Regular Article

Investigation of Antiulcer and Antioxidant Activity of *Juniperus phoenicea* L. (1753) Essential Oil in an Experimental Rat Model

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Juniperus phoenicea is a tree of the Cupressaceae family that is popularly known in the south of Tunisia because of its wide application in herbal medicine, including the use of its leaves to treat many diseases such as diarrhea, rheumatism, and intestinal disorders. The aim of this study was to evaluate the ulceroprotective and antioxidant activity of essential oil extracted from the leaves of *J. phoenicea* (EOJp) against hydrogen chloride (HCl)/ethanol-induced ulcers in rats. The antiulcer activities of 50, 75 and 100 mg/kg body weight (b.w.) EOJp were investigated on 0.3M HCl/ethanol-induced ulcers in rats. The essential oil yield was 0.69% with 48 compounds; α -pinene was the principal component (20.24%). *In vivo* pretreatment with EOJp given orally provided dose-dependent protection against HCl/ethanol-induced gastric ulcers in rats. Furthermore, pretreatment with EOJp significantly decreased malondialdehyde (MDA) content and increased the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The activity of the anti-ulcerogenic EOJp could be from synergistic antioxidant and anti-secretory effects. Oral use of EOJp has excellent preventive effects on induced gastric ulcers comparable to those of the proton pump inhibitor (PPI) omeprazole.

Key words *Juniperus phoenicea*; essential oil; hydrogen chloride (HCl)-induced ulcer; ethanol-induced ulcer; antioxidant; antiulcer effect

Gastric ulcers are part of a chronic relapsing disease that is well known to be a polyetiologic chronic disease.¹⁾ The disease results from an impairment of the balance between aggressive and protective factors.^{2,3)} Various factors, such as stress, the use of cigarettes and alcohol, and steroidal and non-steroidal drugs, can impair this balance,^{4,5)} through contributing to the loss of the mucosal epithelial lining in the gastric wall.⁶⁾ Although many classic drugs are available to treat gastric ulcers, these drugs do not offer permanent treatment, and studies to discover such a treatment are on-going. The current medicinal treatment of peptic ulcers is generally based on the inhibition of gastric acid secretion by H₂-blockers, anti-muscarinics and omeprazole, as well as on acid-independent therapy provided by sucralfate and bismuth.⁷⁾

In recent years, herbal medicines have triumphed as a diverse popular therapy and are emerging as an alternative to the available synthetic drugs.^{8,9)} In fact, frequent consumption of certain fruits and vegetables has been associated with a reduced risk of developing cancer, particularly in the breast and colon.^{10,11)} Therefore, the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Many medicinal plants exhibit antiulcer activity and were therefore found useful for treating peptic ulcers.¹²⁾

Red juniper (*Juniperus phoenicea*) is widespread in Americas, Asia, Europe and Africa. It has strong resistance to wind erosion and anthropic pressure.^{13–15)} This species is characterized, like other junipers, by its red fruit, its bristled juvenile branched and less powerful port and the bushy appearance of its cylindrical branches, as well as by its absence in high

mountains at altitudes above 2200m. In traditional medicines, several *Juniperus* species are used as remedies against many diseases. In fact, cones, twigs and especially the young shoots are administered by infusion because they have diuretic, stomach and digestive effects.^{16–20)} *J. phoenicea* is also used as a decoction against diabetes, diarrhoea and rheumatism, while dried and powdered fruit can cure skin ulcerations and abscesses.^{21–23)}

Chemical compounds are incorporated into pharmaceutical preparations, especially for antiseptic use, which is attributed to the presence of essential oils.^{24,25)} However, there is no information in the literature about the anti-ulcer effects of the essential oil of *J. phoenicea* (EOJp) nor was there any mention for other *Juniperus* species. Therefore, this study aims to investigate the chemical profiles and ulceroprotective and antioxidant activities of the essential oil of this plant against hydrogen chloride (HCl)/ethanol-induced ulcers in rats.

MATERIALS AND METHODS

Plant Material The leaves of *J. phoenicea* were collected on Mount Orbata (Jbel Orbata) near Sened (Gafsa, Tunisia). The plant material was authenticated by a Taxonomist, El Mokni Ridha (Laboratory of Botany and Plant Ecology; Faculty of Sciences of Bizerte, Bizerte, Tunisia) and the voucher specimens were deposited at the herbarium of the Laboratory of Medicinal Plants (National Agronomic Institute of Tunisia INAT, Tunisia) for future reference. No specific permission was required to collect plants from these locations, and the field studies did not involve endangered or protected species.

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Extraction and Chromatographic Analysis of EOJp

The essential oil from dried powdered *J. phoenicea* leaves was isolated by steam distillation in a Clevenger-type apparatus according to Procedure III of the Yugoslav Pharmacopoeia IV.²⁶⁾ The essential oil yield was 0.69% (w/w). Freshly isolated essential oil was a yellow liquid that has an intense, necrotic odour.

The GC-MS analysis of the essential oil from leaves of *J. phoenicea* were performed using a Varian GC-MS (GC Varian CP 3800; MS Varian Saturn 2200) equipped with a VF-5ms fused silica capillary column (30×0.25 i.d., film thickness 0.25 µm). An electron ionization system with ionization energy of 70 eV was used for GC-MS. Inert helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and injector temperature were set at 250°C. In fact, the oven temperature was programmed from 50 to 150°C at 10°C/min, then held isothermal for 6 min and finally raised to 250°C at 10°C/min. Samples (1 µL of *J. phoenicea* essential oil diluted in hexane 10%) were manually injected in the splitless mode and the relative percentage of the essential oil constituents was expressed as percentage by peak area normalisation.²⁷⁾

Identification of chemical compounds of the essential oil was based on GC retention time on VF-5 capillary column. Varian Workstation software was used in computer matching of mass spectra with those of standards.

Preparation of Drug Solution To prepare the appropriate stock solution of the drug, *i.e.*, 50 mg/mL, 75 mg/mL and 100 mg/mL, 100 g of the air dried leaves of *J. phoenicea* were steam distilled for 3 h. For the pharmacological tests performed here, the complete essential oil was emulsified in vehicle (0.1% Tween 80 aqueous solution) before it was administered to the animals. The doses were orally administered by selecting the appropriate concentration of the stock solution. After dissolved in vehicle, omeprazole has been given orally to the reference control group (6 rats) in doses of 20 mg/kg body weight.

Effect of EOJp on HCl/Ethanol-Induced Ulcers in Rats

Effect of orally administered essential oil of *J. EOJp* on HCl/ethanol (60:40, 15N)-induced ulcers in rats were investigated.

Animals

Adult male Wistar rats ($n=60$), weighing 170–190 g, were cared for in compliance with the code of practice for the care and use of animals for scientific purposes. Approval for these experiments was obtained from the Medical Ethical Committee for the Care and Use of Laboratory Animals of Pasteur Institute of Tunis, Tunisia (approval number: LNFP/Pro 152012).

Before any procedures were performed, all animals were kept for two weeks under the same laboratory conditions (temperature of $25\pm2^\circ\text{C}$ and relative humidity of $70\pm4\%$). They

were housed in polypropylene cages under pathogen free, uniform conditions of light and dark cycles (12 h each), and received a nutritionally standard diet (SICO, Sfax, Tunisia) and tap water.

Acute Toxicity of EOJp

A total of 24 rats were randomly divided into four groups ($n=6/\text{group}$). The first group served as a normal control group (C). Groups two (D1), three (D2) and four (D3) were treated with EOJp dissolved in a 0.1% tween, 80% aqueous solution at dose levels of 150, 200, 250 mg/kg, respectively. All animals were observed for toxic symptoms and mortality for 72 h.²⁸⁾

Administration of EOJp on HCl/Ethanol-Induced Ulcers in Rats

A total of 36 rats were divided into six treatment groups ($n=6$) and fasted for 24 h prior treatment; water was allowed *ad libitum*. The treatment of these groups was performed in two steps. First, the groups were treated with vehicle, EOJp dissolved in 0.1% tween 80 aqueous solutions or omeprazole (Omp) in distilled water. Omeprazole was used as a standard antiulcer drug, which acts as a gastric anti-acid output.²⁹⁾ The group (C) was kept as a control group without any treatment, while all other groups were fasted for 24 h and administered an oral dose of 0.5 mL of vehicle (0.1% tween 80 aqueous solution). One hour after the first treatment, the mice were treated with 80 mg/mL of a solution containing 0.3 M HCl/ethanol (60:40, 15N). One group (EtHCl) received a solution of HCl/ethanol, (60:40, 15N) alone. One group (Omp) was treated with omeprazole (20 mg/kg/body weight (b.w.)) and groups G1, G2, and G3 were treated with EOJp at the doses of 50, 75 and 100 mg/kg/b.w., respectively, dissolved in a 0.1% tween 80% aqueous solution (Table 1).

The animals were sacrificed under ether anaesthesia 1 h after the mice were treated with HCl/ethanol, and the stomachs were excised and inflated with saline injection (2 mL) to determine the ulcer index. Throughout the experiments, the stomachs were excised and photographed. They were then opened along the greater curvature; the gastric juice and mucus covering each stomach were then carefully collected into clean tubes. The samples were analysed for the gastric liquid volume, pH value, and mucus weight. Each stomach was then rinsed with saline solution (0.9%) and photographed, and the extent of the lesions were measured (mm^2) and taken as the ulcer index according to the method of Khan.³⁰⁾ All animals were treated humanely and all experiments were performed in the morning and in accordance with the guidelines provided by the Institutional Animal Ethics Committee.

Macroscopic Gastric Lesion Evaluation

The gastric mucosa of each rodent was gently scraped using a glass slide, and the obtained mucus was weighed using a precision electronic balance.³¹⁾ After washing with normal

Table 1. The Experimental Design and Specifications

Groups number	Description	Pre-treatment	Treatment
C	Control group	Control without any treatment	(–)
EtHCl	Ulcer control group	Half milliliter of vehicle (0.1% tween 80 aqueous solution)	(80 mg/mL) HCl/ethanol
Omp	Reference control group	Omeprazole 20 mg/kg	(80 mg/mL) HCl/ethanol
G1	Experimental group 1	Complex 50 mg/kg	(80 mg/mL) HCl/ethanol
G2	Experimental group 2	Complex 75 mg/kg	(80 mg/mL) HCl/ethanol
G3	Experimental group 3	Complex 100 mg/kg	(80 mg/mL) HCl/ethanol

saline, gastric lesions were quantified and ulcers were scored according to the method used by Dashputre and Naikwade.³²⁾

The ulcer scores were as follows:

- 0: normal coloured stomach.
- 0.5: red coloration.
- 1: spot ulcers.
- 1.5: haemorrhagic streak.
- 2: deep ulcers.
- 3: perforation.

The mucosal lesion area (mm²) was measured by planimetry using a transparent grid. The ulcer index (UI) for each rat was taken as the mean lesion area (mm²).

The percentage of ulcer inhibition was determined as follows:

%inhibition of ulceration

$$= \frac{(\text{Ulcer index EtHCl} - \text{Ulcer index treated})}{\text{Ulcer index EtHCl}} \times 100$$

Where UI_{EtHCl} is the ulcer index of the rats treated with HCl/ethanol, (60:40, 15N) and UI_{treated} is the ulcer index of the rats treated with EOJp or Omp and HCl/ethanol (60:40, 15N).

Assessment of Oxidative Stress in Tissue After the macroscopic analyses, the gastroprotective effect of EOJp was assessed from the malondialdehyde (MDA) and reduced glutathione (GSH) as well as the activities of enzyme antioxidants the super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in gastric tissues.

Preparation of Homogenate

The stomach tissues were grinded with liquid nitrogen in a mortar. The grinded tissues were weighed and homogenized with 4.5mL of buffer solution of potassium phosphate (pH 7.4). The mixtures were homogenized on ice, using an ultraturax, for 15min; then, the mixtures were filtered and centrifuged at 4°C. The supernatant were used to determine the enzymatic activities and MDA assays.

Estimation of Protein

The protein content of the gastric tissue was determined by the Folin Lowry Method using bovine serum albumin as a standard.³³⁾ Reduced glutathione GSH was estimated by the method indicated by Sedlak and Lindsay.³⁴⁾ The GPx activity was determined according to the method described by Sazuka *et al.*³⁵⁾ The SOD activity was estimated according to the method described by Misra and Fridovitch.³⁶⁾ The CAT activity was measured according to a report by Aebi.³⁷⁾ The GST activity was assayed with the method according to Habig *et al.*³⁸⁾ by adding 1-chloro-2,4-dinitrobenzene (CDNB). The lipid peroxide content in gastric tissues was determined by thiobarbituric acid reaction as described by in Ohkawa *et al.*³⁹⁾ The lipid peroxide concentration was expressed as the nmol MDA/mg protein.

Histological Studies After digital pictures of each stomach were obtained, the tissues were immersed for 48h at 4°C in the fixative solution (10% formaldehyde, in phosphate buffer, pH 7.6). For histological assessment, sections were obtained at intervals of 5µm and stained in haematoxylin-eosin solution (H&E). Tissue preparations were observed and microphotographed under a light Leica DM 2500 microscope.

Statistical Analysis A descriptive statistics using a software program SPSS 18.0 were used to describe the data,

which were presented as mean±standard deviation (S.D.). Data obtained were initially examined to confirm normal distribution and homogeneity of variance. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's test, and differences between treatments were considered significant for $p < 0.05$.

Table 2. Chemical Composition of Essential Oils Extracted from *J. phoenicea* Harvested in Southern Tunisia Using Analysis by GC-MS

Composants	Amount	RI
Androst-4-en-3-one	0.215	708
Hexadecanoic acid	0.587	717
δ-Cadinene	2.944	727
Podocarp-7-en-3-one	0.289	730
Caryophyllene oxide	0.106	732
β-Elemene	2.152	733
1,5,5-Trimethyl-6-methylene	4.702	745
Androstan-3-ol	0.016	750
Retinol	0.370	753
Octadecanoic acid	0.248	757
Ledene alcohol	0.237	758
Bornyl chloride	0.123	759
Aristolen epoxide	0.245	768
1-Naphthalenol	0.885	771
Terpinolene	4.227	773
Tricyclene	0.357	776
Longifolene-(V4)	2.880	783
Eucayptol	0.403	787
τ-Murolol	0.805	791
Murolan	0.119	792
Himachala-2,4-diene	0.141	796
Cyclohexane	3.332	802
Humulane-1,6-dien-3-ol	0.373	803
δ-Silienne	0.010	805
2,3-Dihydroxypropyl elaidate	0.411	808
p-Cymène	2.001	814
Cubenol	0.486	816
2-Cyclohexen-1-ol	0.151	825
Bornyl acetate	0.131	827
D-Limonene	0.440	828
1R,4S,7S,11R-2,2,4,8-Tetrame	3.791	831
Santolina triene	0.265	836
Isopulegol acetate	0.337	842
Bicyclosquiphellandrene	1.770	845
Cyclohexene	1.024	846
1,3,8-p-Menthatriene	0.039	847
Azulene	3.366	849
α-Pinene	20.245	851
Camphene	0.256	852
Ylangene	0.021	860
Myrcene	3.253	861
α-Cubebene	0.253	862
trans-Oxyde de linalool	3.727	865
Copaene	0.973	870
β-Pinene	5.683	880
Naphtalene	6.659	885
3-Carene	0.091	887
Thujopsene	0.330	896

RI=retention index.

RESULTS

Chemical Compositions of EOJp The essential oil yield of *J. phoenicea* from Gafsa is 0.69%, as illustrated in Table 2. It mainly consists of α -pinene (20.24%), which is accompanied by naphthalene (6.65%) of β -pinene (5.68%), terpinolene (4.22%), linalool (3.72%), myrcene (3.25%), *p*-cymene (2.00%) and bicyclosesphellandrene (1.77%).

Effects of EOJp on HCl/Ethanol-Induced Ulcers in Rats In the acute toxicity study, no deaths or toxic symptoms were observed during the test period. As a part of this pharmacological study, EOJp was investigated for its acute and general toxicity in rodents. It's interesting to note that the equilibrium between the therapeutic *versus* toxicological effects of a drug is a vital parameter in assessing its applicability in relation to pharmacological action.⁴⁰⁾ None of the rats (D1, D2 and D3) experienced any toxicity or mortality, and there were no abnormal physiological or behavioural changes, nor alterations in the body weight, at any time during the 14d of observation (Fig. 1).

EOJp treatment (50, 75 and 100mg/kg b.w.) caused a dose-dependent reduction in the HCl/ethanol-induced gastric lesions, decreasing the ulcer index and percentage of inhibition, especially at doses of 100mg/kg for G3 rats (98.94%) (Table 3). In this study, omeprazole protected the gastric mucosa against HCl/ethanol-induced ulceration in the Omp group (66.35%).

The results of histological observation of EOJp on gastric ulcers induced by the HCl/ethanol solution are shown in Fig. 2. In fact, there were severe injuries in the gastric mucosa (haemorrhagic necrosis of the gastric mucosa) of the EtHCl group (Figs. 2c, d) compared to the control group (Figs. 2a, b). Exfoliation and epithelial oedema of the submucosal layer with deformed glands have also been observed (Figs. 2c, d) whereas moderate injuries were observed in the gastric mucosa. Stomach of rats that were pretreated with omeprazole (20mg/kg) (Figs. 2e, f). However, stomach of G1 rats that were pretreated with EOJp (50mg/kg) (Figs. 2g, h) has shown minor injuries in their gastric mucosa (Figs. 2g, h) whereas stomach of G2 rats that were pretreated with EOJp (75mg/kg) has shown a very mild or even the absence of injury in the gastric mucosa (Figs. 2i, j). No injuries have been observed in the gastric mucosa of the rats G3 stomach that were pretreated with EOJp (100mg/kg) (Figs. 2k, l).

The gastric HCl/ethanol toxicity results in macroscopic redness of the mucous membrane; haemorrhagic lesions and

a significant reduction in the mucus quantity were observed. The acidity of the gastric content in experimental animals pre-treated with EOJp (G1, G2 and G3) was significantly decreased compared to that of the ulcer control group EtHCl ($p<0.05$). The mucus production of gastric mucosa also increased significantly ($p<0.05$) in G2 and G3 rats that were treated with 75 and 100mg/kg, respectively, compared to the ulcer control group EtHCl (Table 3). The EtHCl group received HCl/ethanol (80mg/mL) and had a low level of mucus (91.06 ± 0.80 mg), which is contrary to the G1 and G2 groups (having received 50 and 75mg/kg of EOJp, respectively) that in turn had secretion that was similar to that of the control C group. The G3 group has the highest level of secreted mucus (141.16 ± 0.99 mg) and lowest ulcer index (0.23 ± 0.21 mm²) (Table 3).

Pre-treatment of G2 and G3 groups of rats with EOJp (75 and 100mg/kg, respectively) significantly induced positive changes (Fig. 2). The stomachs of all rats treated with a higher dose of EOJp (100mg/kg) revealed only slight congestion; otherwise, the shape of the stomach was normal. There was a clear change in the gross appearance of the gastric mucosa compared to the group that was treated acidified EtHCl. Furthermore, administering EOJp to rats reduced the gastric ulceration induced by acidified ethanol.

Biochemical Analysis The levels of lipid peroxidation products and GSH and enzymatic antioxidant activity in the gastric mucosa are shown in Table 4. We observed that the ethanol intoxication/HCl (60:40, 15N) significantly decreased ($p<0.01$) the rate of antioxidants, SOD, CAT, GPx and GSH, and increased the MDA levels, compared to control rats (C). Comparing pre-treating rats with omeprazole (20mg/kg b.w.) (Omp) and EOJp, the analysis of the homogenate groups (50, 75 and 100mg/kg b.w.) showed that the level of lipid peroxidation had a clear, significant reduction in the G1, G2 and G3 groups compared to that of EtHCl. Furthermore, there was improvement (G3) compared to the control group C and Omp group. Moreover, we observed a significant increase in the GSH levels and antioxidant enzymes activities. Therefore, the MDA levels decreased compared to the EtHCl group, while the SOD, GSH, GPx, GST and CAT levels were increased (Table 4).

DISCUSSION

The chemical composition of the essential oil of red juniper has been studied in various regions, such as Morocco,^{41,42)}

Table 3. Measurement of the Total Number of Ulcers, Ulcer Index, Inhibition Percentage, Mucus and pH

Groups	pH	Mucus weight (mg) (n=6)	Total no. of ulcers (mean $\pm$ S.D.) (n=6)	Ulcer index (mm ²) (n=6)	% Ulcer inhibition
C	6.28 $\pm$ 0.37 ^{■c}	127.92 $\pm$ 0.74 [■]	—	—	—
EtHCl	2.61 $\pm$ 0.14*** ^c	90.12 $\pm$ 0.76*** ^c	14.83 $\pm$ 0.75*** ^c	22.36 $\pm$ 0.64*** ^c	—
Omp	4.36 $\pm$ 0.77*** [■]	128.41 $\pm$ 1.44 [■]	4.5 $\pm$ 0.54*** [■]	7.52 $\pm$ 0.55*** [■]	66.35*** [●]
G1	4.55 $\pm$ 0.54*** [■]	106.16 $\pm$ 1.47*** ^{■c}	4.16 $\pm$ 0.75*** [■]	4.59 $\pm$ 0.9*** ^{■c}	79.45*** ^{●c}
G2	4.93 $\pm$ 0.1*** ^{■c}	125.66 $\pm$ 0.81*** ^{■c}	0.83 $\pm$ 0.75 ^c	1.24 $\pm$ 0.53*** ^{■c}	94.31*** ^{●c}
G3	5.05 $\pm$ 0.08*** ^{■c}	141.16 $\pm$ 0.99*** ^{■c}	0.28 $\pm$ 0.21 ^c	0.23 $\pm$ 0.21 ^{■c}	98.94*** ^{●c}

All values are expressed as mean $\pm$ S.D. of measurement of 6 rats in each group. Mean difference is significant at the $p<0.05$ level (ANOVA followed by HSD Tukey test). * $p<0.05$ compared with respective control group. ** $p<0.01$ compared with respective control group. *** $p<0.001$ compared with respective control group. [■] $p<0.05$ compared with respective EtHCl group. [●] $p<0.01$ compared with respective EtHCl group. ^{■c} $p<0.001$ compared with respective EtHCl group. ^a $p<0.05$ compared with respective Omp group. ^b $p<0.01$ compared with respective Omp group. ^c $p<0.001$ compared with respective Omp group.

Fig. 1. Body Weight Gain in Rats Treated Orally with Vehicle (EOJp: 50, 75 and 100mg/kg) for 14d

The results are given as the mean $\pm$ S.E.M., $n=6$. ANOVA, $p<0.05$ between groups in the same day.

Fig. 2. Photographs of the Whole, Dissected and Sectioned Stomachs from Rats That Underwent Ethanol-Induced Gastric Ulcers

Sections were stained with haematoxylin and eosin. Magnifications: b and d ($\times 20$); f, h, j and l: ($\times 40$); a, c, e, g, i and k: magnifications are not available since the photos of the stomach have been taken using a Sony-Cybershot DSC-W350 digital camera. a, b: Control group of a normal stomach. c, d: The EtHCl group was negative for stomach findings; there were severe injuries in the gastric mucosa (haemorrhagic necrosis of the gastric mucosa). Also shown are micrograph exfoliation and epithelial oedema (ede) of the submucosal layer with deformed glands. e, f: Stomach of rats that were pretreated with omeprazole (20mg/kg), and moderate injury was observed in the gastric mucosa. g, h: stomach of G1 rats that were pretreated with EOJp (50mg/kg); minor injuries can be observed in the gastric mucosa. i, j: stomach of G2 rats that were pretreated with EOJp (75mg/kg); very mild or even the absence of injury can be observed in the gastric mucosa. G3 (k, l) stomach of rats that were pretreated with EOJp (100mg/kg); instead of flattening the stomach, no injury could be observed in the gastric mucosa.

Table 4. Measurement of the Gastric Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), Glutathione Peroxidase (GPx) and Glutathione Transferase (GST) in HCl/Ethanol Ulcerated Rats

Groups	Lipid peroxidation nmol MDA/mg protein	Protein concentration ($\mu\text{g/mL}$)	GSH ($\mu\text{mol/mg protein}$)	SOD (U/mg protein)	CAT $\mu\text{mol H}_2\text{O}_2$ consumed/min/ mg protein	GPx $\mu\text{mol GSH/}$ mg protein/mL	GST nmol of CDNB conjugate formed/min/ mg protein
C	0.323 $\pm$ 0.046 ^{■c}	20.96 $\pm$ 0.51 [■]	2.46 $\pm$ 0.09 ^{■c}	3.57 $\pm$ 0.3 [■]	7.65 $\pm$ 0.34 [■]	1.96 $\pm$ 0.05 [■]	1.29 $\pm$ 0.45 [◆]
EtHCl	0.635 $\pm$ 0.045 ^{***c}	12.01 $\pm$ 0.30 ^{***c}	0.82 $\pm$ 0.06 ^{***c}	1.82 $\pm$ 0.12 ^{***c}	2.18 $\pm$ 0.18 ^{***c}	0.94 $\pm$ 0.06 ^{***c}	0.84 $\pm$ 0.08 ^{*c}
Omp	0.413 $\pm$ 0.051 ^{**■}	20.72 $\pm$ 0.44 [■]	1.94 $\pm$ 0.06 ^{***■}	3.32 $\pm$ 0.34 [■]	7.45 $\pm$ 0.45 [■]	1.82 $\pm$ 0.12 [■]	0.93 $\pm$ 0.05
G1	0.392 $\pm$ 0.039 [■]	22.23 $\pm$ 0.26 ^{***■c}	2.50 $\pm$ 0.25 ^{■c}	4.05 $\pm$ 0.1 ^{***■c}	8.02 $\pm$ 0.47 [■]	2.05 $\pm$ 0.08 [■]	1.21 $\pm$ 0.18
G2	0.293 $\pm$ 0.037 ^{■c}	24.88 $\pm$ 0.45 ^{***■c}	2.78 $\pm$ 0.18 ^{***■c}	4.13 $\pm$ 0.14 ^{***■c}	8.32 $\pm$ 0.29 ^{■c}	2.21 $\pm$ 0.26 ^{■a}	1.44 $\pm$ 0.26 ^{■c}
G3	0.232 $\pm$ 0.018 ^{***■c}	26.63 $\pm$ 0.51 ^{***■c}	2.98 $\pm$ 0.04 ^{***■c}	4.31 $\pm$ 0.17 ^{***■c}	8.95 $\pm$ 0.06 ^{***■c}	2.38 $\pm$ 0.32 ^{***■c}	1.91 $\pm$ 0.09 ^{***■c}

All values are expressed as mean $\pm$ S.D. of measurement of 6 rats in each group. Mean difference is significant at the $p < 0.05$ level (ANOVA followed by HSD Tukey test). * $p < 0.05$ compared with respective control group. ** $p < 0.01$ compared with respective control group. *** $p < 0.001$ compared with respective control group. ◆ $p < 0.05$ compared with respective EtHCl group. ● $p < 0.01$ compared with respective EtHCl group. ■ $p < 0.001$ compared with respective EtHCl group. ^a $p < 0.05$ compared with respective Omp group. ^b $p < 0.01$ compared with respective Omp group. ^c $p < 0.001$ compared with respective Omp group.

Algeria,⁴³⁾ Corsica,⁴⁴⁾ Spain,⁴⁵⁾ Portugal,^{45,46)} Greece,⁴⁵⁾ Egypt,^{47,48)} Saudi Arabia⁴⁹⁾ and France.⁵⁰⁾ In Tunisia, the chemical characterization of essential oils of the aerial part of *J. phoenicea* from different regions has also been studied.^{51,52)} However, the chemical composition of essential oils and antioxidants in leaves as well as their healing and anti-ulcerogenic properties has not previously been studied. The essential oil yield of *J. phoenicea* from Gafsa is 0.69%. It is higher than Medenine (0.5%) (South of Tunisia)⁵²⁾ and Greece (0.21%) and is close to that of Spain (0.66%).⁴⁵⁾ The chemical composition does not differ greatly from Medenine's *J. phoenicea* essential oil (South of Tunisia), which is very rich in α -pinene (59.11%)⁵²⁾ and of Greece and Spain, which are richer in myrcene (4.5 and 4.0%, respectively) and β -phellandrene (3.5 and 5.9%, respectively) and poor in *p*-cymene (0.80 and 0.40%, respectively).⁴⁵⁾

In terms of its phytochemical constituents, *J. phoenicea* generally contains major classes of secondary metabolites, such as flavonoids, phenolic compounds and terpenoids. These metabolites have several biological activities, such as antibacterial, anti-inflammatory, antiviral, insect repellent, sedative, flavouring and herbicide activities.⁵³⁾ Under physiological conditions, mucus from goblet cells or mucocytes protects the gastric mucosa against the incisive action of hydrochloric acid and the digestive action of proteolytic enzymes in gastric juice. In fact, Ruchebuch⁵⁴⁾ confirmed that at this level, where prostaglandins play a stimulatory role in the gastric mucus secretion, there is a feedback mechanism that inhibits the gastric acid secretion of gastrin (responsible for secretion of the hydrochloric acid *via* somatostatin and gastric inhibitory peptide (GIP)). As described in other studies, the oral administration of ulcerogenic agent for the control group produced clear mucosal damage, which was characterized by multiple bleeding with red stripes of different sizes along the long axis of the glandular stomach.^{55,56)} Acidified ethanol is an aetiological factor; its excessive ingestion gives rise to gastritis, which is characterized by oedema of the mucosa, subepithelial haemorrhage, cellular exfoliation and infiltration of inflammatory cells.⁵⁷⁾ In histological sections, EOJp could protect the gastric tissues from HCl/ethanol injury by enhancing gastric mucus production, healing the ulcer craters and keeping a near normal epithelial cells arrangement.

Ulcerogenic agents, such as HCl and EtOH, are responsible for damage and necrosis of the gastric mucosa and deple-

tion of their defence mechanisms.⁵⁸⁾ Of all these changes, the most prominent are increased capillary permeability and the production of free radicals,⁵⁹⁾ which damage cell membranes, attract neutrophils and initiate inflammatory responses.⁶⁰⁾ From histological results of the present study, we suggest that pre-treatment with EOJp inhibited leukocyte infiltration into the gastric wall thereby protecting the gastric mucosa. In fact, an erosion of the gastric epithelial cells, widening of the inter-glandular spaces and infiltration of the neutrophils in the ulcerated zones have been observed in ulcers HCl/ethanol-induced rats, whereas EOJp exhibited a protective effect. The activation and infiltration of neutrophils seem to be involved in the initial process that forms lesions.⁶¹⁾ In the same context, neutrophil accumulation in the gastric mucosa induces micro-circulatory abnormalities.⁶²⁾ This is defined by the histological results of the present study, where a marked reduction of lesions in the gastric mucosa and cellular influx was observed. In fact, histological study revealed that there is erosion of the gastric epithelial cells, widening of the inter-glandular spaces and infiltration of the neutrophils in the ulcerated zones, whereas EOJp exhibited a protective effect against HCl/ethanol-induced ulcerogenesis in rats.

Compared to controls, the ethanol intoxication significantly decreased the SOD, CAT, GPx and GSH levels as well as increased the MDA levels. Inversely, the SOD, CAT, GPx, GST and GSH activities were significantly elevated by the administration of omeprazole and EOJp in the treated rats, suggesting the preventive effect of EOJp against ulcers induced by HCl/Ethanol. Omeprazole is the first of a new class of drugs that inhibit gastric secretion by altering the activity of H⁺/K⁺-ATPase.^{63,64)} On the other hand, omeprazole has been effective in reducing oxidative stress in the HCl/ethanol model; it is not charged and can cross cell membranes.^{63–65)} It accumulates in the acid space of the parietal cells because it is a weak base. It becomes a cationic thiol-reactive sulfenic acid and/or sulfenamide that binds to groups to form disulphide cysteinyl-S by acid catalysed rearrangement.⁷⁾ Our results corresponded to an earlier report that showed a depletion of sulfhydryls in HCl/ethanol-induced gastric lesions.⁶⁶⁾ In normal conditions, gastric cells possess a powerful antioxidant defence system, including SOD, CAT, GSH-Px and GST, which play a protective role against xenobiotics.⁶⁷⁾ In the present study, the administration of HCl/ethanol significantly ($p < 0.01$) decreased the activities of SOD, CAT and GSH-Px enzymes compared

to control rats. In contrast, pre-treatment of ethanol-ulcerated rats with EOJp preserved the activities of the aforementioned antioxidant enzymes to near normal and decreased the lipid peroxidation product (MDA) activity as dose-dependent mass production of free radicals. In fact, the increase in MDA may suggest a possible mechanism of tissue damage through reactive oxygen intermediates.⁶⁸⁾ These free radicals are highly toxic and can cause direct damage to proteins, DNA and lipid membranes.⁶⁹⁾ This leads to loss of the membrane fluidity and ion transport as well as impaired membrane integrity and, finally, the loss of cell functions.⁷⁰⁾ Reduced glutathione is one of the most abundant non-enzymatic antioxidant biomolecules that is present in the tissues.⁷¹⁾ Its functions are to act as a substrate for GPx and GST, to remove free oxygen species, such as hydrogen peroxide (H₂O₂) (superoxide anions and alkoxy radicals), and to maintain membrane protein thiols.⁷²⁾ The non-availability of GSH decreases the activities of GSH-dependent enzymes, GPx and GST, and/or renders these enzymes inactive and/or less active.⁷³⁾ This study has shown that a medicinal plant probably antagonizes the aggressive factors, which play an important role in the pathogenesis of gastric ulcers, while augmenting the defensive mucosal factors that protect the gastric mucosa from injury. Similar observations were found by Guesmi *et al.* using essential oil of *Thymys algeriensis* in the treatment of gastric ulcer induced by HCl/ethanol.⁷⁴⁾

In this study, we conclude that the oral administration of the essential oil of *Juniperus phoenicea* has potent anti-ulcer activity, which justifies the ethnomedical claims about its significant gastroprotective effect, as evaluated by the significant antioxidant activity because it reduces the MDA level and increases the GSH, GST, GPx, CAT and SOD levels. This effect may be related to an increase in the gastric mucosal defence mechanisms. The results of our study revealed histopathological maintaining the integrity of the mucosa. As a result, the protective effect of the essential oil and its low toxicity requires further study to elucidate the mechanism of action and isolate the active principles.

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