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Evaluation of Antidiarrheal Activity of the Ethanol Extract *Leucaena leucocephala* (Lam) de Wit Seed

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Abstract

BACKGROUND: *Leucaena leucocephala* belongs to the Leguminosae/Fabaceae family. *L. leucocephala* seeds contain alkaloids, flavonoids, and tannins which according to the previous research have antidiarrhea activity.

AIM: This study was investigate the antidiarrheal activity of the ethanol extracts of *Leucaena leucocephala* (Lam) de Wit seeds induced by oleum ricini and intestinal transit methods for rats.

MATERIALS AND METHODS: *L. leucocephala* seeds were extracted by maceration with 80% ethanol. Evaluation of antidiarrheal extract activity was performed by induction of oleum ricini and intestinal transit methods. The extract at dose doses of 50, 100, 200, and 400 mg/kg BW was orally administered to the animals 1 h after induction by oleum ricini. Then diarrhea time, frequency, consistency, stool weight, and duration of diarrhea were observed every 30 min for 6 h. In determining the intestinal transit method, a percentage of the distance of the Chinese ink determined. This study was used positive control as Loperamide (1 mg/kg BW) and 0.5% Na-CMC as a negative control.

RESULT: In diarrhea induced by castor oil, *L. leucocephala* seed extract at doses of 100, 200, and 400 mg/kg bw has been shown to significantly delay the onset of diarrhea, reduce diarrhea frequency, stool weight and duration of diarrhea compared with Na CMC as a negative control ($p < 0.05$). The extract at a dose of 400 mg/kg bw did not differ significantly from loperamide as positive control ($p > 0.05$). In this study, *L. leucocephala* extract reduced the distance traveled by Chinese ink in the intestine but only at a dose of 400 mg/kg bw which has a comparable activity with loperamide significantly. The antidiarrheal activity of extract showed at a dose dependent manner.

CONCLUSION: The ethanol extract of *L. leucocephala* seeds has antidiarrheal activity which supports its use in folk medicines.

Introduction

Diarrhea is a disease whose incidence is still quite high, according to the WHO there are 2 billion cases of diarrheal disease that occur every year in the world and 1.9 million children are declared dead due to diarrhea every year according to data obtained from UNICEF. The total data obtained represent 18% of all deaths that occur in children under 5 years, which means more than 5000 children almost die every day due to diarrhea. There are 78% of cases of diarrheal disease that occurs from all child deaths due to diarrhea in Africa and Southeast Asia [1]. Diarrheal disease causes more than 5–8 million deaths worldwide each year at the age of <5 years [2].

Diarrhea is a common problem for people who suffer from frequent and watery stool. Most cases of diarrhea are caused by disruption of water and electrolyte transport in the intestine. The mechanism of diarrhea occurs due to increased intestinal osmotic pressure (thus causing water retention in the lumen); electrolyte secretion which causes excessive water in the intestinal lumen; fluid and protein exudation out of the mucosa; and changes in intestinal peristaltic thereby accelerating transit. This usually occurs from various interrelated processes, which cause an increase in stool volume and weight accompanied by percent water content [3]. Most cases of diarrhea due to intestinal irritation by pathogens that affect the intestinal mucosal layer can result in increased secretory yield. This irritation affects intestinal peristaltic increment which causes a lot of fluid and electrolyte to be wasted. Individuals who have severe diarrhea can die [4].

The plant *Leucaena leucocephala* (Lam.) de Wit according to the previous research has various activities in medicine such as antidiabetic, antioxidant, antibacterial, and cancer preventive [5], [6]. In Thailand *L. leucocephala* leaves are used for diarrhea medicine[7]. The people of Mexico and Zimbabwe utilize *L. leucocephala* leaves for animal feed which increase livestock milk production [8], [9]. In Peru, bark and flowers of *L. leucocephala* are used as antiseptics [10]. *L. leucocephala* contains alkaloids, saponins, flavonoids, tannins, mimosin, leukanin, proteins, fatty acids, and fiber [6], [10], [11], [12]. Flavonoids also have an antidiarrheal effect by inhibiting intestinal motility thereby reducing fluid and electrolyte secretion [13]. Tannin compounds are as astringent, tannin works as

astringent by wrinkling the surface of the small intestinal mucosa and stimulating water absorption in the lumen. This condition can ultimately reduce diarrhea [14]. This study was constructed and aimed to investigate the ethanol extract of *L. leucocephala* seeds as antidiarrheal using two different methods; these include castor oil-induced diarrhea and intestinal transit methods.

Materials and Methods

Plant collection and Extraction

The seeds of *L. leucocephala* (Leguminosae) were collected from Batang Kuis, Deli Serdang, Sumatera Utara, Indonesia). The plant identified and authenticated by the Indonesian Institute of Sciences (LIPI) Bogor – Indonesia (approval number 904/IPH.1.01/If.07/V/2019). The preparation of L. *leucocephala* semen extract was carried out by maceration of 1200 g of simplisia powder with 80% ethanol solvent. Soak for the first 6 h while stirring occasionally, then let stand for 18 h. Maserati are accommodated and then separated by sedimentation, then filtered. The search process is carried out twice. The Maserati were collected. Then, the filtrate was concentrated at 40 ± 2°C in vacuum evaporator and thickened by heating in waterbath at 40 ± 2°C.

Animals

The use of rats was approved by the Animal Research Ethics Committees of Universitas Sumatera Utara. The experimental animals used were male white rats weighing 150–200 g divided into six groups and each group of five rats. Before being used, the rats were acclimatized for 7–14 days with the environmental conditions of the experiment with 12 h of light and 12 h of darkness.

Castor oil-induced diarrhea

Evaluation of antidiarrheal effects caused by castor oil is carried out with the method described by Yuandani and Nazira [15]. Rats were divided into several groups, which included negative controls, positive controls, and treatment groups. Before the investigation, all animals were not given food for 18 h but could consume water. Each rat was given 2 ml castor oil, then after 1 h the rats were given 0.5% Na-CMC suspension (50 mg/kg BW) orally as a negative control group, loperamide suspension (1 mg/kg BW) as a group positive control, and *L. leucocephala* extract (50, 100, 200, and 400 mg/kg BW) orally for each group. The rats were placed in cages which have been layered with weighted paper and observed every 30 min after treatment for 6 h. The parameters observed include the onset of diarrhea, the frequency of diarrhea, the consistency and weight of stool and the duration of diarrhea.

Intestinal transit method

The anti-motility activity of extract was investigated using transit intestinal methods as described previously by Yuandani and Nazira [15]. The rat groups was divided into negative control, positive control, and treatment groups. Before the investigation, all animals were not given food for 18 h but could consume water. Each rat was given 2 ml castor oil, then after 1 h the rats were given 0.5% Na-CMC suspension (50 mg/kg BW), loperamide suspension (1 mg/kg BW), and *L. leucocephala* extract (50, 100, 200, and 400 mg/kg BW) orally for each group and 1 ml of Chinese ink was given orally 1 h after treatment. After 1 h was given Chinese ink, all animals were sacrificed by dislocation of the cervical vertebrae. Animals are dissected and their intestines are carefully removed. We measured intestinal length through which Chinese ink markers range from pylorus to ileocecal valve of each animal. Then from each animal calculated the percent of the distances traveled by Chinese ink markers the intestine length was calculated. The peristaltic index has been calculated using the following formula:

$$\text{Peristaltic index} = \frac{\text{Distance traveled by chinese ink}}{\text{Total small intestine length}} \times 100$$

Statistical analysis

Data from test observations analyzed using Statistical Package for the Social Sciences (SPSS). The data are presented as mean ± standard error of the mean (SEM) and analyzed using a one-way analysis of variance (ANOVA) and followed by Tukey's *post hoc* test. $p < 0.05$ was considered to be significantly different.

Results

Castor oil-induced diarrhea

Investigation of antidiarrheal activity induced by castor oil, the observed responses include the initial time of occurrence, frequency, duration of diarrhea, the weight, and consistency of stool. Table 1 shows that all doses of extracts were able to delay the onset of diarrhea compared with negative control. *L. leucocephala* at 400 mg/kg BW (120.40 ± 4.106) activity in delaying the onset of diarrhea was observed when compared to the positive control group (118 ± 3.980) had activity that was not significantly different loperamide ($p > 0.05$)

Table 1: Activity of extract *L. leucocephala* seeds on diarrhea induced by castor oil

No.	Treatment	Onset (minutes)	Frequency (times)	Duration (minute)
1.	Na-CMC 0,5%	56.60 ± 3.059+	6.80 ± 0.374+	285 ± 5.394^{+}
2.	Loperamide 1 mg/kg BW	118.80 ± 3.980*	3.60 ± 0.245*	176.4 ± 7.820*
3.	*L. leucocephala* extract 50 mg/kg BW	61.80 ± 6.028^{+}	6.20 ± 0.374+	256.40 ± 6.096*,+
4.	*L. leucocephala* extract 100 mg/kg BW	80.40 ± 4.578*,+	5.60 ± 0.510+	220.40 ± 4.675*,+
5.	*L. leucocephala* extract 200 mg/kg BW	96.20 ± 3.397*,+	4.80 ± 0.374*	190.20 ± 4.913*
6.	*L. leucocephala* extract 400 mg/kg BW	120.40 ± 4.106*	3.80 ± 0.374*	178 ± 4.669*

Data are presented as mean ± SEM (One way ANOVA followed by Turkey's *post hoc* tests), for each group n=5. *p < 0,05 compared by 0,5% Na CMC as control negative, +p < 0.05 compared to loperamide 1 mg/kg BW as control positive.

as positive control. *L. leucocephala* also decreased the frequency of diarrhea (3.8-5.6 times) except at the extract (50 mg/kg BW) compared to the negative control did not differ much (6.8 times). The observation of *L. leucocephala* (400 mg/kg BW) had activity that was not significantly different from positive control (p > 0.05). Based on the observations, the investigation of the duration of diarrhea, the *L. leucocephala* groups with doses of 200 and 400 mg/kg BW reduced the duration of diarrhea which when compared with the loperamide group did not differ significantly (p > 0.05), while in the Na CMC suspension group 0.5% obtained the longest time in this study.

Stool consistency and weight are determined by weighing the stool weight of animals and observing the stool consistency in the form of solid, mushy and watery stool. Ethanol extract of *L. leucocephala* seeds shows activity that reduced stool weight by varying the consistency of the stool at dependent manner dose. In the dose group, 400 mg/kg BW had activity that was comparable to loperamide (p > 0.05). Observation results of stool consistency and weight are shown in Figure 1.

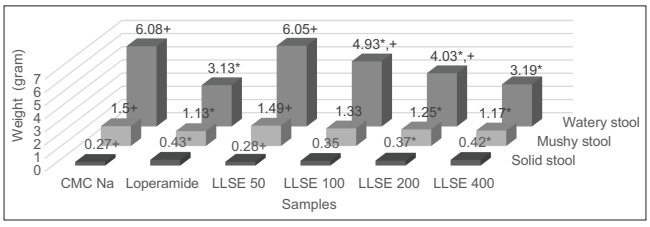

*Figure 1: The weight of stool was differentiated according to the consistency of stool on diarrhea induced by castor oil index. Each bar shows the total number of watery, mushy, and solid stools of L. leucocephala seed extract (LLSE). Data presented as mean + SEM, n = 5. *p < 0.05 compared to negative control (Na CMC 0.5% Kg/bw, + p < 0.05 compared by loperamide 1 mg/kg bw as positive control (One-way ANOVA followed by Turkey's post hoc tests)*

Gastrointestinal motility test

L. leucocephala activity against intestinal motility tested by the intestinal transit method, where Chinese ink is an indicator for determining activity in inhibiting intestinal peristalsis, as shown in Figure 2. *L. leucocephala* shows its activity in inhibiting intestinal peristalsis in rats that have been induced by oleum

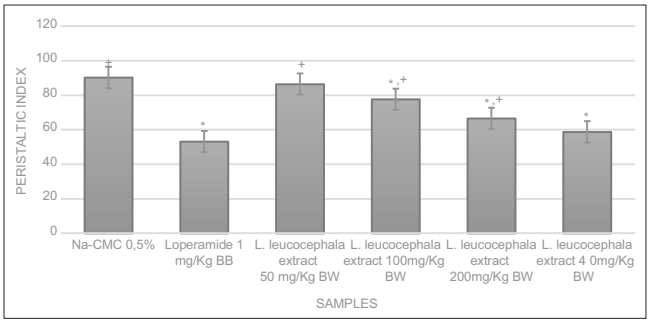

*Figure 2: The peristaltic index of the extract ethanol L. leucocephala seeds activity on intestinal transit test of rat administered with Chinese ink. Data presented as mean + SEM, n = 5. *p < 0.05 compared to negative control (Na CMC 0.5%), +p < 0.05 compared by positive control as loperamide 1 mg/kg bw (One-way ANOVA followed by Turkey's post hoc tests)*

ricini in line with increasing doses. Based on the results of statistical analysis *L. leucocephala* dose 400 mg/kg (58.74 ± 2.208) BW showed activity comparable to loperamide 1 mg/kg bw (53.15 ± 1.792) as positive control (p > 0.05).

Discussion

Diarrhea is defined as defecation with formless or liquid stool with a frequency of more than 3 times in 24 h [3]. Investigation of antidiarrheal effects is carried out by observing the initial time of diarrhea, frequency of diarrhea, weight, and consistency of stool as well as the duration of diarrhea that occurs using oleum ricini (castor oil) as induction of diarrhea in rats. Castor oil which produces active metabolites in the form of ricinoleic acid can cause diarrhea by increasing peristaltic activity in the intestinal mucosa so that it will cause changes in the intestinal mucosal cell permeability to fluids and electrolytes [16]. Oleum ricini was given 1 h before administration of *L. leucocephala* extract. The investigation *L. leucocephala* is stated to have antidiarrheal activity, if the initial time of diarrhea is longer than the negative control and if the diarrhea is faster, then the antidiarrheal activity indicated was weak activity. Based on Table 1, it can be seen the change in the average time (minutes) of animals experiencing diarrhea in each treatment group. CMC-Na 0.5% suspension group showed the fastest average initial time of diarrhea compared to other groups and *L. leucocephala* dose 400 mg/kg BW had significant activity (p > 0.05) with loperamide suspension as positive control.

Loperamide (loperamide hydrochloride) was used this study as a positive control group because it acts directly on opioid receptors in the circular and longitudinal intestinal mucosa. Similar to other μ receptor agonists, loperamide inhibits intestinal peristalsis and prolongs transit time in the digestive system.

Loperamide also stimulates the reabsorption process and changes water and electrolyte transportation. Thus, loperamide can reduce the volume of stool and reduce fluid and electrolyte loss [17].

Investigation of the frequency of diarrhea performed that showed the more diarrhea occur, the antidiarrheal activity will be weaker, and vice versa the less the frequency of diarrhea, the antidiarrheal activity will be stronger. Based on Table 1, there was a change in the average frequency of diarrhea from each treatment group. Negative control group showed the highest average frequency of diarrhea. The *L. leucocephala* group showed the average frequency of diarrhea getting smaller with increasing doses. *L. leucocephala* extract of 400 mg/kg BW showed a smaller average frequency compared to other groups but did not differ significantly when compared to control positive group based on the results of statistical tests obtained.

The investigation of the *L. leucocephala* was declared to have an antidiarrheal effect if the stool weight obtained was smaller than the negative control group. The heavier the stool weight was gained, the antidiarrheal effect indicated was weak activity. The negative control group showed the heavier of stool compared to other treatment groups. *L. leucocephala* group decreased weight of watery stool.

The duration of diarrhea was investigated by observing the animal from the initial time of diarrhea with a watery or flaccid consistency until it returns to the stool with solid consistency. Negative control group showed the longest time compared to other groups. The *L. leucocephala* extract showed decreasing the duration of diarrhea at dose-dependent manner. The positive control group showed the shortest diarrhea time compared to other groups.

L. leucocephala antidiarrheal effect with the intestinal transit method, it was found that *L. leucocephala* has an antidiarrheal effect in inhibiting intestinal motility. *L. leucocephala* dose 400 mg/kg BW showed percent of peristaltic index comparable to positive control, where *L. leucocephala* dose 400 mg/kg BW reduced the percentage of distance travelled by Chinese ink marker and decrease the amount of watery stool compared to the negative control group. Chinese ink is used as an indicator to determine the movement of intestinal motility by observing how far it traverses in the small intestine [15].

In the previous studies, *L. leucocephala* was reported to contain secondary metabolites such as alkaloids, flavonoids, tannins, and steroids [6], [18] Some of these metabolites have antidiarrheal effects as antimotility, antisecretory, and antibacterial properties such as tannins, flavonoids, and saponins [19]. Tannin compounds contained in the extract can reduce peristalsis and intestinal secretions [20]. In addition, flavonoids are also known to inhibit gastrointestinal motility and electrolyte secretion [14], [21].

Conclusion

L. leucocephala seed of the ethanol extract decreased the onset of diarrhea, frequency of diarrhea, consistency, and weight of stool and duration of diarrhea in rats after induced by castor oil. In addition, the extract reduced gastrointestinal motility which indicated by value of peristaltic index. *L. leucocephala* has potential to be developed as new anti-diarrheal agent. However, further studies are required to elucidate its mechanisms to treat diarrhea.

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