Original Research Article

Effect of Ajwa Date (*Phoenix dactilyfera L.*) extract in aspirin induced peptic ulcer model

Hendri Busman [1,*], Winda Trijayanthi Utama [2], Rizki Hanriko [3], Jason Mikail Amper [3], Indriyani [1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Bandar Lampung, Lampung, Indonesia; [2]Department of Public Health, Faculty of Medicine, Bandar Lampung, Lampung, Indonesia; [3]Department of Pathology, Faculty of Medicine, Bandar Lampung, Lampung, Indonesia

***Corresponding author:** Hendri Busman, Department of Biology, Faculty of Mathematics and Natural Sciences, Bandar Lampung, Lampung, Indonesia; E- mail: hendri_busman@yahoo.com*

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ABSTRACT

Dates have been used to ameliorate various gastrointestinal diseases. This study aimed to evaluate the gastroprotective effect of ajwa dates extract on rats induced by aspirin. Rats were divided into five groups using a completely randomized design. Group 1 received only 300 mg/kg BW aspirin. Group 2 received 25.2 mg/kg BW omeprazole and aspirin 300 mg/kg BW. Groups 3, 4, and 5 received 250, 500, and 1.000 mg/kg BW ajwa dates extract and 300mg/kg BW aspirin, respectively, during 6 h. At the end of the examination, the average gaster damage score was 1.36, 0.24, 0.96, 0.68, and 0.80, in groups 1, 2, 3, 4, and 5, respectively. The 500 and 1.000 mg/kg BW doses of ajwa dates extract significantly reduced the gaster damage score. It is suggested that the extract of the ajwa date could be utilized as a gastroprotective agent.

***Keywords*:** Aspirin; Ajwa date extract; gastroprotective

INTRODUCTION

Peptic Ulcer (PU) is a multifactorial disease occurring in the gastrointestinal system that affects many people worldwide [1]. It is estimated that the prevalence of the disease is around 5-10 %, with an incidence rate of 0.1 % to 0.3 % each year [2].

Patients with this disease might show abdominal discomfort, nausea, emesis, bleeding, and weight loss. Mucosal damage

up until the submucosa is identic with this disease [3]. PU can be caused by several factors, which are *Helicobacter pylori (H.pylori)* infection, alcoholism, stress, poor diet, and usage of Non-Steroid Anti Inflammation Drugs (NSAID) [4].

The long-term usage of NSAIDs can cause damage to the digestive tract, from the esophagus to the rectum. Complications in the upper digestive tract are estimated to be six times more likely than in the lower digestive tract. One of the complications caused by the usage of NSAIDs is PU.

PU is associated with the drugs' mechanism and inadequate mucosal protection [5]. Aspirin is an NSAID regularly used to alleviate inflammation, fever, and pain. This drug is used to treat diseases such as *rheumatoid arthritis* and prevent cardiovascular thrombosis [6]. Long-term drug usage is associated with erosion, bleeding, ulceration, and perforation of the stomach mucosa. PU is found most in aspirin users [7]. PU is caused by the drug's mechanism, which inhibits endogenous prostaglandin synthesis, which protects the stomach mucosa from external factors; inhibition of prostaglandin leaves the stomach mucosa more prone to inflammation and bleeding [8].

The treatment of PU mainly revolves around synthetic drugs such as proton pump inhibitors, histamine blockers, and antacids [9]. One of the examples of a proton pump inhibitor is Omeprazole [10]. These drugs cause side effects such as joint pain, changes in heart rate, hematopoietic changes, gynecomastia, impotence, and systemic alkalosis, along with the prices of these drugs [11]. Therefore, the search for an alternative treatment has garnered attention [12]. Several products have been found to alleviate PU, one of which is using dates [13].

Dates *(Phoenix dactylifera)* is a plant grown in the middle eastern part of Asia and several African countries. Phytochemical analysis of the fruit contains *anthocyanin, phenolics, sterols, carotenoids, procyanidins*, and *flavonoid*s. These compounds have various positive effects such as free radical scavenging, antioxidant effects, anti-inflammatory and gastroprotective effects [14]. Antioxidants can prevent stomach mucosa cell damage caused by free radicals due to aspirin's long-term usage [15]. Musa's research shows that rats with PU induced by alcohol given dates show protection in stomach mucosa ulceration [16]. Research showed that at doses 250 mg/kg BW and 1.000

mg/kg BW showed the date extract's optimum antioxidant activity [17].

The date fruits is rich with active flavonoid such as quercetin, isoquercetin, luteolin, appgenin and rutin [18]. Hamad *et al*. showed that the total flavonoid content in date fruits is 2.79 mg/100 g with quercetin (1.35 mg/100 g) as the most dominant flavonoid. The Ajwa date fruit's antioxidant effect is its contents: phenols, melatonin, carotenoid, and vitamins. Flavonoid acts as an antioxidant by suppressing free radicals [19]. Ajwa date fruits are considered a natural agent that can contribute to the treatment of PUs through the modulation of several pathways. Through this study, it could probably place as a safe alternative or adjuvant therapy for PUs patients.

MATERIALS AND METHODS

Study Design

This study is an experimental research that uses a randomized control method with a posttest-only design. A 30 male *Spraque dawley* rats weighing range 150-200 g, aged 10-16 weeks, were housed under standar 12 h light and 12 h dark condition with free access to water and food. Rats were randomly selected and divided into five groups either control (untreated PU and Omeprazole treated PU) or experimental group (low, medium, and high Ajwa date extract). All procedures were performed in compliance with the Faculty of Medicine's ethical research committee, the University of Lampung, with approval number is 144/UN26.18/PP.05.02.00/2021.

Induction of PU using aspirin

All rats received an oral dose of freshly prepared aspirin, 300 mg/kg BW, in sterile aquadest. Aspirin can cause damage in the stomach, mild dyspepsia, abdominal pain and duodenum, and PU. 100 mg tablets of aspirin were used [21].

Ajwa Dates Extract Treatment

Rats received an oral dose of freshly prepared Omeprazole and Ajwa dates extract. One group (PU + Omeprazole) received a 25,2 mg/kg BW. The second group (PU + Low) received a low dose of 250 mg/kg BW extract. The third group (PU + medium) received a medium dose of 500 mg/kg BW extract, and another group (PU + High) received a high dose of 1.000 mg/kg BW extract. All doses were given as single doses, 30 min after rats received aspirin.

Sample Collection

After 6 h, rats were randomly assigned to either control or experimental groups. Rats were euthanized using ketamine (80-100

mg/kg BW). The gaster was collected and fixed in 10 % phosphate-buffered formalin.

Histological Examination

The gaster was fixed for 48 h in 10 % phosphate-buffered formalin at room temperature. The histopathological preparations were made with Mayer Hematoxylin staining carried out following the protocol prepared by the Department of Pathology, Faculty of Medicine, University of Lampung. The degree of histopathological change, to know the gastric damage, was assessed using a scoring system according to Hanriko *et al.* (2018), i.e., score 0 for normal gaster, score 1 for mild damage, score 2 for moderate damage, and score 3 for severe damage [20].

Statistical Analysis

The gastric damage score data were tested using the Kruskal Wallis test followed by the Mann Whitney test. All tests were performed at a 95 % confidence level.

RESULTS

As expected, aspirin induction significantly increases the gastric damage score (untreated PU groups), while the administration of ajwa date extract significantly reduced the gastric damage score, with the highest decrease occurring in the medium doses group. However, the damage scores decrease in the experimental group was still lower than the Omeprazole treated PU group (Table 1).

Tabel 1. The percentage of decrease in gastric damage score

Experimental Group	Gastric Damage Score	Difference of Score with C-Group	Decrease Percentage (%)
PU	1.36 ± 0.260		
PU + Omeprazole	0.24 ± 0.089*	1.12	82
PU + Low	0.96 ± 0.260	0.40	29
PU + Medium	0.68± 0.389*	0.68	50
PU + High	0.80± 0.283*	0.56	41

*Indicates the significant difference with PU group based on the Mann Whitney test with α=5 %

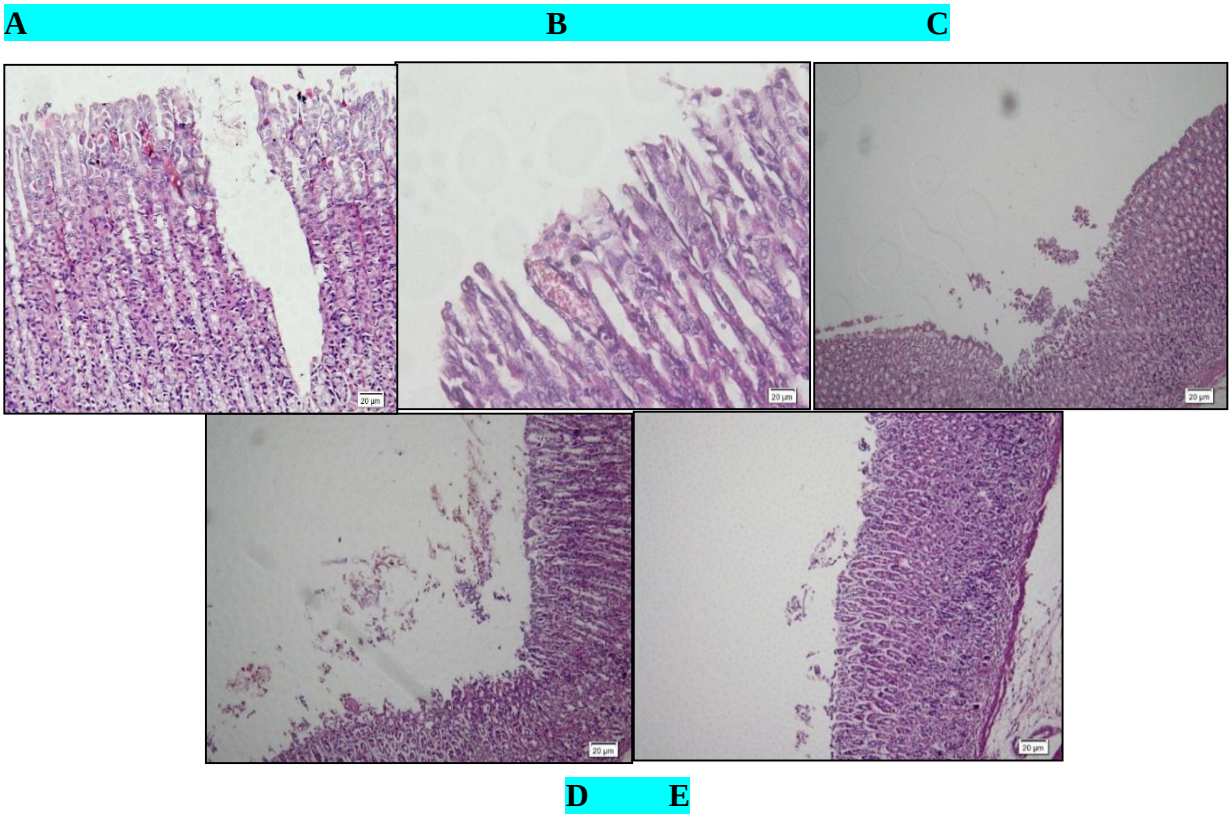

Figure 1. Histological study of the gaster with Mayer Hematoxylin staining. Untreated PU (A) showed epithelial ulceration and desquamation. PU+Omeprazole (B) showed minimum epithelial desquamation. Ajwa date treated PU groups (C, D, E, low-medium-and high doses) showed an epithelial erosion and minimum epithelial desquamation, without epithelial ulceration.

DISCUSSION

Medicinal plants play an excellent role in treating some diseases because they offer some efficacy without troubling some dangerous side effects. Ajwa dates have many potential antioxidant agents; they can serve as a protective agent for the gastrointestinal mucosal track by inhibiting ROS.

In the current study, an animal model of peptic ulcers was used. Aspirin was used to induce gastric mucosal damage due to its action mechanism that inhibits prostaglandin production so that gastric mucosal defenses are impaired. It is evident in the current study, where the highest

gaster damage score was seen in the untreated PU group (1.36). In the untreated PU group, desquamation and ulceration of the gastric mucosa were found. The administration of aspirin makes aggressive and defensive factors in the stomach not balanced. Aggressive factors such as neutrophils, ROS, H2O2 can infiltrate the gastric mucosa and cause damage. This result is in line with research conducted by Clara et al. (2012) with aspirin at a 300 mg/kg BW dose, which can cause significant damage to the gastric mucosa [21]. NSAIDs such as aspirin cause gastric damage by inhibition of endogenous prostaglandins by inhibiting the COX enzyme. There are two forms of COX in the body, namely COX-1 and COX-2. COX-1 inhibition reduces blood circulation to the gastric mucosa, reduces mucus and bicarbonic acid secretion, and increases gastric acid levels. COX-2 inhibition will lead to reduced angiogenesis and increased adhesion of leukocytes with cells, which results in microvascular occlusion resulting in disruption of mucosal defenses, induction of oxidative stress, and damage to the mucosa also, aspirin lysis phospholipids in mucosal epithelial cells, which increase mucosal permeability. Therefore, aggressive stomach factors such as gastric acid and aspirin can penetrate the mucosal defenses and cause inflammation and mucosal damage [22]. The release of oxidative stress in the gastric mucosa causes neutrophil infiltration of the gastric mucosa, besides aspirin can cause gastric mucosal damage by increasing levels of H2O2 and OH- and decreasing levels of gastric peroxidation and antioxidants [23].

The PU + Omeprazole group found a decrease in the gaster damage score (0.24). It was obtained with a percentage reduction of 82 % compared to the untreated PU group. In the current study, Omeprazole can inhibit gastric mucosal damage by suppressing gastric acid secretion to damage the gastric mucosal wall. This result is consistent with existing studies that Omeprazole is used as a medication for gastric ulcers. Omeprazole is a proton pump inhibitor. The action mechanism inhibits the parietal cells H + / K + ATP pump, which is the final stage in gastric acid production [24]. Omeprazole can suppress stomach acid production through this process to not irritate and damage the gastric mucosa. The omeprazole effect acts as soon as 1 h after being given orally and maximum effect 2 h after administration.

Ajwa dates extract treatment in the three PU groups showed a decrease in gaster

damage score compared to the untreated PU group. The administration of low doses Ajwa dates extracts obtained an average score of 0.96, a decrease of 29 % compared to the untreated PU group. The administration of medium and high doses of Ajwa date extract significantly reduced the gaster damage score compared to the untreated PU group. The medium doses can reduce the score of gastric damage by 50 % (0.68), while the high doses can reduce gastric damage by 41 % (0,80). Based on this fact, there are indications that the administration of ajwa date palm extract has a protective effect against gastric damage induced by aspirin's administration. Only minimal desquamation of epithelial cells and gastric erosion without gastric ulceration was found in the three treatment groups.

This study results in the protective effect due to flavonoids' content, which has an antioxidant effect in the Ajwa date palm extract. The highest flavonoid content in ajwa date palm extract is quercetin [19]. The protective effect of quercetin is to inhibit oxidative stress; this is done by regulating the effects of oxidants and antioxidants. A study in rat liver found that quercetin can suppress cadmium fluoride-induced oxidative damage. Also, quercetin inhibited oxidative stress damage to gastric epithelial cells, which is in line with this study [25]. Gastric epithelial cell damage caused by Reactive Oxygen Species (ROS) such as H2O2 can be inhibited by quercetin by inhibiting ROS. Quercetin lowers ROS and increases glutathione (GSH) levels. When free radicals enter their forms in the body, Superoxide Dismutase (SOD) captures O2 and converts it to H2O2. This enzyme then converts H2O2 into H2O, so it does not become toxic. This process requires GSH as a Hydrogen donor [26]. Also, quercetin can act as a free radical scavenger; this quercetin can inhibit LDL oxidation in vitro and inhibits damage caused by free radicals [27].

Among the three treatment groups, it was found that medium doses had the highest protective effect. A low dose may not be sufficient to have a significant protective effect compared to medium and high doses. Simultaneously, the medium doses have a higher protective effect than high doses. This result follows the theory, which states the relationship between a substance or drug concentration and the response to a given therapy. The response caused by a substance in low doses will usually increase in proportion to the dose increase. As the dose of the drug increases, the body's

increased response will decrease so that the increase in the dose will no longer show an increase in response. The relationship between a substance or drug concentration and its effect reflects a parabolic rather than linear curve [28]. In this study, it can be concluded that the medium dose is the optimal dose for protection of the gastric mucosa from aspirin induction at a dose of 300 mg/kg BW.

CONCLUSION

Medium and high doses of Ajwa date extract showed significant protective effects on gaster damage, while low doses showed a minimum protective effect. Although it has a significant protective effect, medium and high doses of Ajwa date extract have not been able to match the effect of Omeprazole administration to reduce gaster damage score.

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