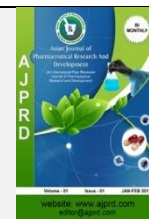


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Research Article

Antidiarrheal Activity of n-Hexane Fraction Seeds of *Leucaena leucocephala* (Lam) de Wit on Rat**Saddam Husein¹, Marline Nainggolan¹, Yuandani²**¹Department of Pharmacy Biology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Sumatera Utara, Indonesia²Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Sumatera Utara, Indonesia**ABSTRACT****Objective:** To evaluate the antidiarrheal activity of the n-hexane fraction of *L. leucocephala* (Lam) de Wit seeds induced by castor oil and intestinal motility methods for rats.**Method:** *L. leucocephala* seeds simplicia powder extracted by maceration with 80% ethanol. Ethanol extract was fractionated with n-hexane solvent. Investigating of antidiarrheal activity of *L. leucocephala* fraction by induction of castor oil and intestinal motility methods.**Result:** The n-hexane *L. leucocephala* fraction reduced diarrhea based on observations of the onset of diarrhea, frequency, consistency, weight of stool and duration of diarrhea compared with negative controls. The n-hexane *L. leucocephala* fraction showed antidiarrheal activity at doses of 200 and 400 mg/kg bw (P <0.05) but differed significantly from loperamide (P <0.05). In the intestinal motility method of the n-hexane fraction doses of 200 and 400 mg/kg bw the activity in inhibiting intestinal motility was significantly different from 0.5% CMC Na (P <0.05).**Conclusion:** The n-hexane fraction of *L. leucocephala* seeds based on antidiarrheal activity testing has the ability to reduce diarrhea.**Keyword:** *Leucaena leucocephala*, Antidiarrhea, Castor oil induced diarrhea, Intestinal motility**ARTICLE INFO:** Received 21 Jan. 2020; Review Completed 20 March 2020; Accepted 23 March 2020; Available online 15 April. 2020**Cite this article as:**Husein S, Nainggolan M, Yuandani Y, Antidiarrheal Activity of n-Hexane Fraction Seeds of *Leucaena leucocephala* (Lam) de Wit) on Rat, Asian Journal of Pharmaceutical Research and Development. 2020; 8(2):10-13.DOI: <http://dx.doi.org/10.22270/ajprd.v8i2.685>***Address for Correspondence:**

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INTRODUCTION

Diarrhea is defined as a condition of increased intestinal emptying and increased water content in the stool. In general, if defecation occurs more than 3 times a day, excretion of faecal with soft/liquid consistency or a combination of both showed an abnormal condition in the defecation process¹. Diarrhea is also characterized by an increase in the frequency of bowel movement, runny stools, presence of blood in the stool, nausea, loss of appetite and weight gain, vomiting, and abdominal pain². Most cases of diarrhea caused by disruption of water and electrolyte transport in the intestine, mechanism of diarrhea can be caused by increasing osmotic pressure in the intestine (thus causing water retention in the lumen); excessive electrolyte and water secretion into the intestinal lumen; exudation of proteins and fluids from the mucosa; and changes in intestinal motility thereby

accelerating transit. In general, there are various processes that affect each other, which leads to an increase in volume and weight of the stool accompanied by a percent of water content³. According to the World Health Organization (WHO) 80% of the world's people are still largely dependent on traditional medicine by using plant extracts as medicine^{4,5}. The use of traditional medicines has long been practiced so that people assume that the use of traditional medicines is relatively safer than synthetic drugs⁶. One of these plants is *L. leucocephala* which contains alkaloids, saponins, flavonoids, tannins, mimosine, leukanin, proteins, fatty acids and fiber^{7,8}. Steroid and flavonoid can inhibit the synthesis of prostaglandin type E2 in the intestine which reduce of diarrhea^{9,10}.

Previous study has reported in Peru, Chinese bark and flower petai are used as antiseptics¹¹. In Thailand, *L. Leucocephala* leaves are used to treat diarrhea¹².

controlling stomach pain from contraception and abortion and lamtoro seed gum has been reported to be beneficial as a binder in tablet formulations^{13,14}.

This study was conducted to evaluate the antidiarrheal activity of the n-hexane fraction of *L.leucocephala* seeds (HFLLS) in white rats with diarrhea induced by castor oil and intestinal motility methods.

Method Material

Plant materials

L. leucocephala seeds are collected from Batang Kuis, Deli Serdang, Indonesia. Identification of *Leucaena leucocephala* (Lam.) de Wit was carried out at the Indonesian Institute of Sciences (LIPI) Jl. Raya Jakarta - Bogor Km 46 Cibinong 16911 Bogor - Indonesia.

Preparation of the fraction

L. leucocephala simplicia seeds powder of 1.2 kg was macerated with 80% ethanol solvent in the first 6 hours soaking, stirring occasionally, then allowed to stand for 18 hours. The results of maceration are accommodated and then Separated by sedimentation, then filtered. This maceration process is repeated twice. Maserates are collected and evaporated¹⁵. Then the fractionation process is carried out using n-hexane solvent.

Animal

The experimental animal used was a healthy male white rat, Wistar strain with a weight of 150-200 grams which was approved by the animal research Ethics Committee of the University of North Sumatra. Rats were divided into 6 groups and each group obtained 5 rats. Before being used, the rats were acclimatized for 7 days under the conditions of the experimental environment and before the experiment began, the rats were fasted for 18 hours but were still given a drink.

Castor oil induced diarrhea

Investigation of antidiarrheal activity induced by castor oil used 5 rats in each positive control group, negative control

group and treatment group. Rats fasted for 18 hours will still be given adequate drinking. Rats were given 2 ml of castor oil to induce diarrhea. One hour later, each group was given 0.5% Na-CMC suspension as a negative control dose of 50 mg/kg bw, loperamide HCl suspension a dose of 1 mg/kg bw as a positive control and the treatment group were given n-hexane fraction at a dose of 50, 100, 200 and 400 mg/kg bw orally. Each rat was put into a glass container with a filter paper mat that had previously been weighed and observed every 30 minutes for six hours. Parameters observed include the onset of diarrhea, frequency of diarrhea, duration of diarrhea, consistency and weight of stool¹⁶.

Intestinal Motility Test

In this method using 5 rats each positive control group, negative control and treatment group. Rats were fasted for 18hours were still given ad libitum water. Rats were given 2ml of castor oil to induce diarrhea. One hour later, each group was given Na-CMC suspension as a negative control with a dose of 50mg/kg bw, loperamide HCl suspension at a dose of 1 mg/kg bw as a positive control and the treatment group was given HFLLS at a dose 50, 100, 200 and 400mg/kg bw orally. After one hour later, 1 ml of Chinese ink (Yamura) was given. After one hour of giving Chinese ink, all animals were sacrificed with cervical vertebra dislocation and their intestines were carefully removed. The distance traveled by the Chinese ink marker is measured from the pylorus to the cecum of each animal. Then from each animal, the percentage of distance traveled by the Chinese ink marker is calculated on the total length of the intestine¹⁶.

$$\text{Peristaltic index} = \frac{\text{Distance traveled by china ink}}{\text{Length of small intestine}} \times 100\%$$

Statistical Analysis

Data from observations of diarrhea activity testing were statistically analyzed by the One Way ANOVA method followed by Post Hoc Tukey HSD test using SPSS (Statistical Product and Service Solution) version 24.

Table 1: Activity of HFLLS on diarrhea induced by castor oil (Data \pm SEM, n: 5)

No.	Treatment	Onset (Minute)	Frequency (Times)	Duration (Minute)
1.	Na-CMC 0,5%	56.60 \pm 3.059 ⁺	6.80 \pm 0.374 ⁺	285 \pm 5.394 ⁺
2.	Loperamide 1 mg/ Kg BW	118.80 \pm 3.980*	3.60 \pm 0.245*	176.4 \pm 7.820*
3.	HFLLS 50 mg/Kg bw	57.80 \pm 1.594 ⁺	6.40 \pm 0,510 ⁺	283.20 \pm 4.779 ⁺
4.	HFLLS 100 mg/Kg bw	61.40 \pm 3.641 ⁺	6.20 \pm 0.583 ⁺	256.80 \pm 4.067* ⁺
5.	HFLLS 200 mg/Kg bw	82.00 \pm 2.775* ⁺	5.40 \pm 0.510	223.00 \pm 3.271* ⁺
6.	HFLLS 400 mg/Kg bw	97.80 \pm 2.557*	4.80 \pm 0.583	198.40 \pm 4.600*

* P< 0, 05 significant with 0,5% Na CMC, + P<0,05 significant with loperamide 1 mg/kg bw

RESULT AND DISCUSSION

Castor oil induced diarrhea

Evaluation of antidiarrheal activity using castor oil as an induction of diarrhea, the observed responses include the onset of diarrhea, the frequency of diarrhea consistency and weight of stool and duration of diarrhea. The onset of diarrhea is determined by observing the first time the animal suffer diarrhea after administration of HFLLS, loperamide and Na CMC. The results showed the activity of the HFLLS in delaying the onset of diarrhea at a dose of

200, 400 mg/kg bw compared with the negative control group and loperamide has a longer time to delay the onset of diarrhea than all groups. The HFLLS also reduced the frequency of diarrhea (4.8-5.4 times) except at a dose of 50 mg/kg body weight which is not much different compared to negative controls (6.8times). Stool consistency and weight are determined by weighing the weight of stool and observing the consistency of the stool in solid, mushy, and watery form. HFLLS shows activity that Reduced consistency and weight of stool at dependent manner dose. Based on observations, the determination of the duration of

diarrhea is shown in Table 1. The dosage of HFLLS at 200 and 400mg/kg bw decreased the duration of diarrhea which when compared with the negative control group was significantly different ($P < 0.05$). The activity of HFLLS in reducing diarrhea by using the above parameters, allegedly due to presence of steroids and flavonoids contained base on phytochemical screening test. Steroids and flavonoids can inhibit the synthesis of prostaglandin type E2 in the intestine which reduced the diarrhea⁹.

Intestinal Motility Test

The activity of HFLLS on intestinal motility was tested by intestinal transit method, where Chinese ink as an indicator to determine activity in inhibiting intestinal peristalsis. The HFLLS showed its activity in inhibiting intestinal

peristalsis of rat that have been induced by castrol oil at certain doses. Based on the results of statistical analysis, doses of 200 and 400 mg/kg bw showed significantly different activities against negative controls ($P < 0.05$), whereas loperamide 1mg/kg bw had better results than the HFLLS 400 mg/kg that can be seen in figure 2. The results of the HFLLS screening phytochemicals test showed that HFLLS contains secondary metabolites such as steroids and flavonoid in which these metabolites had antidiarrheal effects in inhibiting intestinal motility by inhibiting the production of prostaglandin E2, which is known to have an important role in the stimulation of intestinal secretions¹⁷. Prostaglandin inhibition will delay or inhibit diarrhea induced by castor oil¹⁸.

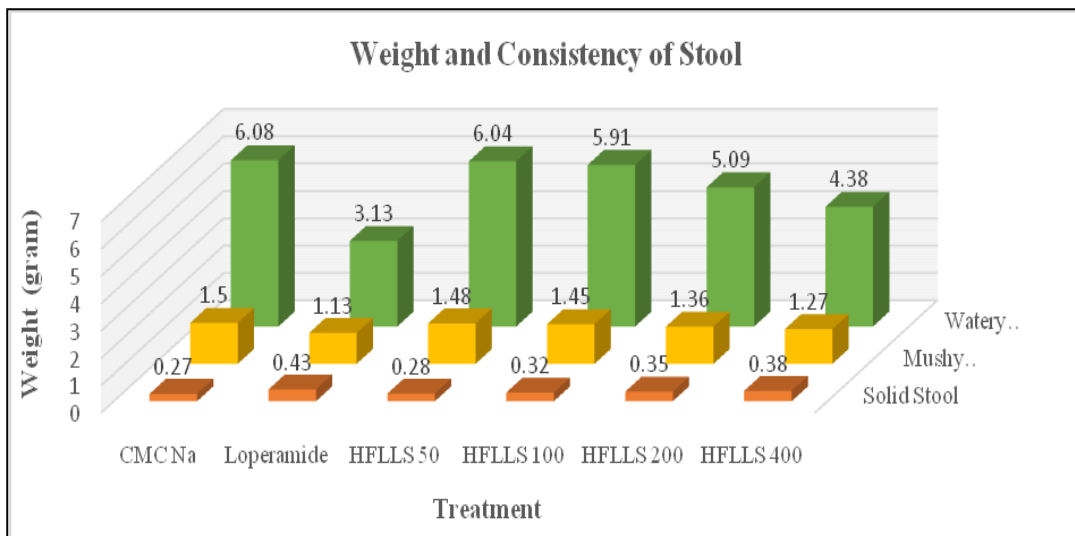


Figure 1: Average stool weight and consistency on diarrhea induced by castor oil

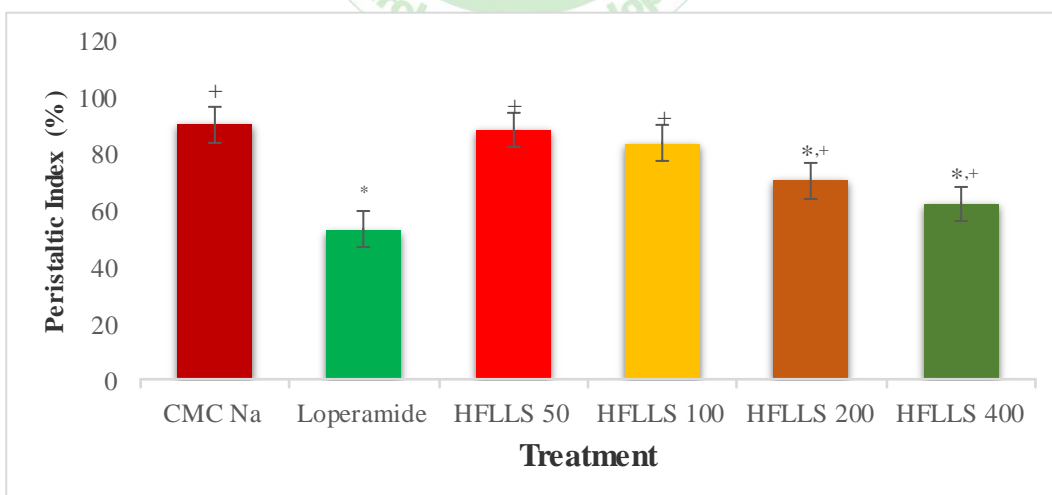


Figure 2 : HFLLS activity on peristaltic index (Data : Mean ± SEM,

* $P < 0.05$ significant with negative control, + $p < 0.05$ significant with positive control)

CONCLUSION

The HFLLS reduced diarrhea base on the onset of diarrhea, diarrhea frequency, duration of diarrhea and the consistency and weight of feces against diarrhea induced

by castor oil. In the intestinal motility method, this fraction has the ability to reduce the peristalsis index by measuring the length of the chinese ink traveled distance on the small intestine.

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