Utilization of banana (*Musa paradisiaca* L.) peel as pectin source as antidiarrheal on castor oil-induced diarrheal Wistar rats model

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**ABSTRACT**

Banana (*Musa paradisiaca* L.) peel, locally name *pisang raja*, contains a high concentration of pectin which is used as antidiarrheal to absorb irritants and increase stool consistency. However, it's use to increase the stool consistency has not been studied, yet. The study aimed to investigate the effect banana peel extract (BPE) administration on stool consistency of diarrheal rat model. It was an experimental study with a post-test only control group design. Thirty male Wistar rats were induced diarrhea with castor oil and then randomized into five groups. Group I as positive control group was given attapulgite at dose of 124 mg/kg BW. Group II as negative control was given 0.5 mL of 1% tween 80. Group III, IV and V as treatment group were given BPE at different doses of 100, 200 and 400 mg/kg BW, respectively. The stool consistency was measured every hour for 4 h by weighing fresh and dry stool weight and then the water content was calculated. Data analysis was conducted using one-way ANOVA and LSD post hoc test. Significantly different in stool consistency between Group III, IV, and V compared to Group II (p<0.05) and between groups Group III and IV compared to Group I (p<0.05) were observed. However, there was no significantly different between group V compared to Group I (p=0.149). In conclusion, BPE can increase the stool consistency of castor oil-induced diarrhea rats. The BPE at the dose of 400 mg/kg BW has similar effect to attapulgite.

**ABSTRAK**

Kulit pisang raja (*Musa paradisiaca* L.) mengandung pektin konsentrasi tinggi yang banyak digunakan sebagai anti diare untuk menyerap zat iritan dan meningkatkan konsistensi tinja. Namun demikian, penggunaannya untuk meningkatkan konsistensi tinja belum diteliti. Penelitian ini bertujuan mengkaji efek pemberian ekstrak kulit pisang (EKP) terhadap konsistensi tinja tikus diare. Penelitian ini merupakan penelitian eksperimental dengan rancangan post-test only control group. Tiga puluh tikus Wistar jantan diinduksi diare dengan minyak jarak dan selanjutnya dibagi menjadi lima kelompok. Kelompok I sebagai control positif diberi atapulgit dosis 124 mg/kg BB. Kelompok II sebagai control negative diberi 0,5 mL tween 80 1%. Kelompok III, IV dan V diberi EKP secara berturut-turut dengan dosis 100, 200 dan 400 mg/kg BB. Konsistensi tinja diukur setiap jam selama empat jam dengan menimbang berat tinja segar dan kering kemudian diukur kadar airnya. Uji statistik menggunakan ANOVA satu jalan dilanjutkan dengan post hoc LSD. Terdapat perbedaan antara Kelompok III, IV, dan V dengan Kelompok II (p<0,05) dan antara Kelompok III dan IV dengan Kelompok I (p<0,05). Namun tidak terdapat perbedaan antara Kelompok V terhadap Kelompok I (p=0,149). Dapat disimpulkan bahwa EKP dapat meningkatkan konsistensi tinja tikus diare yang diinduksi minyak jarak. Ekstrak kulit pisang dosis 400 mg/kg BB mempunyai efek setara dengan atapulgit.
INTRODUCTION

Diarrhea is the eighth leading cause of death worldwide. Based on a survey conducted by World Health Organization (WHO), it was estimated that 1.4 to 2.5 million people in 2015 died due to diarrhea.\(^1\) Diarrhea is also the second leading cause of children under 5 years old death worldwide, which accounts for 525,000 deaths annually.\(^2\) According to WHO, there are approximately 1.7 billion cases of diarrhea in children under 5 years old worldwide each year.\(^2\) In Indonesia, the incidence of diarrhea for all age group in 2013 was 3.5\%.\(^3\) Based on a survey conducted in 2016, there were 6,897,463 cases of diarrhea in Indonesia. However, only 2,544,084 (36.9\%) cases were handled and treated properly.\(^4\)

Diarrhea can cause complications such as dehydration, malnutrition, and even death.\(^5\) Management of diarrhea includes oral rehydration therapy, zinc supplementation, and antidiarrheal drugs such as loperamide, attapulgite, and pectin.\(^6\) Medicinal plants such as guajava leaves, turmeric, tea, and banana pulp have been used traditionally to treat diarrhea in Indonesia. They contain active substances such as pectin and flavonoid which act as absorbent of fecal water content and normalize bowel function.\(^7\),\(^8\)

Extract of banana peel (\textit{M. paradisiaca} L.), locally name \textit{pisang raja}, contains 16.54\% pectin, which can be used as an antidiarrheal alternative.\(^9\) At present, citrus or lemon peel are usually used as a source of pectin due to its higher pectin content. However, banana is the most consumed fruit in Indonesia producing a lot of unutilized peel.\(^10\) Therefore, the availability of the banana peel is unlimited that can be used as main source of pectin. The aim of the study was to investigate the effect banana peel extract (BPE) administration on stool consistency of diarrheal rat model.

MATERIALS AND METHODS

Animals

Thirty healthy male albino Wistar rats weighing 150-200 g were used in this study. The rats were kept in a standard plastic cage and fed with standard pellet diet and water \textit{ad libitum}. They were housed under controlled environmental conditions with a 12-hour light/dark cycle. Before the experiment, the rats underwent acclimatization for 7 days. The experimental cages were prepared with white baking sheet to line the floor of the cages, and wire mesh was placed about 1 cm above the floor.\(^11\)

This study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Diponegoro, Semarang (No. 38/EC/H/FK-RSDK/V/2018). This study was also approved by the Animal Laboratory and Traditional Medicine Laboratory of Faculty of Medicine, Universitas Diponegoro, Semarang.

Preparation of banana peel extract

The unripe banana fruits (\textit{M. paradisiaca} L.) were obtained from a local market in Semarang, Central Java, Indonesia, in May 2018. Banana peels were washed with water, cut and dried under shade. Furthermore, it was processed in oven until it was dry and then crushed into a coarse powder. The coarse powder was soaked in 70\% ethanol in closed Erlenmeyer and kept for 24 h. The mixture was then filtered through filter paper. After filtration, the filtrate was evaporated in a water bath at 80\(^\circ\)C until the ethanol evaporated completely and yielded BPE. The BPE was then kept in a glass bottle to avoid contamination until use.

Observation of stool consistency

On the day of the experiment, 0.5 mL castor oil was administered to each rat to induce diarrhea on the rats. The rats were then divided randomly into
five groups with six rats in each group. Group I as positive control group was given attapulgite at dose of 124 mg/kg BW. Group II as negative control was given 0.5 mL of 1% tween 80. Group III, IV and V as treatment group were give BPE at different doses of 100, 200 and 400 mg/kg BW, respectively. Thirty min after induction, the drug or BPE was administered orally to the respective groups. The stool consistency was measured every hour for 4 h, and the baking sheet was changed every hour. The baking sheet was weighed before use (W₀). The fresh stool was weighed (W₁) and then left in air temperature for 14 h. Followed, the dry stool was reweighed (W₂). The methanol extract, and to a lesser extent the aqueous extract, significantly prolonged the time for diarrhoeal induction; it reduced the frequency of diarrhoea episodes and decreased the propulsion of charcoal meal through the gastrointestinal tract in a dose dependent manner. The aqueous extract did not have any antimicrobial activity at the tested concentration (5 mg/ml). The stool consistency was characterized by its water content which calculated using formula as follow: Water Content (%) = [(W₁-W₂)/(W₁-W₀)] x 100%.

Statistical Analysis

Data were presented as mean ± standard deviation (SD) and analyzed using one-way ANOVA and LSD post hoc test. A p<0.05 was considered to be statistically significant.

RESULTS

The results showed that the administration of castor oil could induce diarrhea in all the rats. The water content of rats stool after BPE administration at all doses (Group III, IV and V) was significantly lower compared with Group II (p<0.05) which received only the vehicle as negative control (FIGURE 1). However, the water content of rat stool after BPE administration at doses of 100 and 200 mg/kg BW (Group III and IV) was significantly higher compared with Group I (p<0.05) which received attapulgite as positive control (FIGURE 1). The BPE administration at dose of 400 mg/kg BW (Group V) could reduce 21.48% of the water content of rat stool compared with Group II. Furthermore, the water content of rat stool of Group V was not significantly different compared with Group I (p>0.05).

FIGURE 1. The effects of BPE on stool water content in castor oil-induced rats. Data were presented as mean ± SD; n=6; *p<0.05 compared to Group II; #p<0.05 compared to Group I.
DISCUSSION

In this study, castor oil was used to induce diarrhea in rats due to its active metabolite, ricinoleic acid, which is irritative for the intestinal lumen. Ricinoleic acid causes the inhibition in Na\(^+\)-K\(^+\)-ATPase activity which then leads to hypersecretion of electrolytes and a decrease in Na\(^+\) and K\(^+\) absorption, which causes secretory diarrhea.\(^{13,14}\) The aim of the current study was to screen the sap of M. paradisiaca for both its secondary metabolites and antidiarrhoeal activity at 0.25, 0.50, and 1.00 mL in rats. Secondary metabolites were screened using standard methods while the antidiarrhoeal activity was done by adopting the castor oil-induced diarrhoeal, castor oil-induced enteropooling, and gastrointestinal motility models. The sap contained flavonoids, phenolics, saponins, alkaloids, tannins, and steroids while cardiac glycosides, anthraquinones, triterpenes, cardenolides, and dienolides were not detected. In the castor oil-induced diarrhoeal model, the sap significantly (P < 0.05) reduced stool consistency.

The results showed that BPE does not show any toxic effect. At dose 400 mg/kg BW, BPE did not cause any death or alter the behavior of the rats. Moreover, at the dose of 100, 200, and 400 mg/kg BW, the BPE could significantly increase stool consistency in a dose-dependent manner compared with the negative control (p<0.05). Even, at the dose of 400 mg/kg BW, the BPE showed no significant differences in the stool consistency compared with the positive control. It was demonstrated that the BPE potency is similar to attapulgite as drug standard.

The BPE can increase stool consistency due to its high pectin content. Siddiqui \textit{et al.}\(^{15}\) reported that as an antidiarrheal drug pectin can absorb and bind water and fat lead to increase stool consistency. Pectin also can remove excess mucus from the digestive system.\(^{15}\) Moreover, pectin can also absorb and bind bacterial toxins and irritants. In this study, pectin in BPE may bind ricinoleic acid of the castor oil causing no into contact with the intestinal surface and no irritation as well as no secretory diarrhea. In addition, pectin in BPE may also bind water lead to increase the stool consistency.

Capasso \textit{et al.}\(^{16}\) reported that pectin in BPE can not be digested and absorbed in the intestines, so it only works locally in the digestive system.\(^{16}\) After reaching the colon, pectin will be fermented by residential colon bacteria into short-chain fatty acids (SCFAs) such as butyrate, propionate, and acetate. These SCFAs then facilitate electrolyte absorption in the colon and serve additional energy lead to increase protein synthesis and oxygen utilization of colon mucosa and increase its protective function. Moreover, these SCFAs have an inhibitory effect on colon motility. Rabbani \textit{et al.}\(^{17}\) reported that the SCFA-colonic mechanism only works on an extract from unripe banana peels. During the ripening process, the pectic materials are hydrolyzed into sugars, which are mostly absorbed in the small bowel. Another study reported that the \textit{M.paradisiaca} L.pulp significantly ameliorate diarrhea symptoms diarrhea in children under five years old. Several mechanisms related to its this effect are its natural phytochemical content, pharmacological activity, and antibacterial activity.\(^{7}\)

In this study, it was used BPE in which its contain not only pectin but also other active substances that could contribute to increasing the stool consistency. Further studies are needed to isolate pectin and evaluate its safety as well as efficacy through preclinical and clinical studies.

CONCLUSION

The BPE (\textit{M.paradisiaca}L.) can increase the stool consistency of diarrheal rats induced by castor oil. At dose 400 mg/
kg BW, it has similar effect attapulgite. Further study will be performed to develop the BPE as new antidiarrhea.

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