ORIGINAL ARTICLE

Laxative Effect of Kersen (*Muntingia calabura*) Leaf Extract with Multilevel Dose on Wistar Rats Induced Gambir

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ABSTRACT

Introduction: Constipation is a gastrointestinal disease that can be reduced with the laxatives. The kersen leaf contains active phytochemical compounds, including flavonoids, tannins, and saponins that have been examined for the laxatives effect. The aim of this study is to prove the laxatives effect of kersen leaf extract in Wistar rats. **Methods:** An experimental study with a posttest-only control group design at Animal Laboratory, Diponegoro University, May 2019. Thirty male Wistar rats, age 2.5 months, 200 grams body weight were randomly divided into 5 groups: K1 was control group; P1 was only induced by gambir (600mg/200 gBW); P2, P3, and P4 were induced with gambir then given kersen leaf extract with oral doses of 250mg/200gBW, 500mg/200gBW, and 1000mg/200gBW. Wistar rats in P1-P4 were induced by gambir for 2 days and 2 milliliters of water were administered 18 hours before treatment to induce constipation effect. The laxative effect was known by observing defecation frequency during 5 hours and feces consistency grouping. This research used One-way ANOVA and Kruskal-Wallis test. **Results:** There was an increased frequency of defecation in P2-P4 (K1=13.20; P1=13.80; P2=14.6; P3=20.0; P4=23.0) in accordance with the laxative effect, although statistically there was no significant difference (p=0.462). The feces consistency with One-way ANOVA test also had no significant difference in each group (p=0.962). **Conclusion:** Kersen leaf has not been said to be effective as laxatives significantly, but it still has potential as laxatives agents.

Keywords: Constipation, Defecation frequency, Feces consistency, Laxative, Kersen

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INTRODUCTION

Constipation is one of the gastrointestinal problems that is common in the world.(1) The prevalence of constipation based on Rome III criteria has been reported from 8,2% to 32,9%, with varying severity levels (2). In Indonesia, according to the International Database US Census Bureau in 2003, 3.857.327 people are diagnosed with constipation (3).

Constipation, perceptions of defecation disorders are characterized by defecation less than 3 times a week or 3 days without defecation and require excessive straining (4). Based on the World Gastroenterology Organization Global Guideline in 2011, constipation is also characterized by pain in the abdomen during defecation, changes in stool consistency to hard, sense of incomplete defecation, large stool size, or difficult stool passage (5). A laxative is a treatment commonly used to treat constipation, works by stimulating the peristalsis of the intestinal wall to facilitate defecation and complete defecation. However, commercially available laxatives with inappropriate doses and use can cause undesirable side effects (6). Therefore, to prevent chemical side effects, herbal treatment is preferable nowadays.

In Indonesia, kersen plant (*Muntingia calabura*) is easy to find but the use of its leaf is still less explored. Kersen leaf contains active phytochemical compounds, including flavonoids (e.g quercetin, rutin, fisetin), tannins, and saponins (7). Several reports on compounds and extracts containing flavonoids have provided evidences for the possibility that flavonoid contributes to the improvement of constipation (8). Based on a 2014 study, the phytochemical compounds

have been examined for the laxatives effect (9). Cactus (*Opuntia humifusa*) water extract contains high levels of flavonoids, and reportedly improves the water content and the fecal pellet number, as well as the histological parameters (10). Also, gastrointestinal disorders such as constipation, indigestion, dysentery and gastroenteritis were significantly improved with *Citrullus colocynthis* (L.) Schrad which is also contains high levels of flavonoids (11). Quercetin has been known as a potential therapeutic strategy for gastrointestinal diseases because it contributes to the stabilization of mast cells and the prevention of histamine release. It promotes gastrointestinal motility and mucin secretion (8). In addition, fiber which is also possessed by kersen can give the form of feces, bind water in the large intestine so that the stool can be released smoothly (12).

Gambir contains glycoside and tannin (catechin) compounds, which are included in the group of polyphenol compounds (13). Excessive use of gambir in normal Wistar rats will cause the absorption of fluid in the intestinal lumen so that it can be used like constipation inducer (14). As an effort to utilize kersen leaf as part of anti-constipation treatment, further research needs to be done. But, there has not been much research on it. Hence, this study aims to prove the effect of multilevel doses of kersen leaf extract as a laxative in Wistar rats induced by gambir (*Uncaria gambir* Roxb.) extract.

MATERIALS AND METHODS

This research was an experimental animal study with a posttest-only control group design. It was conducted for 15 days at Animal Laboratory, Faculty of Medicine, Diponegoro University (Undip), Central Java in May 2019. Thirty male Wistar rats, age 2.5 months, 200 grams body weight were acclimated 1 week before the experiment. Then, all the Wistar rats were randomly divided into 5 groups: K1 was control group; P1 was only induced by gambir (600mg/200 gBW); P2, P3, and P4 were induced with gambir then given kersen leaf extract with infusion orally, sick and inactive rats were excluded. Kersen leaves were dried, but not in direct sunlight.

Fifty grams of dried kersen leaves were heated using a water bath in 200 mL distilled water at 90˚C for 15 minutes. The extract ratio was 1:4, which states that 1 mL of aquadest contained 250 mg of kersen leaves. Accordingly, the infusion can be divided into 250 mg/200 gBW (P2), 500 mg/200 gBW (P3), and 1000 mg/200 gBW (P4) and given to rats orally (15). Wistar rats in P1-P4 induced by gambir for 2 days and 2 milliliters of water were administered 18 hours before treatment. The laxative effect was known by observing the defecation frequency during 5 hours and feces consistency grouping. The data was collected every hour. For consistency measurement, each stool was weighed during those 5 hours, then all the stools were dried in 2 days. The dried stools were weighed and calculated with the consistency formula by the percentage of water content in the stools. All the Wistar rats were still alive until the end of the study.

The data of defecation frequency and feces consistency were processed with Shapiro-Wilk normality test. If the results of the defecation frequency were not normal, data transformation will be needed. The non-parametric statistical difference test, Kruskal-Wallis test, used because the transformation results remained abnormal. The feces consistency distribution results were normal ($p>0.05$). Therefore, One-way ANOVA test used to analyze the data. Significance was stated at $p<0.05$.

RESULTS

This study shows that group P2-P4 had an increased frequency of defecation. The P4 group (1000 mg/200 gBW) had the highest frequency compared to the others. The normality test with the Shapiro-Wilk test of the data showed an abnormal data distribution. Therefore, the Kruskal-Wallis test was used as the statistical test in this study.

The Kruskal-Wallis test results showed that the frequency of defecation of Wistar rats in this study did not have a significant difference in more than two groups. The significance values presented in Table I.

Table I : Results of statistical analysis of laxatives effect measurements

Variable	Group	Mean±Standard Deviation	p*
Defecation frequency	K1	13.20 ± 4.86	
	P1	13.80 ± 5.01	
	P2	14.60 ± 5.45	0.462
	P3	20.60 ± 11.37	
	P4	23.00 ± 9.61	
Feces consistency	K1	66.93 ± 11.33	
	P1	67.29 ±17.38	
	P2	66.76 ± 3.49	0.962
	P3	63.01 ± 4.85	
	P4	65.28 ± 8.02	

*significance for one-way ANOVA test and Kruskal-Wallis test = $p<0.05$

Figure 2 shows there were unstable scores for feces consistency. P2 (250 mg/ 200gBW) had higher consistency score than P3 (500 mg/200 gBW) that had lower consistency score than P4 (1000 mg/200 gBW). This graph also showed that P1 had the highest consistency score which was induced by gambir. The normality test with the Shapiro-Wilk test of the data showed normal data distribution. Therefore, one way-ANOVA test was used as the statistical test in this study.

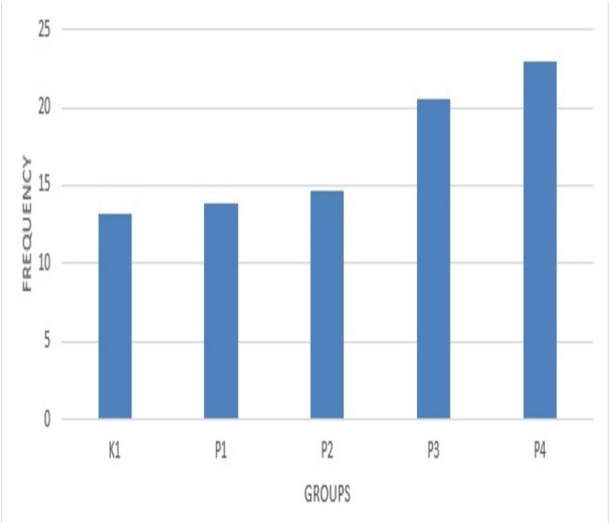

Figure 1 : Comparison of Mean of Defecation Frequency of Subjects in Each Group

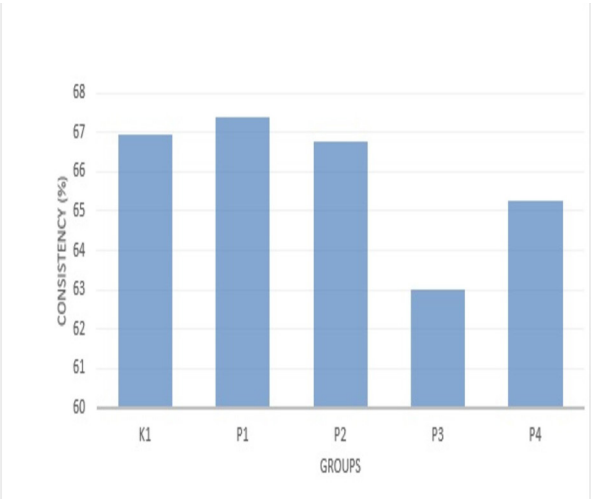

Figure 2 : Comparison of Mean of Feces Consistency of Subjects in Each Group

The statistical test results for feces consistency in each group with one-way ANOVA showed an insignificant difference with a significance value presented in Table I.

DISCUSSION

Gambir was used in many general traditional medicinal including treatments for wounds and ulcers, fevers, headaches, gastrointestinal illnesses and bacterial/ fungal infections (13). Gambir contained glycoside and tannin (catechin) compounds that the previous study showed excessive use of gambir in normal Wistar rats could be used like a constipation inducer (13,14). In the P1 group, which was induced by gambir as a constipation inducer at a dose of 600 mg, it showed a higher defecation frequency than the control group (K1) and the highest consistency feces score compared to other groups. Ester Nuraini *et al* (2016) also demonstrated that the induction of 600 mg gambir for 2 days had not provided an optimal constipation effect in rats (16). However, Saputra *et al* (2016) stated that there was a significant constipation condition after rats were induced by gambir at a dose of 600 mg for 2 days (17). It could be due to the optimal parameters of constipation were still subjective. The excessive drinking water during the induction period or the less effect of gambir also could be caused. Fluid balance is a major function of the gastrointestinal system, such as to facilitate the movement of the intestine (colon) and as a lubricant to help the metabolic waste moves along the colon (18).

Kersen leaves contained active compounds, including flavonoids (e.g quercetin, rutin, fisetin), tannins, and saponins. These compounds would cause fluid and electrolyte secretion in the intestinal lumen which stimulated intestinal peristalsis and quercetin contributes to the stabilization of mast cells and the prevention of histamine release, thus defecation frequency and feces consistency could be affected (8,19). In P2-P4 groups which were given multilevel doses of kersen extraction showed an increased defecation frequency. P4 group which was given 1000 mg/ 200gBW kersen extraction had the highest score for defecation frequency compared to the others. The results obtained were in accordance with the laxative effect, however, there was no significant difference statistically ($p<0.05$). It could be due to the effective doses for kersen as a laxative has not been reached. Effective dose (ED50) means doses that give around 50% of the maximum possible drug effect that often proves to be sufficient (20). However, kersen has potential as laxative agents which can be seen from an increase in the percentage of defecation frequency results for each treatment group. Therefore, further research of kersen as potential laxatives agents are necessary to be investigated.

Our study shows the P2 group (250 mg/ 200gBW) had a higher consistency score than P3 (500 mg/200 gBW) that had a lower consistency score than P4 (1000 mg/200 gBW). Whereas, P1 had the highest

consistency score which was induced by gambir. This is inconsistent with the statement that laxatives should be increasing feces consistency as also stated in research by Aprilia (2015) (21,22). The impairment of intestinal function in binding water could be the cause (15).

In this study, the percentages of active phytochemical compounds in kersen extract by infusion was not measured. From the previous study, kersen contains flavonoids (e.g quercetin, rutin, fisetin), tannins, and saponins. Different environment and plant constituents can affect the result (23).

CONCLUSION

Kersen leaf has not been said to be effective as laxatives significantly, but it still has potential as laxatives agents. Therefore, the further research of kersen as potential laxatives agents are necessary to be investigated. Phytochemical tests, the effective dosage of kersen leaf extraction, and other extraction methods need to be done in further research.

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