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NATURAL DRUGS

HEALING EFFECT OF HYDRO-ALCOHOLIC EXTRACT OF *EPHEDRA PACHYCLADA* BOISS. IN EXPERIMENTAL GASTRIC ULCER IN RAT

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Abstract: *Ephedra pachyclada* Boiss. (family Ephedraceae) is a medicinal plant very frequently cited as acting against gastrointestinal disorders in ethno-pharmacological inventories of the Kerman region of Iran. This study was done to evaluate the effect of hydro-alcoholic extract from the stems of *E. pachyclada* for treatment of gastric ulcers induced by ethanol in Wistar rats. Experimental treatments were the hydro-alcoholic extract of *E. pachyclada* (250, 500, and 1000 mg/kg, orally), omperazole as standard drug (20 mg/kg, orally), and control group. Ulcer index in mm^2 and histological examination were evaluated. On 3, 6, 9 and 12 day after treatments, the hydro-alcoholic extract of *E. pachyclata* (1000 mg/kg) produced 51, 72, 98.8 and 100% and omperazole also produced 53, 79, 93 and 100% curative effect for gastric mucosal damage in ethanol model, respectively. The results of the histopalogical analysis indicated the hydro-alcoholic extract of *E. pachyclada* at 1000 mg/kg was effective in experimentally healing rat ulcers. *E. pachyclada* accelerated ulcer healing in rats and, thus supports its folk medicine use by Kerman people.

Keywords: *Ephedra pachyclada*, ulcer, hydro-alcoholic extract

The family Ephedraceae has the only genus *Ephedra* L., which consists of about 50 species of perennials and shrubs in the world. *Ephedra* L. generally grows wild in arid and semiarid climates and distributed mainly in the temperate zones of Europe, Asia and North America. They are called "joint-pine", "joint fir", "sea grape", "mormon-tea" or "shrubby horsetails" in English, and "ormak", "rish boz" or "alijonak" in Persian (1). This genus is commonly used by the Chinese people as a folk medicine for treatment of allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, and nasal congestion. The main chemical compounds have been identified and isolated from *Ephedra* extract is alkaloids group such as ephedrine, pseudoephedrine, and norpseudoephedrine (2-4). In flora of Iran, 12 species of *Ephedra* has been reported (1). *Ephedra pachyclada* Boiss. ("Hoom" or "Hooma" in Persian) is distributed from Sinai to Pakistan, the Zagros range in Iran and extending south to Yemen and Oman. Lee and Lee (5) reported that quinaldic acid isolated from the stems of *E. pachyclada* had antibacterial activity against *Clostridium difficile* and *C. perfringens,* while had no effect on the growth of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *L. casei*.

Gastric ulcer is a major gastrointestinal disorder, which occurs due to an imbalance between the offensive and defensive factors (6, 7). Various factors, including stress, smoking, nutritional deficiencies, *Helicobacter pylori* and ingestion of non steroidal anti-inflammatory drugs increase the gastric ulcer incidence (8, 9). Current chemical drugs for treatment of peptic ulcer are associated with many side effects, tolerance to drugs and occurrence of relapse. These problems are the basis for developing of new drugs with herbal origin (10). In folk

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knowledge, medicinal plants are used for treatment of various disorders with little information of their pharmacological uses (11). For example, *E. pachyclada* popularly known as "Khimok" used for treatments gastric disorders by Kerman tribal, in Central Iran (12).

To our knowledge, no documented reports on the effect of hydro-alcoholic extract of *E. pachyclada* on gastric ulcers are available. Therefore, the aim of this study was to evaluate the protective effect of *E. pachyclada* hydro-alcoholic extract against gastric ulcers induced by ethanol in rat.

MATERIALS AND METHODS

Plant extract preparation

The stems of *E. pachyclada* were collected from Kerman, Iran in June 2011. The sample of plant was identified by regional floras and voucher specimen was deposited at the Herbarium of Agriculture and Natural Resources Research Center of Kerman province, Iran (No. 7667). The stems of *E. pachyclada* were dried at room temperature (30°C) and then powdered. The hydro-alcoholic extract was obtained by maceration of the crude plant powder with ethanol/water (70/30) for four days in a chamber temperature (35 ± 5°C). The hydro-alcoholic extract was filtered using a sterile cloth sheet, then dried under reduced pressure at temperature below 45°C with rotary evaporator and gave a dark red residue with yield 7%. The extract samples were stored in universal bottles and refrigerated at 4°C prior to use.

Determination of total phenolic content

Total phenolic compound content in extract was determined by Folin–Ciocalteu method with some modifications (13). In this method, 0.5 mL of the sample was mixed with 2.5 mL of Folin-Ciocalteu phenol reagent. After 5 min at 37°C, 2 mL of saturated Na_2CO_3 (7.5%) solution was added to the mixture and it was made up to 10 mL by adding deionized distilled water. The mixture was kept for 120 min at room temperature in the dark. The absorbance was measured at 765 nm against the reagent blank with a Shimadzu UV–Vis spectrophotometer (Shimadzu Corp., Japan). Gallic acid (Sigma, G-7384) was used as the reference standard. The total phenolic content is expressed as mg of gallic acid equivalents per gram of extract on dry basis.

Experimental animals

Male Wistar rats (200-250 g) of three months were used. The animals were housed in standard environmental conditions of temperature (22 ± 3°C), humidity (60 ± 5%) and a 12-h light/dark cycle. During experimental time, rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*. The rats were used for the experiment after one week of acclimatization period. All the procedures were approved by the Medical Ethics Committee of Shahrekord University of Medical Sciences.

Ethanol induced ulcer

After fasting for 48 h and supplied with sucrose 8% during the fasting period, each animal received 1 mL of absolute ethanol (99.6%) orally (14). The experimental treatments were submitted to the treatments with vehicle, omeprazole (20 mg/kg), hydro-alcoholic extract (250, 500, and 1000 mg/kg) 1 h after induction of gastric injury by absolute ethanol.

Determination of ulcer index

On 3, 6, 9 and 14 days after induced ulcer, ulcer area were measured in mm^2 by tracing the wound boundaries on a transparent paper. The animals were sacrificed 1 h later using an overdose of ether, and the stomach removed and observed for ulcers in the glandular and nonglandular region (15). The surface area of each lesion was measured and scored as described by Tan et al. (16). The ulcer index for each rat was taken as the mean ulcer score. The percentage of curative ratio (%CR) was calculated as described by Nguelefack et al. (17) using the following formula:

$$\% \text{ CR} = \frac{\% \text{ US(c)} - \text{US(t)}}{\text{US(c)}} \times 100$$

where US(c) = ulcer surface area in control and US(t) = ulcer surface area in treated animals.

Histopathology analysis

On 3, 6, 9 and 14 day after induction, the experiment was terminated and the ulcer area was removed from the surviving rats for histological analysis. The samples were fixed in 10% formaldehyde solution and were embedded in paraffin wax. A 5 µm thickness sections were stained with hematoxylin and eosin and observed for the histopathological changes under light microscope (Olympus BX51).

Statistical analysis

The results were expressed as the mean ± SD. The difference between the mean ulcer indexes of experimental groups were analyzed using ANOVA and followed by Tukey test; $p \leq 0.05$ was considered

Table 1. Effect of the treatments on ulcer index.

	Ulcer index (mm^2)			
Treatments	3rd	6th	9th	14th
Control	321.66 ± 17.90† b‡	251.00 ± 26.83 b	285.66 ± 7.85 c	314.66 ± 17.75 b
Standard drug (omperazole)	150.00 ± 33.20 ab	51.00 ± 11.84 a	19.66 ± 8.19 a	0.00 ±0.00 a
Extract 250 mg/kg	139.00 ± 43.31 a	104.66 ± 12.81 a	13.00 ± 3.05 a	6.00 ± 2.08 a
Extract 500 mg/kg	137.33 ± 36.33 a	75.33 ± 7.42 a	4.00 ±1.732 a	0.33 ± 0.33 a
Extract 1000 mg/kg	157.66 ± 56.29 b	70.00 ± 25.54 a	3.33 ± 1.20 a	0.00 ± 0.00 a
ANOVA	$p \leq 0.05$	$p < 0.01$	$p < 0.01$	$p < 0.01$

†Each value represents the mean ± S.D. n = 3. ‡Means with different letter in a row are statistically significant at 5% level probability.

Table 2. Effect of the treatments on curative ratio (*% CR*).

	Curative ratio (*% CR*)			
Treatments	3th	6th	9th	14th
Control	-	-	-	-
Standard drug (omperazole)	98	93.1	79.6	53.3
Extract 250 mg/kg	99	95	58.3	54.7
Extract 500 mg/kg	100	98.5	70	57.3
Extract 1000 mg/kg	100	98.8	72.1	51

statistically significant. All data processing was performed with SPSS software version 17.

RESULTS AND DISCUSSION

Ulcer index

The effect of treatments was investigated on experimental gastric ulcers induced by ethanol in rats (Table 1) Statistically, the percentage of ulcer index indicated that there were significant differences between treatments on 3, 6, 9 and 14 days after induced ulcer (Table 1). The ulcer index in control group was highly significant ($p < 0.01$) in comparison with the other groups during the experimental course (Table 1). The results indicated that omeperazole and the hydro-alcoholic extract at 500 and 1000 mg/kg indicated 99.8, 100 and 100% healing effect, respectively, after 14 days of experiment (Table 2).

Histopathology investigation

At 3rd day of treatment, all groups showed grossly severe mucosal ulceration, necrosis and hemorrhage. Ulcers in different forms and sizes were dispersed mainly in glandular and less in nonglandular part of stomach. The mucosal stomach was hyperemic and the margin ulcers were edematous and swollen. Hyperemia was more evident in the control groups than in the others. Histopathologically, necrosis and slaughing of gastric mucosa were occurred and erosions and ulcers in different degrees affected mucosal stomach. Lamina propria showed hyperemia and hemorrhage. Inflammatory cells, especially neutrophils and a few eosinophils, infiltrated around the marginal ulcers. Submucosal layer was edematous and thickened by a lot of fibrin exudates and hemorrhage (Fig. 1). At 6th day, the hyperemia and hemorrhage were decreased in all treated groups, especially in omeperazole treated group. Various sizes of ulcers and erosions were observed yet and their surfaces were covered superficially by a fibrinonecrotic layer. The control group showed severe hemorrhage and hyperemia with extended ulcers. Granulation tissue was developing at the base of ulcerated areas. Lamina propria and submucosal layer showed moderate hyperemia and inflammatory cells were scattered in these layers. At 9th day, except of control group, mucosal hemorrhage and

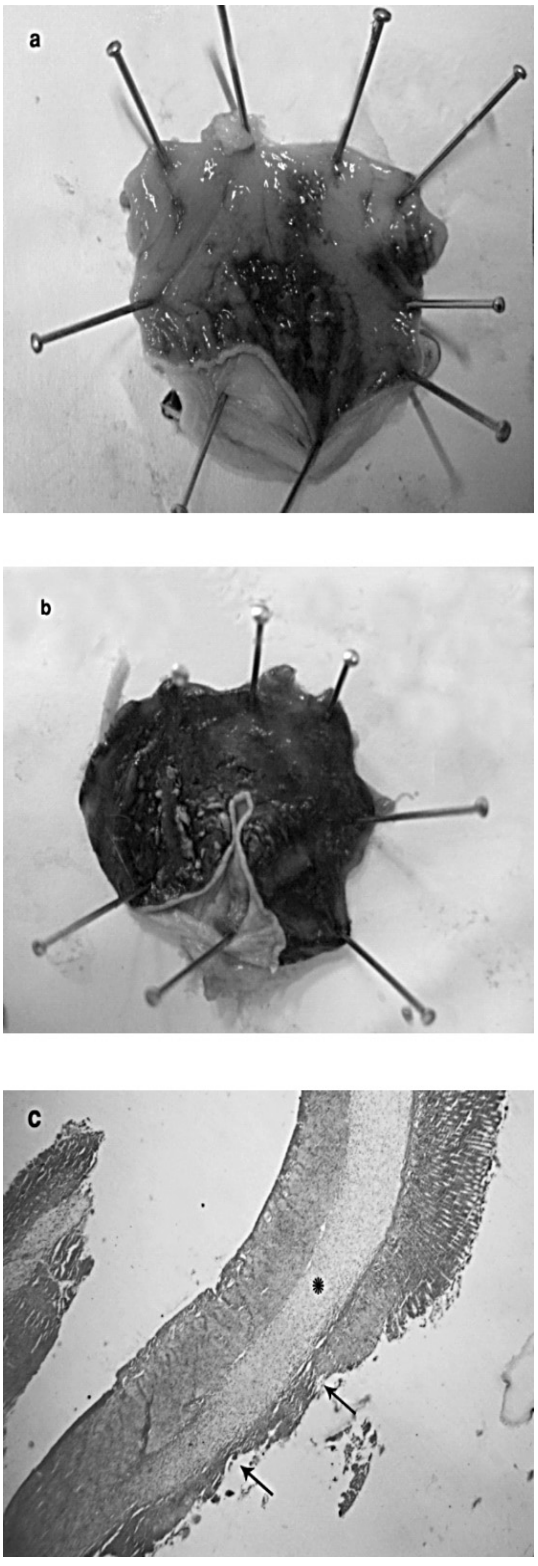

Figure 1. Gastric erosions, ulcers and hemorrhage induced by ethanol in rat; **a**) rat treated with *E. pachyclada* extract (1000 mg/kg), **b**) control group (saline), **c**) histopathologic features of stomach show mucosal ulcers (arrows) and thickening of submucosal layer by fibrin accumulation and inflammatory cells (asterisk) (magnification 40×, hematoxylin + eosin)

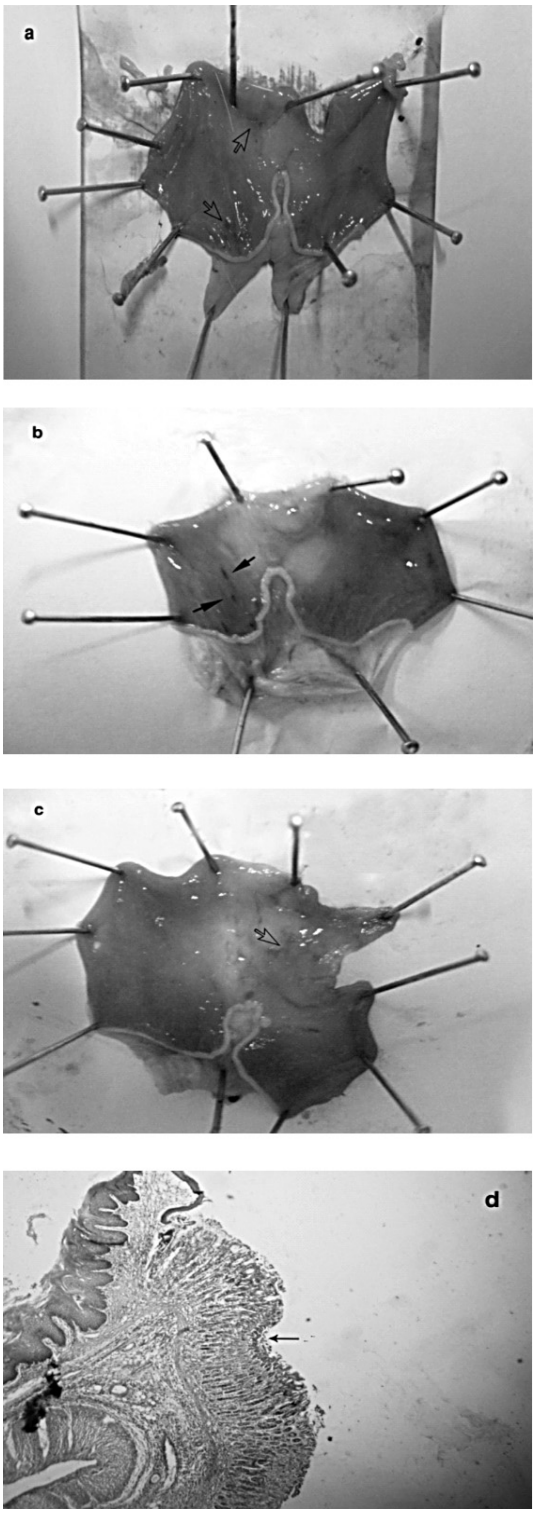

Figure 2. Ethanol-induced ulcers in rat's stomach 9 days after treatment; **a-c**) small erosions (arrows) in rat treated with *E. pachyclada* extracts at 1000, and 250 mg/kg and omeperazole (20 mg/kg), respectively (arrows). Treated group with the extract (250 mg/kg) show more erosion and ulcer in the stomach (arrows), **d**) histopathologic features of stomach show superficial erosion in the central part of healed ulcer (arrow) (magnification 100×, hematoxylin + eosin)

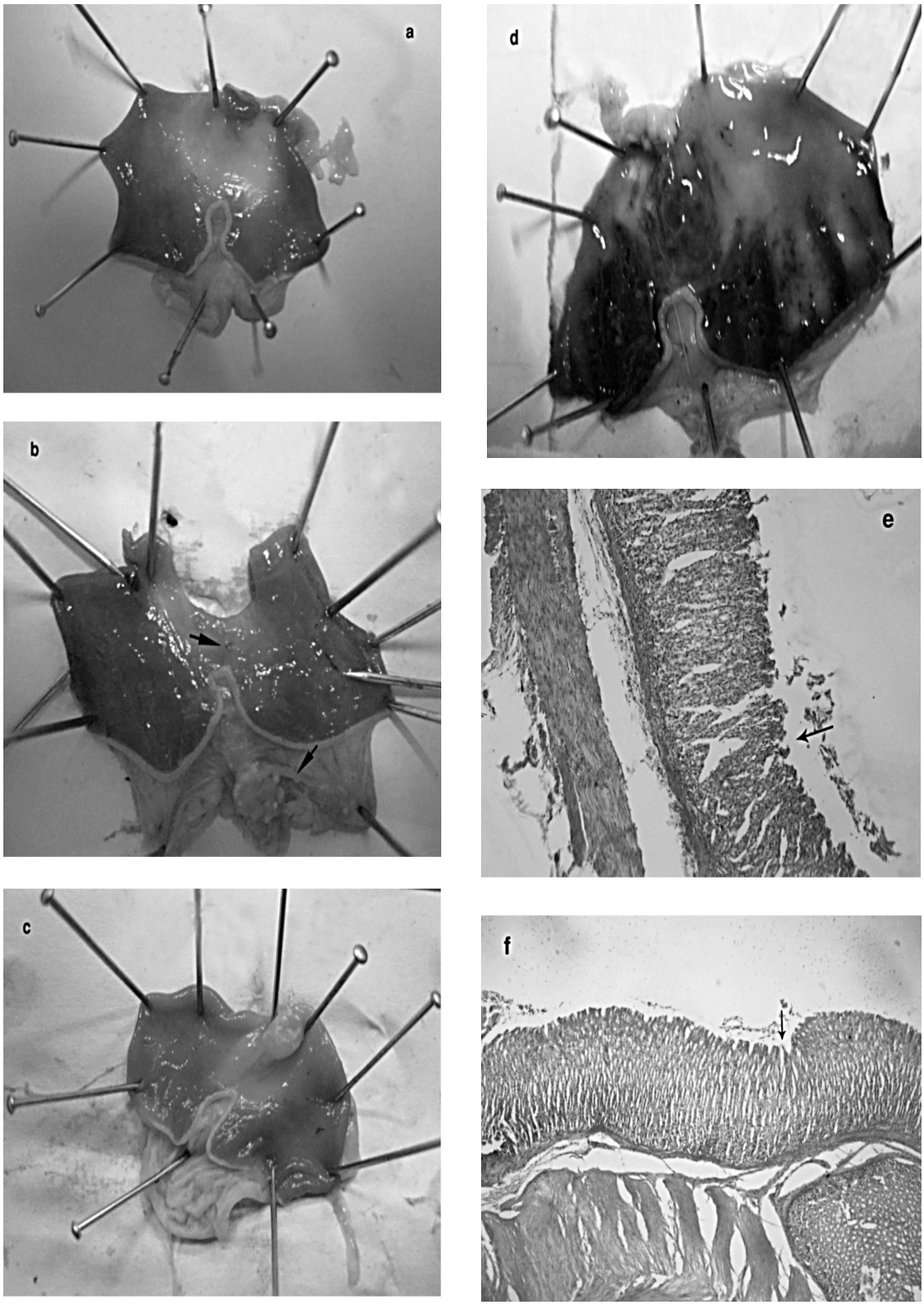

Figure 3. Ethanol-induced ulcers in rat's stomach 14 days after treatment; **a-c**) rat treated with *E. pachyclada* extracts at 1000, and 250 mg/kg and omeprazole (20 mg/kg), respectively. Macroscopic small erosions and ulcers are visible in treated rat with 250 mg/kg extract (arrows), **d**) control group (saline), **e**) histopathologic features of stomach treated with *E. pachyclada* extract (1000 mg/kg) show repair of ulcerated area (arrow) (magnification 100×, hematoxylin + eosin), **f**) histopathologic features of stomach in rat treated with *E. pachyclada* extract (250 mg/kg) show superficial erosion in the central part of healed ulcer (arrow) (magnification 100×, hematoxylin + eosin)

hyperemia were decreased remarkably in the extract groups (500 and 1000 mg/kg) and omeprazole group. The ulcerated lesions were healed but small erosions remained. The extract group at 250 mg/kg, showed more hemorrhage and hyperemia, and presence of some ulcers were noticeable. Thickness of gastric mucosa was variable in different areas and treated rats had thin-walled stomachs. Microscopic analysis showed well developed regeneration of epithelium in ulcers margin and throughout the ulcerated areas. New formed gastric glands had simple columnar epithelium and their lumens were dilated. The superficial erosions were located in the central portion of the healed ulcers (Fig. 2). The base of healed ulcer was formed by the dense connective tissue scar. In the submucosa, a great number of newly formed vessels were noticeable in the extracts treated groups especially at the higher doses. At 14th day, no gastric erosions or ulcers were found in the rats which received the extract (1000 mg/kg) and omeperazole but very small erosions were visible yet at dose 250 and 500 mg/kg of extract. Control group showed severe hemorrhage and mucosal damage (Fig. 3). We observed a thicker regenerative mucosa in treated animals for 14 days when compared to the control group. The thickness of regenerated areas was not the same in different parts of mucosa. In all groups, there were infiltration of a few lymphocytes and eosinophils in submucosal layer and the base of healed regions.

This study investigated the healing activity of the hydro-alcoholic extract of *E. pachyclada* on gastric ulcers induced by ethanol in rats. In experimental studies, intragastrical ethanol was used as the most common ulcerogenic agent that creates severe gastric hemorrhagic erosions and ulcers (18). The pathogenesis of gastric lesions induced by ethanol is multifactorial, including depletion of gastric wall mucus content (19), disrupting its barrier, stimulating and changes of microvasculature in few minutes after its application. Combination of rapid and severe vasoconstriction and then following vasodilation induces damage to capillaries of mucosal layer (20, 21). There is in agreement that the destructive effects of ethanol on gastric mucosa are results of enhanced lipid peroxidation. The oxygen free radicals cause lipid peroxidation and consequently gastric mucosal lesions that are induced by some agents such as indomethacin, alcohol and aspirin in rats (22).

Liquorice and vitamin E in rats are reduced under ethanol treatment and caused significant changes in the ulcer index. The presence of these phytochemicals may be responsible for the gastroprotective action of the plant extract. Schmeda-Hirschmann and Yesilada (23) reported that chemical compounds isolated from medicinal plants have varied antiulcer activity. Our results indicated that regenerative mucosa were thicker after 14 days with *E. pachyclada* hydro-alcoholic extract (500 and 1000 mg/kg) in comparison with the extract at 250 mg/kg and control group. Behravan et al. (24) reported that gastric ulcerogenicity of the extract of *Anacardium occidentale* (cashew nut) at the doses of 300, 400 and 800 mg/kg was lower than for the similar doses of indomethacin.

Results of our study indicated that total phenolic content in the extract of *E. pachyclada* was 45 mg of GAE/g dry weight. It has been demonstrated that many medicinal plants or herbs can contain a wide variety of free radical scavenging molecules, such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains) etc. which are effective in healing experimentally induced gastric ulcers (25).

Finally, we can suggest that the hydro-alcoholic extract of *E. pachyclada* was effective in experimentally healing rat ulcers. The hydro-alcoholic extract of *E. pachyclada* at 1000 mg/kg produced a significant gastric ulcer when compared with the negative control and omeperazole (standard drug). Further, the results of current study prove the anti-ulcer effect and suggest that it may be due to antioxidant mechanism of action.

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