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Contributed Paper

Gastroprotective Effects and Antioxidant Activities of *Paederia pilifera* Hook.f. Root Extract

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ABSTRACT

Gastric ulcer is a type of gastrointestinal diseases, which affects people all over the world. Modern gastric ulcer drugs have various severe side effects and show less efficacy. Thus, more effective and safer anti-ulcer agents are needed. Reactive oxygen species have important roles in the pathogenesis of various diseases, including gastric ulcers. The gastroprotective effects and the antioxidant activities of *Paederia pilifera* Hook.f. (Rubiaceae) root extract were examined on ethanol-induced gastric ulcers in rats. The effects of the plant extract and omeprazole on ulcer index, percentage inhibition, antioxidant activities and lipid peroxidation were evaluated. Antioxidant properties of the plant *in vitro* were also determined. Root extract of *P. pilifera* exhibited high total phenolic and ascorbic acid contents and showed free radical scavenging activities. Pretreatment with *P. pilifera* extract at all doses (250, 500, 750 mg/kg BW) and omeprazole exhibited a significant reduction in gastric mucosal lesions. The percentage inhibition in the rats treated with the extract was dose dependent. Pretreatment with *P. pilifera* extract and omeprazole showed a significant decrease in lipid peroxidation and an increase in the activities of catalase (CAT) and superoxide dismutase (SOD) enzymes and glutathione (GSH) levels. The cyto-protective and antioxidant properties of the extract found in this study could be due to the presence of the bioactive compounds in extract.

Keywords: gastroprotective effect, antioxidant activities, *Paederia pilifera* extract, gastric ulcers, free radical

1. INTRODUCTION

Gastric ulcer is a major gastrointestinal disorder, which affects many people in the world. This disease causes deep lesions penetrating through the muscularis mucosa. It is well known that the development of gastric ulcers occurs due to an imbalance between various aggressive factors (ethanol, NSAIDs, smoking, stress, leukotrienes, reactive oxygen species) and endogenous defensive factors (mucus-bicarbonate barrier, mucosal blood flow, prostaglandins, enzymatic and non-enzymatic antioxidants). Gastric damage caused by ethanol and NSAIDs leads to an overproduction of the reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals

[5,13]. There is evidence of the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as gastrointestinal disorders and gastric ulcers. ROS attacks essential cell constituents, such as proteins, lipids, and DNA, and induces lipid peroxidation in the cell membranes [24]. Enzymatic and nonenzymatic antioxidants, including vitamin C, catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) play an important role in the prevention of the toxicity of ROS and in gastric damage. Although many modern drugs on the market have been widely applied in the prevention and treatment of gastric ulcers, most of these drugs have various severe side effects and have low efficacy. It is believed that herbal drugs have few side effects, are effective and less expensive than synthetic drugs. Many medicinal plants listed in ancient pharmacopoeias and in older herbal books have a reputation for treating gastric disorders, including gastric ulcers. In certain scientific literature, a large number of medicinal plants with gastric antiulcer potential have been reported. It is also known that medicinal plants contain a wide variety of antioxidants, such as phenolic compounds and vitamin C, which minimize gastric injuries in experimental animals [1, 2, 6, 27].

Paederia pilifera Hook.f. (Rubiaceae), known in the Thai language as *Kra-pang-hohm*, is a medicinal plant distributed throughout Thailand. It is a vine climbing plant with leaves that are 6-15 cm long and 2-6 cm wide. The opposite, green leaves may release a strong fetid odor when bruised. This plant can be found in the deciduous forests and tropical rainforests throughout India and Southeast Asia. In Thai herbal pharmacopoeias, this plant has been used in the treatment of gastrointestinal disorders such as diarrhea, gastiritis, food poisoning, dyspepsia, jaundice and hyperbilirubinemia [29]. Although *P. pilifera* has been used to treat numerous aliments, its gastroprotective effects and antioxidant activities based upon scientific studies, have not yet been published. The aim of our work is to determine the gastroprotective effects and antioxidant activities of *Paederia pilifera* root extract on the ethanol-induced gastric mucosal damage in rats.

2. MATERIALS AND METHODS

2.1 Animals

Male and female albino Wistar rats (*Rattus norvegicus*) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Thailand. Animals were housed in a temperature controlled (25±1°C) room with a 12-h dark and 12-h light cycle. The rats were fed *ad libitum* with a standard pellet diet and had free access to water. All procedures involving the animals were conducted with strict adherence to the guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of the Biology Department, Faculty of Science, Chiang Mai University (protocol no. Re 002/12).

2.2 Plant Preparation

The roots of the *Paederia pilifera* Hook.f. were collected from Chiang Mai Province, Thailand in April 2012. The plant material was identified by a botanist from the Department of Biology, Faculty of Science, Chiang Mai University. A voucher specimen (WP 3137) was deposited at Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand. The root was washed with water, cut into small pieces, dried in an oven at 60°C and then ground into a fine powder. One hundred grams of the root powder was subjected to continuous hot extraction in a

soxhlet apparatus with 50% ethanol (700 ml) as a solvent. The extract was evaporated under reduced pressure using a rotary evaporator and then freeze-dried respectively.

The extract was subjected to qualitative phytochemical screening according to the method of Rasool *et al* [22].

Determination of total phenolic and, ascorbic contents and antioxidant activity of the extract

Total phenolic content was quantitatively determined by the procedure described by Singleton and Rossi [28]. Ascorbic acid content of the root extract was determined according to the method of Schlessier *et al* [25]. The scavenging effect of the extract on the DPPH radical was examined based on the method of Brand-William *et al* [9]. The ABTs assay was measured by the method of Re *et al* [23].

2.3 Acute Toxicity Test

Female albino rats weighing 100 to 120 g were used for the acute toxicity study. The study was carried out as per OECD guideline 423[20]. No adverse effects or mortality were detected in the rats at up to 2 g/kg, p.o., during the 24-hour observation period. Based on the results obtained from this study, and on the recommended daily intake in humans, the determined dose for gastroprotective activity was fixed to be 250, 500 and 750 mg/kgBW.

2.4 Study of The Gastroprotective Effect and Antioxidant Activity of *Paederia pilifera* Using an Ethanol Induced Gastric Ulcer Method

2.4.1 Gastroprotective study

The effects of *Paederia pilifera* root extract and omeprazole on the ethanol-induced gastric damage were determined. Thirty male Wistar rats weighing between 180-200 g were divided into 5 groups with 6 rats in each group. Group 1 rats were a negative control group that received 1 ml of distilled water. Group 2 rats received 8 mg/kg body weight of omeprazole and served as a positive control group. Groups 3, 4 and 5 rats received root extract at doses of 250, 500 and 750 mg/kg body weight, respectively. The animals were given the root extract orally for a period of 15 days. On the 15th day, thirty minutes after pretreatment with the extract and drug, gastric ulcers were induced with absolute ethanol (5ml/kg body weight) in all rats, which had been fasted overnight. The animals were later sacrificed by an overdose of ether and their stomachs were immediately excised. The stomachs were opened along the greater curvature and washed with normal saline solution.

2.4.2 Assessment of ulcer index and percentage inhibition

The ulcer lesions were examined and scored (mm^2) under light microscopes. The opened stomachs were cut in half and the right part was scraped with a glass slide to obtain samples of gastric mucosa. The samples were stored at 4°C for subsequent antioxidant activity assay. Ulcer index and percentage inhibition were calculated using the following formulas:

$$\text{Ulcer index (UI)} = \frac{\text{total ulcer score}}{\text{Number of animals ulcerated}}$$

$$\text{Percentage Inhibition} = \frac{\text{UI of ethanol treated group-UI of pretreated groups} \times 100}{\text{UI of ethanol treated group}}$$

2.4.3 Histological evaluation

The left part of each stomach was fixed in 10% buffered formalin. Tissues were dehydrated and processed for histological examination using standard techniques. Sections were cut into 6 μm thickslices and stained with hematoxylin and eosin. The sections were mounted and histopathological

changes were observed under a light microscope.

2.4.4 Estimation of antioxidant activities

The mucosal scrapings were weighed and gastric mucosa homogenates were prepared in 20 mM Tris HCl buffer (100g/L). The homogenate was centrifuged at 10,000 g for 20 minutes using a cold centrifuge. The supernatant was used to measure MDA, GSH, CAT and SOD.

Lipid peroxidation in gastric mucosa was estimated by determination of malondialdehyde (MDA) using the thiobarbituric acid reaction substances (TBARS) test according to the method of Buege and Aust [10]. Superoxide dismutase (SOD) activity was measured according to the method of Xin *et al* [30]. The amount of glutathione (GSH) was estimated according to the method of Sedlak and Lindsey [26]. Catalase activity was determined according to the Goldblith and Proctor method [12]. The protein content was assayed by the method used by Bradford [8].

2.5 Statistical Analysis

Results were expressed in terms of mean ± SD. The statistical significance of differences between groups was assessed using one-way ANOVA followed by a least significant difference test (LSD). A value of $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

The phytochemical analysis confirmed the presence of various components, such as alkaloids, glycones, terpenoids, saponins, phenols and anthraquinones in the root extract of *P. pilifera*. The yield of the extract was 6.71% w/w.

3.1 Determination of Total Phenolic and Ascorbic Contents and Antioxidant Activity of *P. pilifera* Extract

The root extract of *P. pilifera* exhibited a high total phenolic content (58±0.01 mg gallic/g extract). The ascorbic acid content in the extract was found to be 35±0.05 mg/g extract. The free radical scavenging activities of the extract were measured by DPPH and ABTS assays. The extract scavenged the DPPH and ABTS radicals with an IC_{50} value of 17.02 mg/ml and 57.57 mg/ml, respectively.

3.2 Study of Gastroprotective Effects

The oral administration of absolute ethanol induced elongated reddish bands of hemorrhagic erosions in the gastric mucosa (Figure 1b). Mild hemorrhagic erosions developed in the gastric mucosa of the omeprazole treated group (Figure 1c). *P. pilifera* treated groups showed a dose-dependent decrease in hemorrhaging where compared with the ethanol group (Figure 1d,e,f).

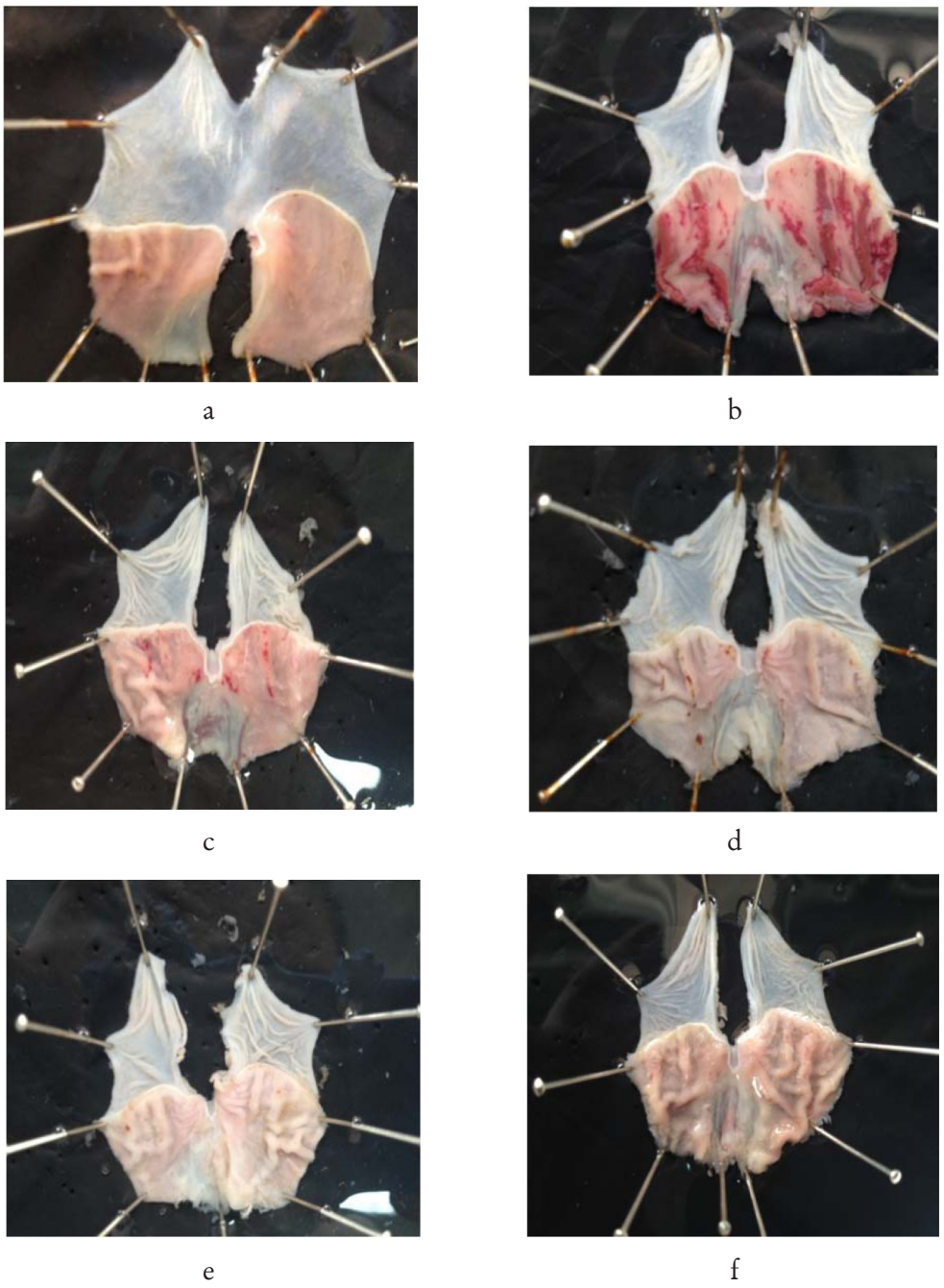

Figure 1. Gross appearance of gastric mucosa in rats: (a) gastric mucosa of a normal control rat (b) gastric ulcerated rat group (c) omeprazole treated group, and (d)(e)(f) pretreatment with *P. pilifera* extract at the doses of 250, 500 and 750 mg/kg BW, respectively.

The gastric ulcerated group revealed the highest ulcer index rating (Table 1). As shown in Table 1, pretreatment with *P. pilifera* extract and omeprazole exhibited a significant reduction in the ulcer index, when compared with the gastric ulcerated rat group ($p < 0.05$). The percentage inhibitions were 75.62%, 81.17% and, 91.03% in rats treated with 250, 500 and 750 mg/kg BW of the extract, respectively, while the rats treated with omeprazole showed a lower percentage of inhibition. Ulcer index and percentage inhibition in the rat treated with the extract were determined to be dose dependent.

Table 1. Gastroprotective effect of *P. pilifera* extract on ethanol-induced gastric lesion in rat.

Experimental Groups	Ulcer index	% inhibition
Normal control	-	-
Gastric ulcerated group	84.09 ± 16.4^{a}	-
P. pilifera 250 mg/kg	20.50 ± 6.40^{b}	75.62
P. pilifera 500 mg/kg	15.83 ± 1.00^{b}	81.17
P. pilifera 750 mg/kg	7.54 ± 1.40^{c}	91.03
Omeprazole 8 mg/kg	31.64 ± 0.50^{d}	62.37

Data are presented as mean ± standard deviation. Differences in superscript letters are considered significantly different ($p < 0.05$).

Gastric ulcers occur due to an imbalance between aggressive factors and endogenous defensive factors. Gastric mucus and bicarbonate secretion, maintenance of mucosal blood flow and prostaglandin production are known to protect the gastric mucosa against damage. It is known that increases of gastric acid secretion, reduced mucosal blood flow, inhibition of prostaglandin production and a decrease of gastric mucus enhance the susceptibility of the mucosa to injuries induced by the aggressive factors [11]. Ethanol is an aggressive factor which induces gastric ulceration. It rapidly penetrates the gastric mucosa, promotes membrane damage and ulcer formation through a destruction of the mucus barrier, increases vascular permeability and damages the capillary endothelium [3, 21]. Ethanol also has the ability to induce the formation of leucotriene, a lipoxygenase derived metabolite of arachidonic acid, and inhibits the agents that enhance the mucosal defensive factors, such as prostaglandin [17]. We showed that the administration of ethanol produced significant gastric damage in rats, while the damage was reduced in the extract pre-treated groups. As shown in Table 1, pretreatment with *P. Pilifera* extract at the doses of 250, 500, 750 mg/kg BW revealed a protective action against ethanol-induced gastric mucosa damage as demonstrated by the reduction of the gastric ulcer area.

This result was confirmed by the histological examination showing a prevention of mucosal hyperemia, edema and epithelium disruption found in gastric ulcer induced rats. Histological observations of ethanol induced gastric damage showed severe disruption of the surface epithelium, hemorrhaging in the mucosa and deep mucosa, edema and neutrophills infiltration of the submucosal layer (Figure 2b). Mild epithelium disruptions and edema of the submucosa developed in the gastric mucosa of the omeprazole treated group (Figure 2c). Pretreatment with *P. pilifera* extract provided better protection of the gastric mucosa than did the drug. This was indicated by a reduction of damaged mucosal epithelium, reduced or absent edema and neutrophills infiltration in the submucosa (Figuresure 2d,e,f).

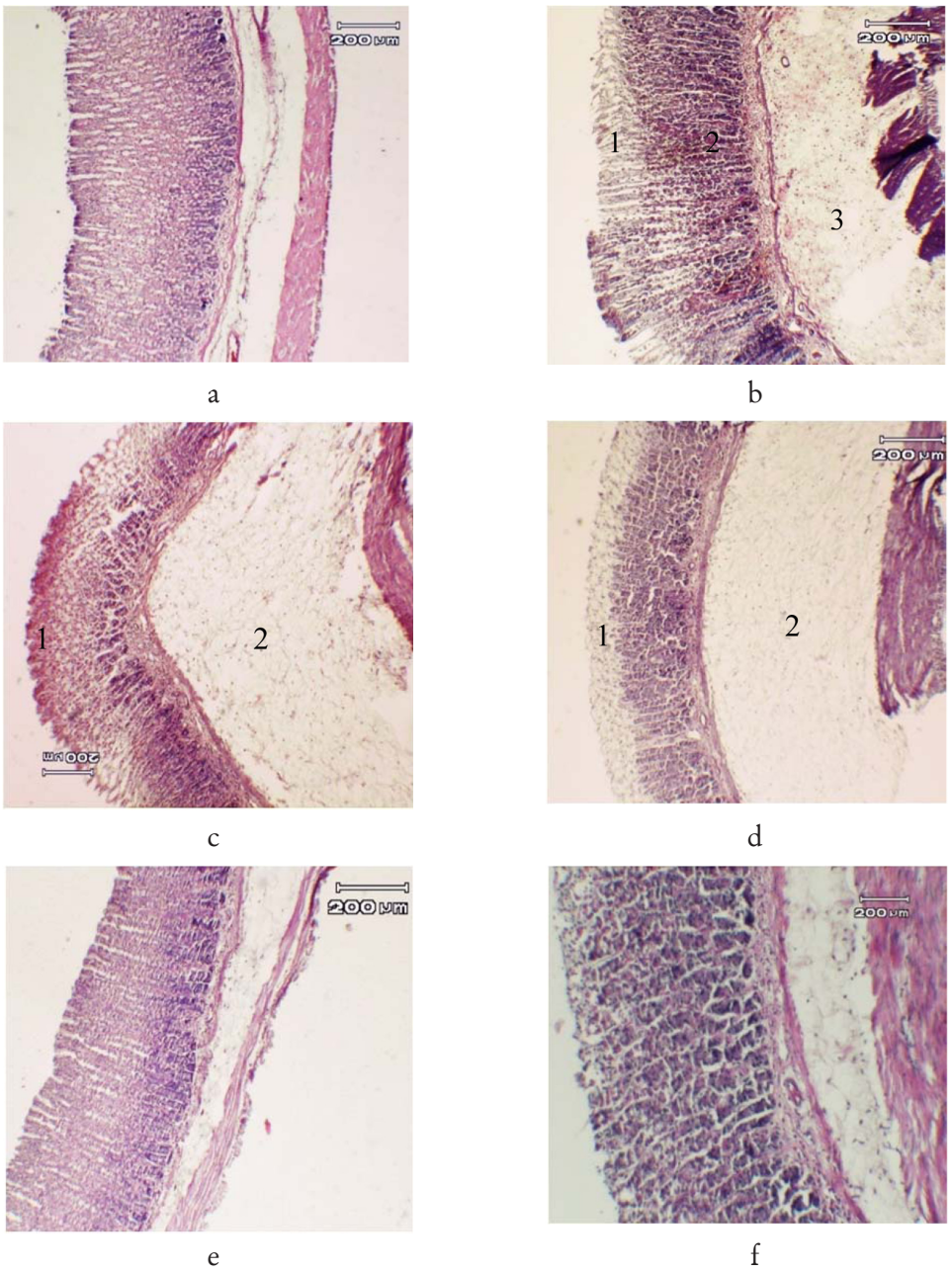

Figure 2. Histological study of gastric mucosa in rats: (a) Normal control rat; (b) gastric ulcerated rat damage showing severe disruption in the surface epithelium (1), hemorrhage in mucosa and deep mucosa (2), edema and neutrophills infiltration of the submucosal layer (3); (c) omeprazole treated rat showing mild epithelium disruptions (1) and edema of submucosa (2); (d) pretreatmant with *P. pilifera* extract at a dose of 250 mg/kg BW showing mild epithelium disruptions (1) and submucosa edema (2); (e) pretreatmant with *P. pilifera* extract at a dose of 500 mg/kg BW; (f) pretreatmant with *P. pilifera* extract at a dose of 750 mg/kg BW.

It is clear that *P. pilifera* extract effectively prevents mucosal damage. The gastroprotective effect of *P. pilifera* extract may be attributed to various compounds in the extract, including phenolic compounds, vitamin C, alkaloids, flavonoids, saponins and glycosides. These compounds have been found to possess antiulcer activities. Flavonoids and phenolic compounds increase mucosal prostaglandin production. Prostaglandins are found to produce a gastroprotective effect by decreasing acid secretion and also increasing gastric mucus and bicarbonate secretion, promoting angiogenesis and triggering mucosal cell proliferation [5]. It is possible that *P. pilifera* extract might be rich in flavonoids and phenolic compounds, and could enhance the function of prostaglandins. Flavonoids have been shown to increase the formation of new capillaries, decrease histamine secretion by the inhibition of histidine decarboxylase and chelate free radicals and reactive oxygen species, while inhibiting leucotriene [16]. The protective activities of saponins are probably due to the activation of the mucus membrane protection factors and the improvement of blood flow [4, 7]. Alkaloids have been reported to inhibit gastric acid secretion in rats [16].

3.3 Estimation of Antioxidant Activities

The effects of the pretreatment with *P. pilifera* extract on enzymatic and nonenzymatic antioxidants in gastric mucosa are presented in Table 2. As compared to normal control rats, ulcerated rats showed significant an increase in the level of MDA in gastric mucosa and a decrease in SOD, CAT activities and GSH levels ($p < 0.05$). Rats pretreated with *P. pilifera* extract at doses of 250, 500 and 750 mg/kg BW and omeprazole showed a significant decrease in lipid peroxidation and an increase in the activities of the antioxidant enzymes, as well as glutathione levels.

Table 2. Effects of pretreatment with *P. pilifera* extract and omeprazole on lipid peroxidation and the antioxidant activities in the gastric mucosa of rats.

Treatment	MDA (μmol/mg protein)	CAT (U/mg protein)	SOD (μmol/min/mg protein)	GSH (nmol/mg protein)
Normal Control	3.68 ± 0.21^{a}	0.0320 ± 0.004^{a}	7.05 ± 0.00^{a}	132.5 ± 3.54^{a}
Ethanol	4.82 ± 0.00^{b}	0.0043 ± 0.000^{b}	0.87 ± 0.12^{b}	14.0 ± 0.00^{b}
*P. pilifera*250 mg/kg	4.53 ± 0.04^{c}	0.0119 ± 0.0026^{c}	2.27 ± 0.24^{c}	55.0 ± 12.73^{c}
*P. pilifera*500 mg/kg	3.96 ± 0.57^{a}	0.0190 ± 0.0089^{c}	2.21 ± 0.15^{c}	61.5 ± 7.78^{c}
*P. pilifera*750 mg/kg	2.95 ± 1.26^{a}	0.0225 ± 0.0082^{ac}	6.45 ± 0.14^{d}	73.0 ± 2.83^{d}
Omeprazole8 mg/kg	4.45 ± 0.35^{ac}	0.0111 ± 0.0010^{c}	1.23 ± 0.15^{e}	53.5 ± 4.90^{e}

Data are presented as mean ± standard deviation. Differences in superscript letters are considered significantly different ($p < 0.05$).

Reactive oxygen species have an important role in the pathogenesis of various diseases, including gastric ulcers. The administration of ethanol results in an overproduction of free radicals, such as superoxide, hydroxyl and peroxyl radicals [18]. The production of free radicals leads to increased lipid peroxidation resulting in an increased MDA level. Preventive antioxidants, such as CAT, SOD and GSH, are the first line of defense against ROS. In the present study, the administration of ethanol significantly increased the levels of MDA and decreased CAT, SOD activities and GSH levels in the gastric mucosa in comparison to the normal control group (Table 2). It is obvious that ethanol caused the gastric injury by reducing the CAT and SOD activities, which were associated with increased oxidative damage. Pretreatment with *P. pilifera* extract and omeprazole showed significant increases in SOD, CAT activities and GSH levels and a decrease in MDA levels. Plant chemical substances, such as phenolic compounds and flavonoids which are present in *P. pilifera* extract have been shown to scavenge free radicals. The root extract of *P. pilifera* exhibited a high total phenolic content, which appears higher than the phenolic content that was detected in other plant extracts that were reported to possess gastroprotective effects [2, 19]. Ascorbic acid is related to the positive effects on antioxidant systems [15]. It acts as a chain breaking antioxidant, which impairs the formation of free radicals in the intracellular substances throughout the body. The qualitative determination of ascorbic acid in the extract of *P. pilifera* shows that it is a good source of ascorbic acid. This extract was found to possess antioxidant activity when tested in DPPH and ABTS assays. Thus, it is possible that the antioxidant activity of the root extract contributes to its anti-gastric ulcer activity. The extract components, known for their antioxidant and antiulcer activities, have a high potential for antioxidant activity and the gastroprotective effect.

4. CONCLUSION

The results of our study provide additional support for the traditional use of this plant as a gastric antiulcer drug. The root extract of *P. pilifera* has antioxidant activity and gastroprotective effects on ethanol-induced gastric ulcers. The cytoprotective and antioxidant properties of the extract are due to the presence of one, or a combination of bioactive compounds in the root. This finding could lead to the further isolation and pharmacological study of new therapeutic compounds against ulcers. Therefore, further studies are required to investigate the active compounds and the mechanisms of action of *P. pilifera* extract.

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