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Evaluation of Gastroprotective Effect from *Phaleria macrocarpa* Fruits Extract on Gastric Ulcer in Male Wistar Rats

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Abstract

BACKGROUND: The long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) triggers gastric mucosal damage and causes ulcers. Meanwhile, studies showed that Mahkota Dewa fruit *Phaleria macrocarpa* contains secondary metabolites of flavonoids and tannins that can protect the gastric mucosa.

AIM: This study aims to determine the gastroprotective effect of *P. macrocarpa* ethanolic extracts against gastric ulcers in rats induced with acetosal and ethanol.

METHODS: The extracts were obtained by maceration method using 96% ethanol as solvent. Phytochemical screening of *P. macrocarpa* fruit simplicia and extract was carried out using standard methods. The male rats used were divided into seven groups for each test with ethanol and acetosal induction. Each group consisted of five rats, namely, normal control, ulcer control, vehicle control, positive control (sucralfate 360 mg/kg body weight [BW] for ethanol induction method or omeprazole 3.6 mg/kg BW for acetosal induction method), and extract doses of 100, 200, and 400 mg/kg BW. All groups were given treatment for 7 days except normal and ulcer controls group. On day 6, rats were fasted for 36 h and induced with acetosal or ethanol except normal control. In ethanol induction, the animal was sacrificed after 10 h of oral ethanol administration. While in acetosal induction, the animal was sacrificed 6 h later after oral administration of acetosal. The stomach section was taken for macroscopic, microscopic parameters and gastric acid secretion examination.

RESULTS: The results of phytochemical screening showed that the extract contained flavonoids, tannins, saponins, alkaloids, and glycosides. In acetosal-induced ulcers, the administration of one dose of the extract reduced the number and score of ulcers, repair epithelial cells, increase pH, and total gastric acidity. Furthermore, the percentage of ulcer inhibition at the extract dose of 400 mg/kg BW was 91.91 ± 3.74% in ethanol induction and 59 ± 13.08% in acetosal.

CONCLUSION: The ethanolic extract of *P. macrocarpa* has a gastroprotective effect on acetosal-induced and ethanol-induced gastric ulcer rats.

Introduction

Gastric ulcers occur due to an imbalance between the aggressive factors of gastric acid and pepsin with mucosal defense factors [1]. The digestive tract is covered by a mucous membrane containing bicarbonate which protects the gastric tissue from the corrosive properties of gastric acid and pepsin. In the pathogenesis of gastric ulcers, the main factors are infection with *Helicobacter pylori* bacteria, use of nonsteroidal anti-inflammatory drugs (NSAIDs), and stress [2], [3].

The long-term use of NSAIDs triggers gastric mucosal irritation and bleeding [3], [4], and induces ulcers by inhibiting the cyclooxygenase enzyme in prostaglandin biosynthesis [5], [6], [7], [8]. In addition, it also induces mucosal damage by increasing the production of reactive oxygen free radicals and exerts a cytotoxic effect on epithelial cells, thereby making the mucosa more susceptible to damage [9], [10], [11]. The previous studies showed that ethanol produces ulcers in the gastric mucosa through impaired microcirculation [12], [13], inhibition of prostaglandin synthesis, and reduced mucus production [14]. Meanwhile, animal models of gastric ulcers with ethanol induction represent agents that act directly on the gastric mucosal lining such as food [15].

Phaleria macrocarpa (Scheff.) Boerl. or Mahkota Dewa fruit is a native plant from Papua Island, Indonesia, and contains high levels of alkaloids, flavonoids, and polyphenols [16], [17], [18]. The previous studies showed that quercetin, melatonin [19], and naringenin have gastroprotective effects [20]. *P. macrocarpa* has various pharmacological activities as an anti-inflammatory, antioxidant, anticancer, antidiabetic, antihypertensive, and hepatoprotective [18], [21]. The gastroprotective potential of this plant is due to the high content of secondary metabolites of flavonoids [22], [23], [24] and other polyphenols such as tannins [25], [26], triterpenes, and steroids [27], [28], [29], [30], which protect cells

from damage [24], [25], [31]. The protective effect is relevant to the condition of the ulcer which is called gastroprotective, however, its impact on *P. macrocarpa* has not been scientifically reported [32]. Therefore, this study aims to determine the gastroprotective potential of Mahkota Dewa fruit extract against ulcers induced with NSAIDs and ethanol.

Materials and Methods

Plant material

Fresh fruit of *P. macrocarpa* was from Pancur Batu Subdistrict, Deli Serdang District, Sumatra Utara Province, Indonesia. The sample was identified at the Herbarium Medanense (MEDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (Identification Number 4153/MEDA/2019). The skin, shell, and seeds of the fruit were separated from the flesh. Subsequently, the skin was separated by peeling with a knife, the flesh was cut into small pieces, cleaned, weighed to obtain the wet weight, and dried in a drying cabinet at a temperature of 30–40°C. The dry ingredients obtained were ground with a blender to obtain fruit pulp powder.

Extract preparation

P. macrocarpa fruit simplicia was put into a chamber and mixed with 75 parts of 96% ethanol solvent. The mixture was left protected from light for 5 days and stirred frequently. Subsequently, the results of maceration were scattered, squeezed, washed with sufficient 96% ethanol solvent to obtain 100 parts, then left protected from sunlight for 2 days, and deposited or filtered to obtain macerate. The macerate was concentrated using a rotary evaporator to obtain a thick extract [33].

Phytochemical analysis

Phytochemical screening of simplicia and ethanolic extract of *P. macrocarpa* fruit was carried out using standard methods to examine the secondary metabolites [34].

Experimental animal

The animal used was a male albino Wistar rat aged 8–15 weeks weighing ± 150–200 grams, which was from the Pharmacology Laboratory of Faculty of Pharmacy, Universitas Sumatera Utara. They were acclimatized 1 week before the experiment and given standard pellet feed and water *ad libitum*.

Acetosal-induced gastric ulcer experimental protocol

This experimental protocol was based on Mutmainah *et al.* [35] with slight modifications. The rats have fasted for 24 h before induction, however, they were allowed to drink. They were also given acetosal suspension solution at a dose of 800 mg/kg body weight (BW). The test animals were divided into seven groups, each group consisting of five rats as follows:

Group I: No treatment (negative control)
Group II: Given 0.5% carboxymethyl cellulose (CMC) suspension (vehicle control)
Group III: No treatment (ulcer control)
Group IV: Sucralfate suspension was given 360 mg/kg BW (positive control</AQ15>)
Group V: Given extract suspension at a dose of 100 mg/kg BW
Group VI: Given extract suspension at a dose of 200 mg/kg BW
Group VII: Given extract suspension at a dose of 400 mg/kg BW

All groups of rats were given treatment for 7 consecutive days orally except Groups I and III. On day 6, all treatment groups were fasted for 36 h and were given ulcer induction with acetosal at a dose of 800 mg/kg BW orally except Group I. The animals were sacrificed under anesthesia 10 h after induction.

Ethanol-induced gastric ulcer experimental protocol

The test protocol referred to Sowndhararajan *et al.* [36] and Sattar *et al.* [37] with modification. The animals were divided into seven groups, where each group consists of five rats, namely:

Group I: No treatment (negative control)
Group II: Given 0.5% CMC suspension (vehicle control)
Group III: No treatment (ulcer control)
Group IV: Omeprazole suspension 3.6 mg/kg BW (positive control)
Group V: Given extract suspension at a dose of 100 mg/kg BW
Group VI: Given extract suspension at a dose of 200 mg/kg BW
Group VII: Given extract suspension at a dose of 400 mg/kg BW

The treatment was given for 7 days except for Groups I and III. After being given treatment on the 6th day, the rats fasted for 36 h and ethanol induction P.A (99.5%) was given orally at a dose of 2 ml/200 mg except Group I. The animals were sacrificed using anesthesia after 6 h of induction.

Animals were dissected and gastric organs were taken to examine the test parameters. The protocols were approved by the Animal Research Ethics Committee, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (approval

letters number 00185/KEPH-FMIPA/2019 and 00186/KEPH-FMIPA/2019).

Macroscopic observation

The dissected rats were drained of gastric fluid contents, while the stomach was isolated and opened for further cleaning with 0.01 N NaCl solution. Subsequently, the stomach was spread on a flat surface to observe the ulcers formed. The number of ulcers and severity were recorded with the following score: 0 = No ulcer, 0.5 = reddish spots, 1 = ulcer area ≤ 0.5 mm^2, 2 = ulcer area 0.5–2.5 mm^2, 3 = ulcer area 2.5–5 mm^2, 4 = ulcer area 5–10 mm^2, and 5 = ulcer area ≥ 10 mm^2 [38].

The length and width of the ulcer and the gastric area were measured using a caliper. The total area of the ulcer was added up and seems more reddish than the normal gastric area.

The protection ratio was from the gastric ulcer index data calculated by the following formula [39]:

$$Ulcer\ index = \frac{Ulcer\ area \times 100\%}{Total\ gastric\ area}$$

The percent ulcer inhibition was calculated using the formula below:

$$\%\ ulcer\ inhibition = Induced\ ulcer\ control\ index - \frac{Treatment\ ulcer\ index \times 100\%}{Induction\ control\ ulcer\ index}$$

Histopathological observation

Preparation was carried out at the Histology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, Indonesia. Clean gastric tissue that had been cut was fixed in 10% formalin solution for slide preparation. Subsequently, the slides were observed under a light microscope with ×10 with hematoxylin and eosin staining.

Examination of gastric acid secretion

The boundaries between the gastric-esophagus and gastric-duodenum were clamped before the stomach was isolated. The pH of gastric fluid was measured with a pH meter [39], [40] and 3 ml of distilled water was added into the gastric and shaken carefully. The gastric fluid was collected, centrifuged for 10 min at 3000 rpm and the supernatant was taken and three drops of phenolphthalein were added. Titration was carried out using 0.1 M sodium hydroxide (NaOH) solution until the test color changed to light pink, which indicated a pH of 7.0. Meanwhile, the volume of NaOH required for the titration was used to determine the hydrogen ion concentration. The gastric mucus of each rat was obtained by gradually scraping the mucosa with a glass slide and weighed using an electronic scale [41].

$$Total\ acidity\ [40] = \frac{NaOH\ volume \times Normality \times 100 \times mEq\ L^{-1}}{0.1}$$

Statistic analysis

All outcome data were presented as mean ± standard error mean and analyzed using the one-way analysis of variance method with the SPSS 22.0 program. This was followed by the Tukey post-test, where $p < 0.05$ was considered statistically significant.

Results and Discussion

Phytochemical screening results

The results of the phytochemical screening showed that the simplicia powder and ethanolic extract of *P. macrocarpa* contained alkaloids, glycosides, flavonoids, tannins, and secondary metabolites of saponins (Table 1).

Table 1: Phytochemical screening result of *Phaleria macrocarpa* simplicial powder and ethanolic extract

Secondary metabolites	Simplicia powder	Ethanolic extract
Alkaloids	+	+
Glycosides	+	+
Flavonoids	+	+
Tannins	+	+
Saponin	+	+
Triterpenoids/steroids	−	−

Notation: (+) contains the secondary metabolites, (−) does not contain the secondary metabolites.

Based on these results, the simplicia and ethanolic extract of *P. macrocarpa* fruit contained tannins, saponins, alkaloids, flavonoids, and glycosides. This is in line with the previous study [17], [18], [42] which stated that *P. macrocarpa* have a high content of alkaloids, flavonoids, and polyphenols [17], [18], [19]. Moreover, the flavonoid quercetin, melatonin [20], and naringenin have gastroprotective effects [21], while the ethanolic extract of the fruit has a relatively high content of flavonoids [17], [19]. This compound is effective as an antioxidant by inhibiting various oxidation reactions and reducing hydroxyl, superoxide, and peroxyl radicals [19], [43], [44], [45].

Gastric gross macroscopic observation

The results of the macroscopic study showed that the administration of *P. macrocarpa* ethanolic

extracts (PMEE) gave a significant reduction in scores, area, and number of ulcers (Figure 1). There were no ulcers in normal rats (normal control group), while absolute ethanol-induced ulcers in the control have the highest mean number of ulcers compared to other groups. Furthermore, a decrease in the mean number of ulcers indicated a positive ulcer healing effect because the positive control group has the lowest mean number of gastric ulcers. The mean number of ulcers in the extract-administered group decreased as the extract dose increased compared to the ulcer control group (Table 2).

The severity of the ulcer showed that the test group had a decrease in the mean ulcer score, which indicated a reduction in the area compared to the negative control. The three doses of *P. macrocarpa* ethanolic extract had a mean ulcer index that was significantly different from the induction group and the vehicle group. Meanwhile, the decrease in the gastric ulcer index showed the effectiveness of the gastric ulcer healing process. Based on these results, the lower the ulcer index, the better the healing effect of gastric ulcers (Figure 2a and b).

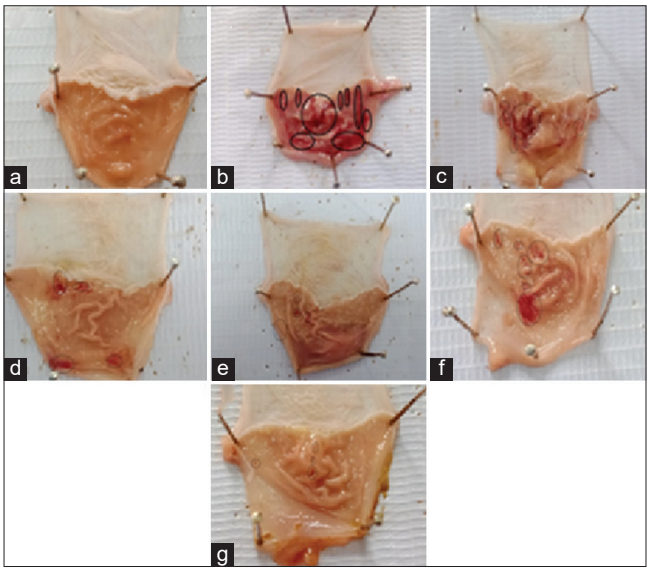

Figure 1: The macroscopic view of the gastric ulcer of rats induced by absolute ethanol. (a) Normal control group; (b) ulcer induction control group; (c) vehicle control group; (d) positive control group (omeprazole); (e) P. macrocarpa ethanolic extract (PMEE) dose of 100 mg/kg BW group; (f) PMEE dose of 200 mg/kg BW group; (g) PMEE dose of 400 mg/kg BW group. Circle marks indicate ulcers

The secretion of gastric acid plays an important role in the formation of gastric ulcers. Meanwhile, substances such as proton pump inhibitors that can suppress secretion are believed to accelerate the healing process of gastric lesions or inhibit the formation of mucosal injury [46]. In this study, omeprazole was used as the positive control group, which showed the

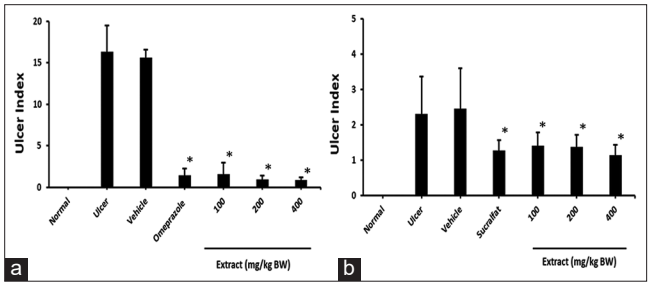

*Figure 2: Ulcer index of treatment of P. macrocarpa extracts on gastric damage caused by (a) ethanol and (b) acetosal in rats. *Significantly different with ulcer control group at p < 0.05*

ability to suppress gastric acid secretion and is assumed to accelerate the healing process of gastric lesions or inhibit mucosal wound formation [47].

Based on the percentage of protection parameters, the PMEE can protect the gastric mucosa induced by ulcers with absolute ethanol or acetosal. Furthermore, the three extract doses showed the same percentage of protection as the positive control group, respectively. In ethanol-induced ulcers, the percent protection of PMEE doses of 100, 200, and 400 mg/kg BW gave 79.22 ± 7.87%, 86.47 ± 7.57%, and 91.91 ± 3.74%, respectively, compared to omeprazole, which was 93.20 ± 4.19% (Figure 3a). Meanwhile, the highest percentage of protection from PMEE in acetosal-induced ulcers was indicated by a dose of 400 mg/kg BW of 50.59 ± 13.08% (Figure 3b). From Figure 4, it is deducted that the gastroprotective effect shown by the administration of PMEE was due to the strengthening of mucosal defense factors [48].

From Figure 4a, PMEE showed the ability to increase gastric mucus production on ethanol-induced induction. These results are in line with the previous study, where the extract of Mahkota Dewa prevents gastric ulcers due to indomethacin in rats by increasing gastric mucus levels [49]. This is because mucus forms a gel that covers the mucosal surface and physically protects it from abrasion [1], [50]. Gastric mucus is a factor that plays an important role as a cytoprotective [50] and is regulated through prostaglandin E [51]. Therefore, it is assumed that the inhibition of prostaglandins by acetosal causes a decrease in gastric mucus production (Figure 4b).

Table 2: Macroscopic studies of gastric after treatments

Group of treatments	Number of ulcers		Ulcer score		Ulcer area (mm2)	
	Ethanol	NSAID	Ethanol	NSAID	Ethanol	NSAID
Normal control	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*
Ulcer control	5.60 ± 1.63	6.80 ± 0.58	3.82 ± 0.68	3.72 ± 0.38	169.74 ± 32.27	24.38 ± 9.57
Vehicle control	3.60 ± 1.08	3.00 ± 0.45*	3.31 ± 0.44	3.38 ± 0.58	39.73 ± 10.04*	22.62 ± 10.54
Positive control	2.40 ± 0.24*	2.60 ± 0.24*	0.90 ± 0.29*	2.95 ± 0.35	11.87 ± 6.02*	12.60 ± 2.81*
PMEE 100 mg/kg BW	5.00 ± 0.95	3.20 ± 0.2*	3.46 ± 0.38	3.30 ± 0.45	17.20 ± 12.40*	15.03 ± 4.66*
PMEE 200 mg/kg BW	3.80 ± 1.11*	3.00 ± 0.63*	2.93 ± 0.55	3.00 ± 0.61	10.96+5.83*	14.63 ± 2.90*
PMEE 400 mg/kg BW	2.60 ± 0.68*	2.00 ± 0.32*	2.47 ± 0.24*	2.78 ± 0.38*	7.00 ± 2.51*	11.13 ± 3.26*

BW: Body weight, *significantly different with ulcer control group at P < 0.05.

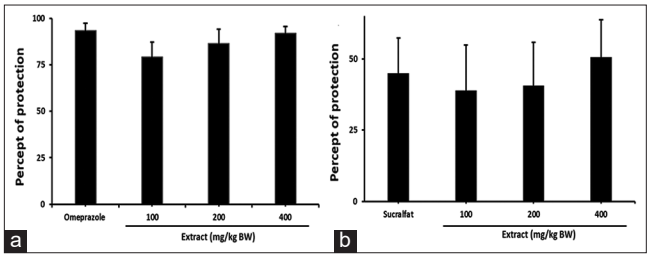

*Figure 3: Percentage of protection of gastric mucosa from P. macrocarpa extract on gastric damage caused by (a) ethanol and (b) acetosal in rats. *Significantly different with omeprazole or sucralfate as positive control group at p < 0.05*

Gastric secretion study

The inhibition of gastric acid secretion is probably the main strategy of most therapeutic agents for treating gastric ulcers [46], [47], [48]. In this study, PMEE showed protection against gastric ulcers by decreasing ulcer number, score, and index. Furthermore, the parameters of gastric secretion volume showed that PMEE can reduce gastric acid secretion in ethanol-induced ulcers.

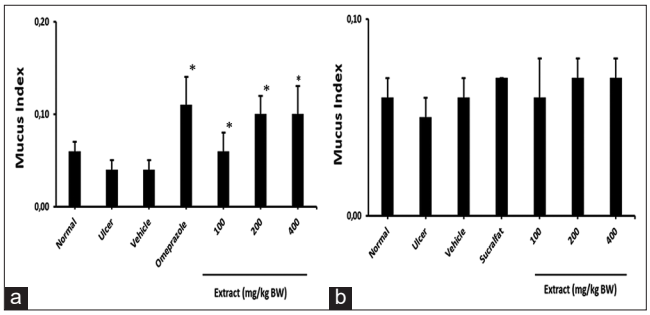

*Figure 4: Mucus index of treatment of P. macrocarpa extracts on gastric damage caused by (a) ethanol and (b) acetosal in rats. *Significantly different with normal control group at p < 0.05*

This indicated that the acidity of gastric juices can be reduced by the administration of the extract *P. macrocarpa* (Table 3 and Figure 5). However, further study on the compounds contained in the extract which are assumed to be responsible for reducing gastric acid secretion is recommended.

Table 3: Gastric secretion after the treatments

Group of treatments	Volume of gastric secretion (ml)		pH of gastric secretion	
	Ethanol	NSAID	Ethanol	NSAID
Normal control	2.32 ± 0.09*		4.92 ± 0.34*	
Ulcer control	4.08 ± 0.37	3.50 ± 0.35	3.80 ± 0.14	2.48 ± 0.12
Vehicle control	3.34 ± 0.19	3.08 ± 0.19	3.90 ± 0.28	3.48 ± 0.14*
Positive control	2.36 ± 0.31*	3.16 ± 0.32	4.74 ± 0.13*	3.24 ± 0.14*
PMEE 100 mg/kg BW	3.16 ± 0.22*	3.48 ± 0.46	4.02 ± 0.39	3.08 ± 0.18*
PMEE 200 mg/kg BW	3.00 ± 0.33*	3.20 ± 0.06	4.42 ± 0.43*	3.44 ± 0.27*
PMEE 400 mg/kg BW	2.84 ± 0.47*	3.04 ± 0.10	4.62 ± 0.44*	3.52 ± 0.20*

BW: Body weight; *significantly different with ulcer control group at P < 0.05.

Gastric histology studies

In the CMC Na control group, histopathological results showed damage and erosion of gastric cells, which were not as extensive as in the induction group. The rats that were given positive control of omeprazole showed more dense gastric mucosal cells than other groups. Moreover, the histopathological results of gastric cells of rats given PMEE at a dose of 100 mg/kg BW showed that the gastric cells were not intact and did not appear to be tightly packed between cells. This showed that at a dose of 100 mg/kg BW, PMEE still damaged epithelial cells in the tissue gastric mucosa, indicating a less gastroprotective effect than omeprazole (Figure 6).

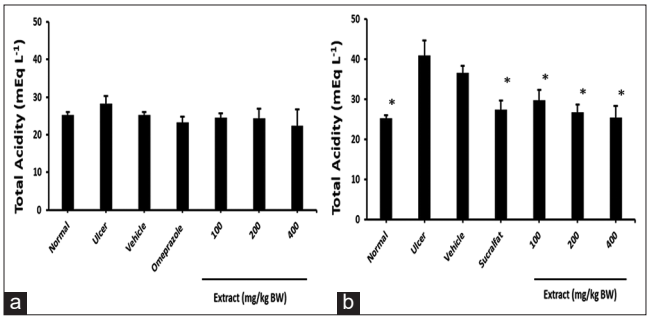

*Figure 5: Total acidity of gastric juices in the treatment of P. macrocarpa extracts on gastric damage caused by (a) ethanol and (b) acetosal in rats. *Significantly different with ulcer control group at p < 0.05*

In the PMEE group, the histopathological testing at a dose of 200 mg/kg BW showed no significantly different results from the 100 mg/kg BW group. Some cells that are not tightly packed showed that the erosion of gastric cells is not completely inhibited. Meanwhile, at a dose of 400 mg/kg BW, there were very few erosions and were denser, which are similar to the gastric of the

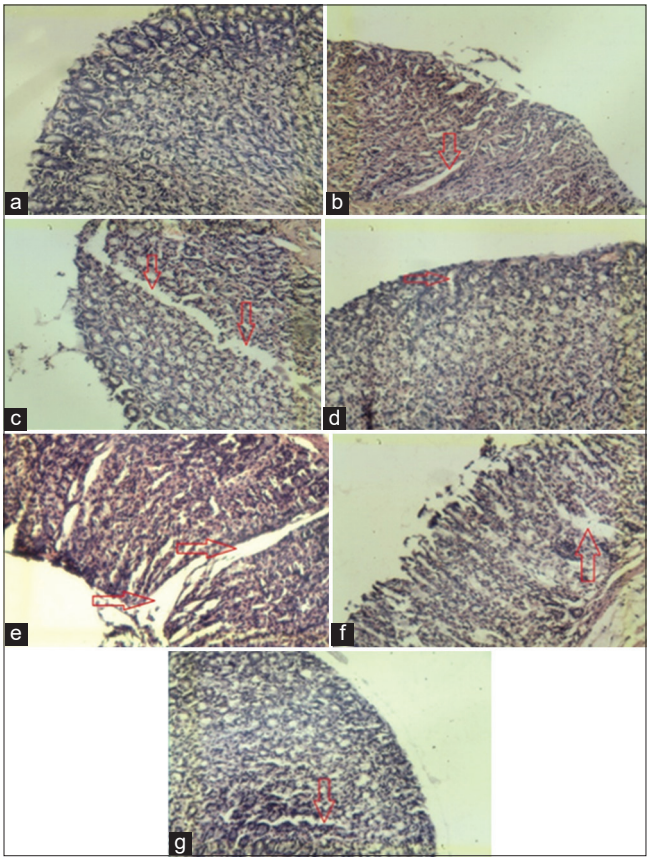

Figure 6: The results of microscopic observations of the rat gastric. (a) Normal control group; (b) vehicle control group; (c) ulcer control group; (d) positive control group (omeprazole); (e) PMEE 100 mg/kg BW; (f) PMEE 200 mg/kg BW; (g) PMEE 400 mg/kg BW). Red arrow indicates erosion

omeprazole group. This showed that the use of PMEE at a dose of 400 mg/kg BW is the best dose to protect the rat gastric mucosal cells from erosion caused by the inducing agent.

The high flavonoid in the extract is a factor that plays an important role in mucosal protection. The compound can change or reduce free radicals and acts as an anti-free radical in the body during the repair of damaged body cells [19], [31], [52], [53], [54]. Furthermore, administration of PMEE at a dose of 400 mg/kg BW can reduce the severity of erosion in gastric cells due to the flavonoid content.

Conclusion

The results showed that the ethanolic extract of *P. macrocarpa* had a gastroprotective effect on white rats induced with a gastric ulcer with acetosal and absolute ethanol. In addition, further study is needed to determine the compounds contained in the extract, which are responsible for providing a gastroprotective effect and the mechanism of action.

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