinal protection and intestinal transit methods, and to determine the effective concentration that gives the best antidiarrheal effect in mice. This study used thirty mice and divided into six groups. The negative control group was given Tween 80 (1%), positive control group was given Loperamide HCl, and four various concentrations of shallot infusion treatment groups (1.25%, 2.5%, 5%, and 10 %). The treatment was given by the oral route. The parameters used in the intestinal protection method were frequency of defecation and feces consistency, while in intestinal transit method the parameters used were the percentage ratio of the marker trajectories compared to the overall length of the intestine. The results showed that the 1.25% concentration of shallot infusion indicated the best antidiarrheal activity and had a smaller ratio or stronger inhibition to intestinal peristaltic compared to all other concentrations, 2.5%, 5%, dan 10%. We concluded that shallot bulbs infusion with a concentration of 1.25% has the most effective anti-diarrheal effect. This research can be continued by measurement of other diarrheal parameters such as stool weight, as well as start and stop time of diarrhea to further clarify the anti-diarrheal potential of shallot bulb infusion.

Keywords: antidiarrheal, infusion, intestinal protection, intestinal transit, shallot

ABSTRAK

Umbi bawang merah telah digunakan oleh generasi terdahulu untuk pengobatan tradisional, salah satunya adalah untuk pengobatan diare. Penelitian dilakukan dengan tujuan mengetahui aktivitas antidiare umbi bawang merah yang diuji dengan metode proteksi intestinal dan transit intestinal. Selain itu bertujuan untuk menentukan konsentrasi efektif dari umbi bawang merah yang mampu menjadi antidiare terbaik pada mencit. Penelitian ini menggunakan 30ekor mencit yang dipisahkan menjadi enam kelompok perlakuan. Kelompok kontrolnegatif dengan pemberian Tween 80 (1%), kontrol positif dengan pemberian Loperamid HCl, dan empat kelompok konsentrasi bertingkat infusa bawang merah (1.25%, 2.5%, 5%, dan 10 %). Rute pemberian perlakuan adalah peroral. Parameter frekuensi defekasi dan konsistensi feses digunakan dalam evaluasi metode proteksi intestinal. Sedangkan perbandingan dari persentase rasio lintasan penanda dengan panjang usus keseluruhan menjadi parameter pada metode transit intestinal. Hasil penelitian menunjukkan bahwa konsentrasi infusa bawang merah 1.25% memiliki aktivitas antidiare paling baik dan memiliki rasio lebih kecil atau lebih kuat menghambat gerak peristaltik usus dibandingkan dengan konsentrasi 2.5%, 5%, dan 10%. Sehingga dapat disimpulkan bahwa infusa umbi bawang merah dengan kosentrasi 1.25% memiliki efek antidiare paling efektif. Penelitian ini dapat dilanjutkan dengan pengujian parameter seperti bobot feses, waktu mulai dan waktu henti diare yang diharapkan dapat memperjelas potensi antidiare dari infusa umbi bawang merah.

Kata kunci: antidiare, bawang merah, infusa, proteksi intestinal, transit intestinal

INTRODUCTION

Diarrhea is a public health problem, especially in developing countries such as Indonesia where the incidence of diarrhea ranging from 200 - 374 cases in 1000 population, and 60-70% of which are children under five years old. the death rate for diarrhea reached 7.4% in Indonesia, while the death rate due to persistent diarrhea was higher, reaching 45% (Rizal et al., 2017).

Diarrhea is an endemic disease with the potential to sporadically develop into a fatal epidemic. Epidemic of diarrhea occurred three times in three provinces during 2016, with 198 cases and six fatalities. The epidemics of diarrhea increased rapidly to 21 incidents in 2017 in 12 Provinces and 17 Regencies, with a total of 1,725 cases and 34 fatalities. In 2018 epidemic diarrhea occurred ten times in 8 Provinces and eight Regencies, with a total of 756 cases and 36 fatalities (Utaminingtyas and Harahap, 2021).

Indonesia has more than 30,000 plant species in its tropical forests, and nine thousand six hundred species are known for their medicinal properties. From time to time, various ethnic groups in Indonesia have used approximately 940 plants believed to have medicinal properties (Siregar, 2017).

The use of herbal plants is traditionally considered safer than modern medicine because herbal medicines have been believed to have fewer side effects than modern ones (Bustanussalam, 2016). Several synthetic antidiarrheal drugs could cause unwanted side effects. Limited access, high prices, and the threat of resistance are also other problems in the use of modern medicinal preparations. In this instance, alternative herbal medicines from safer natural ingredients could be used as a safe and attainable substitution to the use of synthetic medicines (Fokam et al., 2019).

Plants used as antidiarrheal medicine due to secondary metabolite compounds contained, that are active to reduce clinical signs and usually also have antibacterial effect to pathogens causing diarrhea. One plant traditionally used as a herb to treat diarrhea is shallot (*Allium cepa L.*) (Simanjuntak, 2021). Shallot bulbs contain alkaloids, flavonoids, glycosides, essential oils, saponins, steroids, tannins, and triterpenoids which can act as anti-diarrhea and antibacterial (Setiawan et al., 2021). However, scientific evidence to support the pharmacological use of shallot bulbs as anti-diarrhea has been very rare. This study was aimed to determine the antidiarrheal effectiveness of shallot (*Allium cepa L.*) bulbs using the intestinal protection and transit methods and to determine the effective concentration of shallot bulb that gave the best antidiarrheal effect in mice.

MATERIALS AND METHODS

Animal Ethics Clearance

This research received animal ethics approval from the animal ethics commission, School of Veterinary Medicine and Biomedical Sciences, IPB University, with number 010/KEH/SKE/II/2022, on 16 February 2022.

Time and Place of Research

This research was conducted at the Pharmacy Laboratory and Laboratory Animal Management Unit (UPHL), School of Veterinary Medicine and Biomedical Sciences, IPB University (SKHB IPB

University), from November 2021 – March 2022.

Tools and Materials

The equipments used were mouse cages, plastic boxes, wire cage covers, drinking water bottles, digital scales with an accuracy of 0.01 g, filter cloth, gastric sonde, 1 ml syringe, measuring tape, minor surgical tools, blender, tube, parchment paper, stirring rod, measuring cup, measuring flask, infusion pot, thermometer, mortar, and stamper.

The materials used were male mice (*Mus musculus*) DDY strain (*Deutschland Denken Yoken*) aged 2-3 months with 25-30 g of body weight, dried powdered simplicia of shallot bulbs (*Allium cepa L.),* tween-80, distilled water, castor oil (*oleum ricini*), loperamide HCl, paper, Chinese ink, ketamine, xylazine, husk (bedding), mouse feed, and water.

Shallot Bulb Extraction

As much as 10 g of dry shallot powder simplicia was put into an infusion pot, then distilled water was added (100 ml). The infusion pot was heated for 15 minutes, starting when the boiling temperature reaching 90°C. The boiling liquid was poured into a measuring cup, and distilled water was added until the volume showed 100 ml (replacing the distilled water lost due to heating), allowed to drain and was filtered using a filter cloth. The infusion obtained was diluted to a concentration of 1.25%, 2.5%, 5%, and 10% w/v.

Experimental Animals

The mice used were in good health. The feed ration given to mice was a ration according to the nutritional standards of BPOM RI (Food and Drug Supervisory Agency of the Republic of Indonesia). Drinking water was given ad libitum. Mice were kept under normal environmental conditions with a temperature of 25 – 28°C.

Preparation of Tween 80 (1%) Solution

One ml of tween 80 was put in a measuring cup. Aquades were added until the volume reached 100 ml and stired using a stirring bar.

Preparation of Loperamide HCl Solution

Loperamide HCl solution 1.56% of the concentration was prepared by grinding loperamide HCL tablets at a 2 mg/tablet dose. A total of 0.087 g of loperamide HCl powder was added to 1 ml of tween 80 solution and then crushed until homogeneous, and distilled water was added until the volume of 100 ml.

Experimental Design

This study used 30 mice which were used in two methods (intestinal protection and intestinal transit). Mice in each method were divided into six groups of five mice. Mice used in the intestinal protection method were acclimatized for one week before being used in the intestinal transit method. The negative control was given tween 80 solutions (1%), the positive control was given loperamide HCL solution with a concentration of 1.56%, the treatment group of concentration of 10% was given an infusion of shallot bulbs with a concentration of 10%, the group of concentration of 5% was given an infusion of shallots with a concentration of 5%, the group of concentration of 2,5% was given 2.5% shallot bulb infusion, while the group of 1.25% concentration was given 1.25% shallot bulbs infusion.

Intestinal Protection Method

The research was started with one week of acclimatizing the mice. The mice fasted for two hours before the intestinal protection method antidiarrheal test was conducted. The mice that had been fasted were then treated with 1 ml of each treatment set. Mice treated were then given 0.5 ml of castor oil orally 30 minutes after the treatment. Each mouse was then put into a plastic box lined with paper and observed for the frequency of defecation, and the feces consistency were observed every 30 minutes for four hours.

Intestinal Transit Method

Mice used in this method were mice previously used in the experiment using the intestinal protection method. The mice were rested for one week after being used in the intestinal protection method. Mice were then fasted for 18–22 hours before being treated. The mice that had been fasted were then treated with 1 ml of each treatment set. The mice were then force-fed with Chinese ink at a dose of 0.1 ml/10 g BW 45 minutes after the treatment was given. After 20 minutes, the mice were anesthetized using a combination of ketamine-xylazine and killed by the cervical dislocation. The mice were then dissected, and the intestines were prepared. The length of the intestine stained with Chinese ink and the length of the whole intestine were measured. The ratio between the length of the colored intestine and the total length of the intestine was calculated.

Data Analysis

Data on feces consistency, defecation frequency, and the ratio of intestinal stained compared to total intestinal length were processed quantitatively using Minitab 18 with the one-way Analysis of Variance (ANOVA) and the Tukey test method.

RESULT AND DISCUSSION

The antidiarrheal effectiveness test of shallot bulb infusion on the intestinal protection method was carried out by observing parameters of frequency of defecation and feces consistency. The potential effectiveness of shallot bulb infusion antidiarrheal was indicated by a decrease in the frequency of defecation parameters and an increase in feces consistency in the treatment group compared to the control group. The data of defecation frequency and feces consistency were shown in **Table 1**.

Data showed that the negative control group differed significantly from the other treatment groups ($p < 0.05$). The negative control test group showed an average defecation frequency of 6.8 times with an average feces consistency of 17.2. The positive control group obtained an average defecation frequency of 2.6 times with an average feces consistency of 3.4. The 10% concentration group showed an average defecation frequency of 3.6 times with an average feces consistency of 3.6. The 5% concentration group showed an average defecation frequency of 2.2 times with an average feces consistency of 2.6. The 2.5% concentration group showed an average defecation frequency of 2.6 times with an average feces consistency of 2.6. The 1.25%

concentration group showed an average defecation frequency of 1.2 times with an average feces consistency of 1.6. The data showed that all test groups had antidiarrheal activity because they had an average value in both parameters lower than those in the negative control group.

Table 1. Average Stool Frequency and Consistency of Antidiarrheal Test with the Intestinal Protection Method of Shallot (*Allium cepa L.*) Infusion in Mice (*Mus musculus*)

Group	Frequency of Defecation (times)	Feces Consistency
Negative Control Group	6.800 ± 0.447^{a}	17.20 ± 8.90^{a}
Positive Control Group	2.600 ± 2.074^{b}	3.400 ± 2.79^{b}
10% shallot bulbs infusion	3.600 ± 1.517^{b}	3.600 ± 1.517^{b}
5% shallot bulbs infusion	2.200 ± 1.924^{b}	2.60 ± 2.074^{b}
2.5% shallot bulbs infusion	2.600 ± 1.517^{b}	2.60 ± 1.517^{b}
1.25% shallot bulbs infusion	1.200 ± 0.837^{b}	1.600 ± 1.517^{b}

All groups of shallot bulb infusion concentrations had observable antidiarrheal activity because they could reduce the frequency of defecation and improve feces consistency. The shallot bulb infusion group with a concentration of 1.25% showed the best average results in both intestinal transit protection test parameters when compared to other concentrations.

The antidiarrheal effectiveness test of the intestinal transit method was carried out by calculating the percentage of marker passage ratio compared to the total length of the intestine. The antidiarrheal potential of shallot bulb infusion is indicated by a decrease in the percentage of marker passages to the total length of the intestine of mice in the treatment groups compared to the control group. The average results of calculating the percentage ratio of marker passages compared to the overall intestinal length are shown in **Table 2**.

Table 2. Average Results of The Ratio of Antidiarrheal Test Markers using the Intestinal Transit Method of Shallot (*Allium cepa L.*) infusion in mice (*Mus musculus*)

Group	Marker Pass Ratio (%)
Negative Control Group	78.94 ± 3.35^{a}
Positive Control Group	42.99 ± 12.14^{bc}
10% shallot bulbs infusion	48.90 ± 12.94^{bc}
5% shallot bulbs infusion	50.68 ± 12.34^{bc}
2.5% shallot bulbs infusion	53.47 ± 19.06^{b}
1.25% shallot bulbs infusion	27.47 ± 11.04^{c}

The marker passages ratio to overall intestinal length showed that the negative control group was significantly higher than the other treatment groups ($p<0.05$). All groups of shallot bulb infusion with various concentrations could reduce the percentage ratio of marker passages to the total intestinal length. The 1.25% concentration group showed better feces consistency than the average value of the control group and other concentration groups. The 1.25% concentration group had a smaller ratio of marker passage to the total length of intestine, compared to the other concentrations. This indicated that the 1.25% concentration group was more potent in inhibiting intestinal peristalsis than other shallot infusion in other concentrations.

This study used *Oleum ricini* or castor oil as an inductor of diarrhea because lipase enzymes will hydrolyze castor oil in the small intestine to glycerol and ricinoleic acid (Rizal et al., 2017). According to Supborini et al. (2022), ricinoleic acid is a laxative that changes the permeability of the intestinal mucous membrane, accelerates intestinal motility, and releases prostaglandins as a result of inflammation. Prostaglandins will inhibit the absorption of sodium and fluids, thereby increasing fluid secretion in the intestinal lumen and causing the feces consistency to become softer and watery (Yakubu et al., 2015).

A compound that can reduce intestinal motility, protect and prevent the inflammation in the intestinal mucous membrane, absorb poisons and toxins, or provide electrolyte fluids, has a potential to be used to treat diarrhea. Loperamide was chosen in this study as a positive control drug because loperamide can restore the reabsorption-secretion balance of intestinal mucosal cells (Ambari, 2019). Loperamide also inhibits peristaltic movement, prolongs transit time, improves intestinal mucosal absorption of water and electrolytes, reduces feces volume, increases viscosity, and prevents water and electrolyte loss. This is obtained by inhibiting the release of acetylcholine from the myenteric plexus and restoring cells that are in a hypersecretory state back to their normal state (Suproborini et al., 2022).

The shallot bulb infusion in this study was shown to have antidiarrheal activity by reducing the frequency of defecation and improving feces consistency and reducing the ratio of marker passages to total intestinal length. This anti-diarrhea effect might be related to flavonoids and tannins contained in shallot bulbs (Setiawan et al., 2021).

This can be obtained by reducing the extracellular calcium concentration in intestinal smooth muscle, inhibit the action of protein kinase C, cyclic Adenosine Monophosphate (cAMP) phosphodiesterase, and inhibiting the release of acetylcholine, prostaglandins, and histamine (Gálvez et al., 2001).

Tannins have been shown to reduce the rate of intestinal peristaltic and increase the reabsorption of water and electrolytes in the intestine (Yu et al., 2020). Tannins also act as astringents that shrink the pores of the mucous membranes of the intestine so they can reduce water secretion (Gultom et al., 2021; Russo et al., 2018). Tannins have some antioxidant activities that helps protect the large intestine and small intestine in the early phase of chronic diarrhea through the mechanism of protecting the intestine from cell death due to oxidation (Ren et al., 2012).

CONCLUSION

This study indicate that the infusion of shallot (*Allium cepa L.*) bulbs has an antidiarrheal activity. Infusion of shallot bulbs with a concentration of 1.25% had the most effective antidiarrheal effect compared to other concentrations. Further testing of the antidiarrheal properties of shallots with other parameters of observation such as feces weight, diarrhea start and stop time is warranted to further clarify the antidiarrheic potential of shallot bulb infusion.

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