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The gastroprotective effects of arrowroot tuber starch (*Maranta arundinacea* L.) on ethanol-induced gastric damages in rats

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ABSTRACT

Empirically, arrowroot tubers (*Maranta arundinacea* L.) have been widely used in the treatment of gastric ulcers. They are known to contain carbohydrates and flavonoids that play a role in reducing inflammation. This study sought to identify the gastroprotective effects of arrowroot tuber starch (*Maranta arundinacea* L.) on the ulcer index, % protection ratio, and the histopathological image of Wistar rat models of gastric ulcers. The test animals were divided into six groups. Group I was given free access to food and water (normal control), while Group II was given ethanol without treatment (negative control). Groups III, IV, and V were treated with arrowroot tuber starch at the doses of 125, 250, and 500 mg/kg BW, respectively. Group VI was given sucralfate at the dose of 400 mg/kg BW (positive control). All treatments were administered orally for 14 days and followed by 24 hours of fasting. On Day 15, all groups, except for the normal control, were given 96% ethanol orally at the dose of 1 ml/200gr BW. After one hour, they were dissected, and their stomach was removed for further analyses. The results showed that the administrations of arrowroot tuber starch at 125, 250, and 500 mg/kg BW produced ulcer indices of 2, 1.25, and 1.5, respectively, smaller than the negative control (4.25), and % protection ratios higher than the positive control. The histopathological imaging showed that the stomach of rats receiving arrowroot tuber starch at 250 mg/kg BW presented no pathological changes. Based on these findings, the arrowroot tuber starch is proven to have the ability as a gastroprotective agent.

Keywords: arrowroot tuber (*Maranta arundinacea* L.), gastroprotective agent, histopathology

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INTRODUCTION

Gastric ulcer is a disease in the gastrointestinal tract characterized by damages to the mucous membrane lining (Kalyana and Robert, 2007). A variety of drugs, such as H2 blockers, proton pump inhibitors, sucralfate, and bismuth, are used for their curative effects on this disease (Kalyana and Robert, 2007). However, like most other drugs, they have side effects, including impaired kidney function, diarrhea, nausea, and vomiting (Tarigan, 2009).

In vivo tests use ethanol as a necrotizing agent that can easily cause gastric mucosal damage. This substance can release free radicals, increase lipid peroxidase, reduce mucus production, and inhibit prostaglandin synthesis (Rozza, 2011). Arrowroot is one of the tuber plants used in gastric ulcer therapy. This plant contains alkaloids, phenolic compounds, saponins, tannins, terpenoids, flavonoids, and carbohydrates, as well as proteins and amino acids (Shintu *et al.*, 2015). Previous research suggests that when administered at the doses of 125, 250 and 500 mg/kg BW, the ethanolic extract of arrowroot tubers exhibits gastroprotective effects (Pertiwi, 2016). As for this study, it examines arrowroot tuber starch for its possibility of having the same property. Compared with the ethanolic extract, starch is easier, simpler, and cheaper and, therefore, can be produced by the public. Besides, arrowroot tuber starch is known to contain polysaccharides in a large number, and these specific compounds can protect the stomach from damages (Cordeiro *et al*., 2012). In the treatment of gastric ulcers, polysaccharides can also reduce the incidence of side effects from taking commercial medicines (Nascimento *et al*., 2017).

Given these points, the study measures the gastroprotective effects of arrowroot tuber starch on gastric ulcers in rats based on the ulcer index, % protection ratio, and the histopathological image of the murine stomach.

MATERIALS AND METHOD

Materials

Arrowroot tuber was purchased from local market in Gegunung Sendangsari Pengasih Kulon Progo and authenticated by Biological Laboratory, Faculty of Applied Science And Technology, Universitas Ahmad Dahlan (No. 011/Lab.Bio/B/II/2017).

Preparation of arrowroot tuber starch

The arrowroot tubers were cleaned, peeled, and washed with clean water. Then, the crushed or grated tubers were squeezed, dissolved in water, and filtered with a filter cloth. Any suspension passing through this filter was allowed to settle until the starch was formed. This starch was later dried in an oven at 60°C and, then, strained using a sieve with 80/100 mesh. The yield was calculated using the following equation (Kusbandari, 2015).

$$\text{Yield (\%)} = \frac{\text{weight of dry sediment (g)}}{\text{weight of wet } \textit{simplisia} \text{ (g)}} \text{ x } 100\%$$

In the equation, *simplisia* is dried natural ingredients that are used as medicine and have not been exposed to processing mechanisms.

Test animals

The ethical approval for this study, No. 011703024, was granted by the Research Ethics Committee of Universitas Ahmad Dahlan on May 19, 2017. White female Wistar rats weighing approximately 200 g and aged 3 months old were acquired from the Integrated Research and Testing Laboratory (LPPT UGM). After an adaptation process of one week, these test animals were randomly divided into six groups, each consisting of five rats. During the research, all groups were given ad libitum access to food and water.

Induction of gastric ulcer with ethanol

In this study, gastric ulcers in rats were induced by 96% ethanol at a dose of 1 ml/200gr BW (Almasaudi *et.al*., 2016). The test rats were divided into six groups with the following conditions:

Group I (normal) was given food and water only, while Group II (negative control) was given ethanol without treatments. Groups III, IV, and V received arrowroot tuber starch suspension at the doses of 125, 250, and 500 mg/kg BW. The last one, Group VI (positive control) was given sucralfate at the dose of 400 mg/kg BW. Each treatment was carried out orally for 14 days and followed by 24-hour fasting. On Day 15, 96% ethanol at the dose of 1 mL/200 gr BW was administered to Groups II-VI. After one hour, all groups were anesthetized using chloroform, then the stomach was dissected.

Determination of ulcer index and % protection ratio

Gastric damages were determined based on the severity of the induced hemorrhage. These damages are expressed on a scale of 0 to 5. This scoring method (Table I) is a modification of the ulcer index scoring used in Saha *et al*. (2016).

Table I. Macroscopic scoring for the severity of murine gastric damages induced by 96% ethanol

Cross-section of rat stomach	Scores
Normal mucosa	0
Hemorrhage in 0-1% of areas of the gastric mucosa	1
Hemorrhage in 2-5% of areas of the gastric mucosa	2
Hemorrhage in 6-10% of areas of the gastric mucosa	3
Hemorrhage in 11-15% of areas of the gastric mucosa	4
Hemorrhage in >15% of areas of the gastric mucosa	5

The ulcer index (UI) is the average score of each treatment group. Meanwhile, the ability to protect against peptic ulcers is based on the % protection ratio, which was computed using the formula below:

$$\%\ Protection\ Ratio = 100\ \% - \left(\frac{UI\ of\ test\ groups}{UI\ of\ normal\ groups}\ x\ 100\%\right)$$

(Kumar *et al*., 2012)

Histopathological analysis

The histopathological profile was built following the procedures applicable at the Faculty of Veterinary Medicine, Universitas Gadjah Mada. The abdominal organs were washed with 0.9% NaCl and fixed with 10% formalin solution. After the hematoxylin and eosin (HE) staining of the gastric tissues, the stomach was examined under a light microscope by pathologists and researchers. Then, a quantitative histopathological analysis was performed by scoring based on the level of damage (Table II). This scoring method is a modification of the histopathological scoring method in Okatiranti (2011).

Table II. Scoring of gastric damages based on histopathological observation

Level of Damage	Scores
No pathological change	0
Congestion, edema, and inflammatory cell infiltration	1
Erosion, congestion, and inflammatory cell infiltration	2
Hemorrhage, edema, and inflammatory cell infiltration	3
Erosion, hemorrhage, edema, and inflammatory cell infiltration	4

Data Analysis

The results of the histopathological scoring were statistically analyzed with the Kruskal-Wallis test and a post hoc Mann-Whitney test. A p-value<0.05 shows a statistical significance.

RESULTS AND DISCUSSION

The yield of arrowroot tuber starch

The starch was produced through the process of peeling, washing, grating, squeezing, settling, and drying. In this study, the yield of the starch was 17.33%.

Ulcer index and % protection ratio

Several previous studies rely on ethanol as an agent that causes gastric lesions or ulcers because it quickly penetrates the gastric mucosa (Nordin *et al.*, 2014). Another research confirms that ethanol can interfere with gastric acid secretion and decrease mucus secretion (Dashputre and Naikwade, 2011). It can also increase fatty acid peroxidation, induce oxidative stress in cells, and change the permeability of mucous membranes. Further damage develops as hemorrhage forms in mucous and submucosal membranes and cell necrosis and inflammatory cell infiltration occur (Nordin *et al.*, 2014).

Based on the ulcer index and % protection ratio presented in Table III, the three doses of administration of arrowroot tuber starch exhibited better gastroprotective activities than the positive and negative controls. Among these doses, 250 mg/kg BW produced the highest gastric protection ratio. These findings are supported qualitatively by the macroscopic images (Figure 1) that show congestion in Groups II (negative control), III (arrowroot tuber starch= 125 mg/kg BW), V (arrowroot tuber starch= 500 mg/kg BW), and VI (positive control; sucralfate).

Table III. The ulcer index and % protection ratio in rats with gastric ulcers induced by 96% ethanol

Groups	Doses (mg/kg BW)	Ulcer Index	% Protection Ratio
Normal	-	0	100
Negative control	-	4.25	0
Arrowroot tuber starch	125	2	52.94
	250	1.25	70.59
	500	1.5	64.71
Positive control (Sucralfate)	400	2.5	41.18

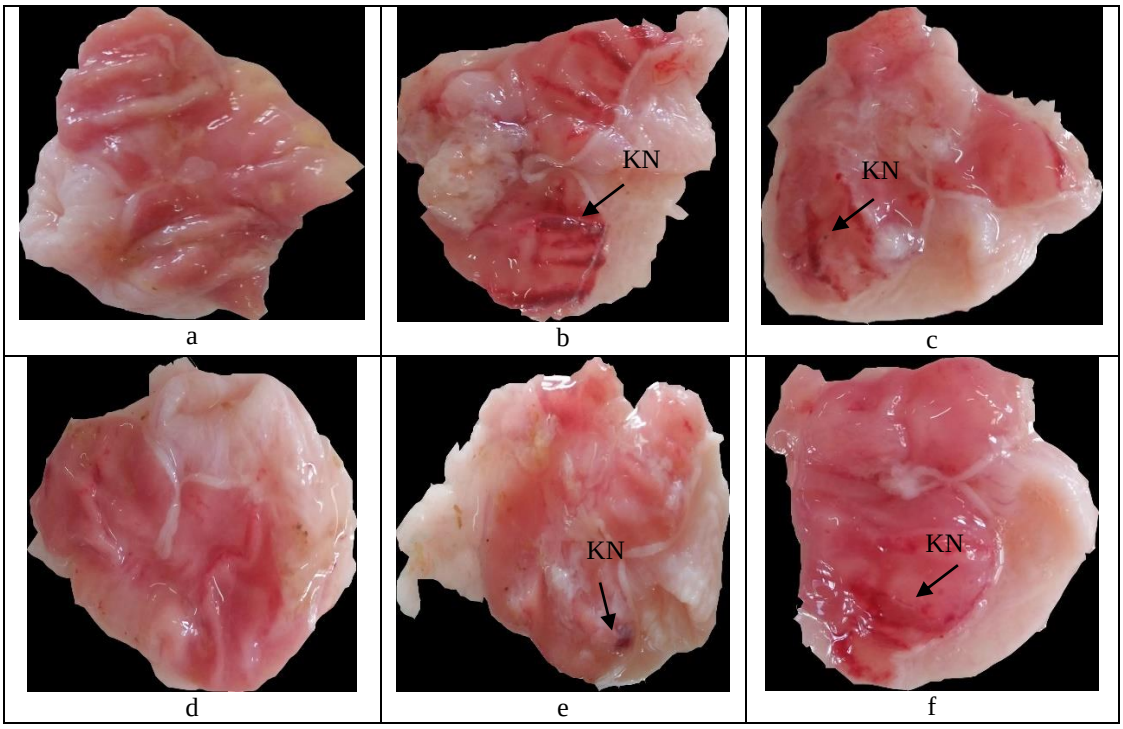

Figure 1. The macroscopic images of rat stomach after gastric ulcer induction with 96% ethanol; (a) Group I or normal control; (b) Group II or negative control; (c) Group III receiving arrowroot tuber starch at the dose of 125 mg/kg BW; (d) Group IV receiving arrowroot tuber starch at the dose of 250 mg/kg BW; (e) Group V receiving arrowroot tuber starch at the dose of 500 mg/kg BW, (f) Group VI or positive control
Note: KN = congestion

Histopathological profile

Table IV shows the average total histopathological scores of all groups of test rats, which also quantify the level of damage to the gastric mucosa, as seen in Figure 2. The Mann-Whitney test results showed that Group IV (arrowroot tuber starch= 250 mg/kg BW) was similar to Group I (the normal control). The average total histopathological scores of both groups were significantly different (sig<0.05) from the negative control (Group II). On the contrary, Groups III (arrowroot tuber starch= 125 mg/kg BW), V (arrowroot tuber starch= 500 mg/kg BW), and VI (positive control; sucralfate) showed no significant difference (sig>0.05) from the negative control. Although insignificant, the administration of arrowroot tuber starch at the dose of 125 mg/kg BW (Group III) had a lower histopathological score than the negative control (Group II). This relatively low score (Group III) and the absence of pathological damages in Group IV (arrowroot tuber starch= 250 mg/kg BW) indicate that the gastroprotective functions of arrowroot tuber starch are effective at the doses of 125 and 250 mg/kg BW. Meanwhile, Group VI (sucralfate) had the highest histopathological score, signifying the most severe gastric damages.

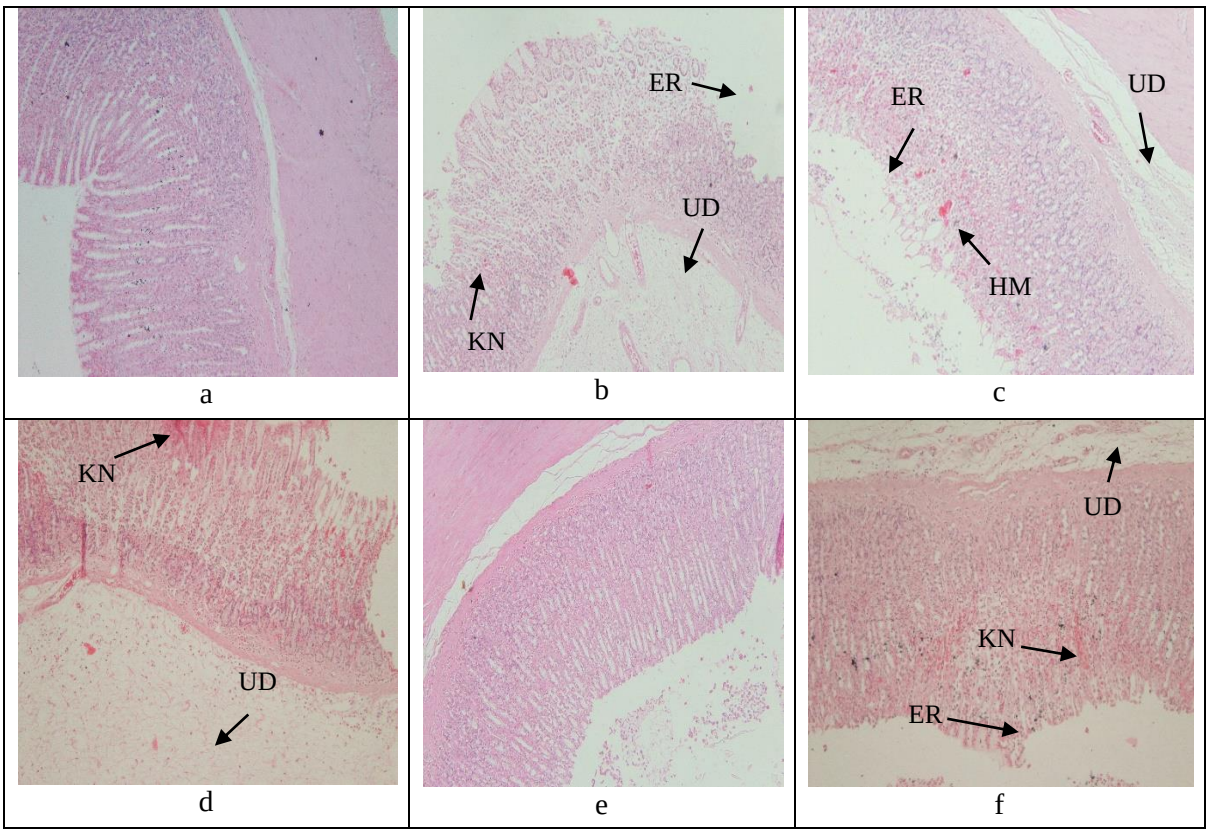

Figure 2. The histopathological images of rat stomach after HE staining with 100x magnification; (a) Group I or normal control; (b) Group II or negative control; (c) Group III receiving arrowroot tuber starch at the dose of 125 mg/kg BW; (d) Group IV receiving arrowroot tuber starch at the dose of 250 mg/kg BW; (e) Group V receiving arrowroot tuber starch at the dose of 500 mg/kg BW, (f) Group VI or positive control

Notes: ER = erosion; HM = hemorrhage; KN = congestion; UD = edema

Table IV. The average total histopathological scores of the test rats

Groups	Doses (mg/kg BW)	X ± SD
Normal	-	0.00 ± 0.00*
Negative control	-	1.33 ± 0.58
Arrowroot tuber starch	125	0.67 ± 0.58#
	250	0.00 ± 0.00*
	500	1.67 ± 1.53#
Positive control (Sucralfate)	400	2.67 ± 1.15#

Notes: The average total histopathological scores of the rat stomach (X) ± SD, *sig<0.05 shows a significant difference with the control, #sig>0.05 shows no significant difference with the control, SD: Standard Deviation, n: 3.

In general, the gastroprotective activities of arrowroot tuber starch are expected to be stronger as the administered dose increases. However, this correlation does not apply to this study. Although the highest dose is 500 mg/kg BW, the gastroprotective effects seem to lessen, although not significantly. The decrease is potentially attributable to various factors, e.g., the occurrence of

saturation at specific doses. Pharmacologically, herbs respond differently from standardized synthesis drugs because they involve many active compounds that can work on one or several targets. With these components, herbs can have both synergistic and antagonistic properties (Syahrir *et al*., 2016). The synergistic effect is likely to rise until its saturation point at a particular concentration, and this explains the non-linear correlation between the dose of arrowroot tuber starch and its gastroprotective effects.

Based on the dose and resultant % protection ratio, the arrowroot tuber starch in this study has higher effectiveness as a gastroprotective agent than its ethanolic extract. At the dose of 250 mg/kg BW, the former results in a protection ratio of 70.59%, while the latter produces 58.84% higher than the positive control (Pertiwi, 2016). This variation involves different active substances between the two preparations, and therefore, the gastroprotective mechanisms they initiate are not similar. The ethanolic extract of arrowroot tubers is rich in flavonoids, but the starch is a polysaccharide composed of 25% amylose and 75% amylopectin (Priamsari and Pujiastuti, 2016). Polysaccharides can bind to the surface of the gastric mucosa, which in turn improves the protective layer. Also, they are known to reduce gastric acid and pepsin secretion, disarm free radicals, and increase mucus secretion (Cipriani *et al*., 2009). Mucus is an essential protective factor because it can prevent the penetration of necrotizing agents (Cordeiro *et al*., 2012). Sucralfate also has cytoprotective activity against ethanol-induced ulcers by lining the gastric mucosa. However, this mechanism is not enough to maintain mucous levels (Nascimento *et al*., 2017).

The histopathological image is used to support the quantitative analysis results, i.e., ulcer index and % protection ratio. Comparison has proven that the macroscopic observation (ulcer index) shows a slightly different result from the microscopic examination (histopathology). Several factors are believed to have caused this, including the sample used for histopathological testing. Instead of using all areas of gastric tissue, this test only investigates the inflamed ones as the samples. In this test, the administration of arrowroot tuber starch at the dose of 250 mg/kg BW shows the most favorable result, as apparent from the absence of pathological change. This dose also presents the most substantial effects on ulcer index and the best % protection ratio.

CONCLUSION

Arrowroot tuber starch has gastroprotective functions as it has been proven to decrease ulcer index and increase protection ratio in the macroscopic analysis of 96% ethanol-induced gastric ulcers in rats. Moreover, the histopathological image confirms that the administration of arrowroot tuber starch can improve the rat stomach after the ulcer induction.

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