Antiulcer activity of Moroccan *Artemisia campestris* L. subsp. glutinosa against ethanol-induced gastric ulcer in Mice.

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Abstract

Artemisia campestris L. subsp. glutinosa is a plant growing in Morocco and widely used in traditional medicine as a beneficial remedy for the digestive system, especially against stomachache gastric ulcer and diarrhea. The present study was carried out to evaluate the antiulcer activity of aqueous extract (AEAc) and hydro-ethanolic extract of this plant (EEAc) using an experimental model not previously tested against ethanol-induced gastric ulcer and acute toxicity in mice. The gastric lesion was assessed by ulcer area, ulcer index, prevention index, histopathological examination, and the evaluation of lipid peroxidation level. Administered of AEAc and EEAc at a dose of 100, 200, 400 mg/kg before ethanol ingestion significantly inhibited gastric ulcers ($p < 0.01$). AEAc and EEAc induced a significant decrease in the ulcer area compared to the control group ($p < 0.01$). The preventive index of different doses of both extracts is almost similar to that of omeprazole. These results were confirmed by a decrease in mucosal thickness and MDA level in the group treated with the plant compared to the control ulcer group. The acute toxicity study revealed no abnormal sign or death to the mice treated with 4 g/kg and 8 g/kg of both extracts. This funding reveals a safe antiulcer activity of Moroccan *Artemisia campestris* L. subsp. glutinosa

Keywords: Acute toxicity, Antiulcer, *Artemisia campestris* L., Gastric Ulcer, Gastro-protective.

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Introduction

Gastric ulcer (stomach ulcer) is characterized by a significant loss of substance from the gastric mucosal membrane layer. It can occur at any age and varies in size from a few millimeters to several centimeters. It is widespread and concerns 10% of the world population. The causes lie in an imbalance between the aggressive gastric juice, the defense mechanisms, and the mucus. The aggressive factors are infection with the bacteria *Helicobacter pylori*, non-steroidal anti-inflammatory drugs (NSAIDs), stress, smoking, and alcohol. On the other hand, defensive factors include, mucus layer, prostaglandins and, bicarbonate (Chai 2011 ; Li et al. 2016). The latter creates a near-neutral pH gradient at the epithelial surfaces of the stomach and provides the first barrier of mucosal protection against luminal acid. The continuous adherent mucus layer is a barrier to luminal pepsin and protects the underlying mucosa from proteolytic digestion (Allen and Flemström 2005). Gastrointestinal hormones regulate gastric acid secretion. Mainly acetylcholine, gastrin, and histamine, which stimulate the parietal cells to secrete acid. In addition, somatostatin inhibits acid secretion and exerts tonic pressure on parietal, enterochromaffin and gastrin cells (Zhang et al. 2020). Current treatments for gastric ulcers include antisecretory, proton pump inhibitors, and antihistamines H_2. However, long-term use of these drugs can cause various side effects (Henry and Langman 1981; Piper 1995; Sheen and Triadafilopoulos 2011). Hence, the need to continue research aimed at discovering natural substances capable of effectively curing gastric ulcers with fewer side effects. Currently, medicinal plants represent interesting therapeutic sources (Bourhia et al. 2019), especially in developing countries. According to WHO estimates 80% of the world's population choose medicinal plants to treat illnesses (WHO 2002). The species *Artemisia campestris* L. (Asteraceae) can be segregated into six subspecies distinguished by their morphological characters and which can be listed as: (a) subsp. campestris L., (b) subsp. glutinosa (Gay ex Besser) Batt., (c) subsp. maritima Arcangeli, (d) subsp. alpina (DC.) Arcangeli; (e) Subsp. bottanica A. N. Lundstrōm ex Kindb, and (f) Subsp. borealis (Pallas) Hall & Clements. However, it has been pointed out that the subspecies occurring in Morocco is an *Artemisia campestris* L. subsp. glutinosa (Besser) that grows in the desert area, stony lands for pastures and in the mountain regions. Moreover, this plant was previously identified in our laboratory based on morphological traits and essential oil profile specifying that it was indeed *Artemisia campestris*. subsp. glutinosa (Besser) Batt (Dib et al. 2017a). The Moroccan traditional pharmacopeia is full of a multitude of herbal

recipes to prevent, cure, relieve or improve the well-being of man (Lahsissene and Kahouadji 2010). *Artemisia campestris* L. is one of Moroccan plants *w*hich is used in the treatment of several diseases such as cancer (Djeridane et al. 2007), diabetes, obesity (Bnouham et al. 2002), hypertension (Boudjelal et al. 2013), allergy, Asthma, diarrhea (Sassi et al. 2007), antispasmodic (Ouelbani et al. 2016) and also against gastric ulcer (Leporatti and Ghedira 2009). Several pharmacological studies on *Artemisia campestris* demonstrate beneficial effects on diseases of the gastrointestinal tract. However, they have shown antitumor activity against HT-29 cells (Akrout et al. 2011). Also, have antidiarrheal and antispasmodic activity (Marghich et al. 2021a ; Marghich et al. 2021b).

This study aims to continue to enhance the beneficial effect of Moroccan *Artemisia campestris* L. subsp. Glutinosa on the digestive tract diseases. In this work, we studied for the first time the Antiulcer activity of *Artemisia campestris* L. subsp. Glutinosa from oriental Morocco against ethanol-induced gastric ulcer in mice and the acute toxicity.

Materials and Methods

Plant

The choice of the plant was based on ethnobotanical studies that mentioned that *Artemisia campestris* L subsp. Glutinosa. is a plant traditionally used by the Moroccan population to treat digestive problems (Fakchich and Elachouri 2014). The aerial part of the plant was collected from a desert area situated between Tendrara and Figuig in Morocco (32°49′48″N, 1°39′36″W). This the plant was identified by Pr. Elachouri Mostafa, and the voucher specimen HUMPOM-151 was kept in the herbarium of the faculty of sciences, university Mohammed Premier Oujda (Morocco).

Extract Preparations

Aqueous Extract

According to traditional usage, the aqueous extract of *Artemisia campestris* (AEAc) was prepared by infusion of 30 g of the aerial part in 300 mL distilled water already boiled at 100 °C, then let cool to room temperature in the dark for 30 min. The aqueous extract was obtained after filtration and evaporation to dryness in vacuum using a rotavapor at 50°C (yield: 19%) and was stored at −20 °C until use.

Hydro-Ethanolic Extract.

25 g of sample was soaked into 250 mL of 50% aqueous-ethanolic solution in the dark at room temperature for 24 h. The mixture was filtrated, and the filtrate was evaporated to dryness using a rotavapor at 50°C to obtain the extract in a yield of 14%. The hydro-ethanolic extract (EEAc) was kept at -20 °C until use.

Animal

Swiss albino mice were provided by the animal facility of the biology department of the Faculty of Sciences-Oujda, Morocco; they were placed under standard conditions (23 ° C ± 2 ° C and 12 h light-dark cycle), with free access to water and food. The animals were kept in their cages for one week before starting the study to allow acclimatization to laboratory conditions. All animals were cared for following the internationally accepted guide for the care and use of laboratory animals published by the United States National Institutes of Health (NIH 2011).

Phytochemical Screening

The qualitative phytochemical analysis was carried out following the standard procedures described by (Shaikh and Patil 2020).

Test for Terpenoids

To 30 mg of each extract was solubilized in 5 mL of distillate water. Then the chloroform was added, and then concentrated H_2SO_4 was carefully added to form a layer. Reddish-brown staining of the interface indicates the presence of terpenoids.

Test for Flavonoids

Using Ammonia test: 5 mL of dilute ammonia solution was added to the extract, and then the concentrated sulfuric acid was added. A yellow coloration indicates the presence of flavonoids.

Test for Tannins

According to Braymer' test: 30 mg of each extract was solubilized in 5 mL of distillate water, and then filtered. Few drops of 0.1% of ferric chloride was added. The observation of brownish green or a blue-black coloration indicate the presence of tannins.

Acute Toxicity Studies in Mice.

The acute toxicity study was evaluated following the recommendations by OECD-Guidelines (OECD 2008). Acute toxicity studies were carried out in Swiss albino mice, weighing 25-32 g each, using a single dose administered orally.

We used five groups of five albino mice (2 males and 3 females in each bath). The mice fasted for 18 h with free access to water.

• The first control group only receives distilled water by gavage.

• The second and the third groups receive 4 g/kg and 8 g/kg, respectively of AEAc (1 mL of extract solution /100 g.bw).

• The fourth and the fifth groups receive 4 g/kg and 8 g/kg respectively of EEAc (1 mL of extract solution /100 g.bw).

Continuous observation of general mice behavior and signs of toxicity and/or death after oral unique treatment for one hour, four hours, and 24 h (Jatsa et al. 2018), and daily for 14 days. Took Bodyweight measurement before (day 0) and after the gavage (day 1, day 7, day 14).

Ethanol-induced Gastric Ulcer in Mice

Acute gastric ulceration was induced by administering absolute ethanol to mice intragastrically by gavage according to the protocol described by Jalilzadeh-Amin et al. 2015 with late modification. The mice were deprived of food 14 hours before each test and water one hour before the experiment**.** Mice were randomly divided into nine experimental groups, each containing five animals. The normal group (that received only vehicle) and the control ulcer group (that received also the vehicle in the first step). The prevention group received 30 mg/kg of Omeprazole (reference drug, dissolved in 0.9% saline). The first three test groups received 100, 200, 400 mg/kg of the AEAc respectively with 1 mL/100 g.bw. The second three test groups received 100, 200, 400 mg/kg of the EEAc respectively with 1 mL /100 g.bw. After one hour of third day, mice were given 80% of ethanol by gavage (0.5 mL / 100 g body weight) to induce the gastric ulcer, except the normal group. After two hours of the ethanol injection, the animals were sacrificed by euthanasia in an ethylic ether chamber. The stomachs were removed following a ventromedial dissection and then were opened according to the greater curvature, washed with physiological solution saline to remove the gastric contents and blood clots. The stomachs were spread on a white paper to assess the extent of gastric damage formed. The observations were carried out using a binocular magnifying glass. Photographs were taken to measure the ulceration area using a software computer. After that, each stomach was dichotomized, with one moiety of stomach immersed in 10% formaldehyde for histological evaluation and gastric tissue, the other moiety stored at −20 °C for Malondialdehyde (MDA) determinations.

Determination of Macroscopic Gastric Damage

Assessment of Damage Gastric Area

The surface of the gastric lesions induced by ethanol in the different mice was carried out using the program (MESURIM_PRO). The results obtained allowed us to calculate the percentage of ulceration according to the following formula:

$$\%\ \text{Ulceration (\% ulcer area)} = \frac{\text{Damage gastric area}}{\text{Stomach area}}\ \text{x } 100$$

Assessment of Mean Scoring

The degree of gastric lesions was organized into scores. The latter was presented as follows the method described by Panda and Khambat 2014.

- 0: normal coloring of the stomach.
- 0.5: red mucosa.
- 1: the presence of ulcerative spots, petechiae.
- 1.5: the presence of hemorrhagic streaks.
- 2: the presence of a true ulcer.
- 3: the presence of perforations

The mean of the ulceration scores of each mouse shows the ulceration index (UI), the latter used to calculate the preventive effect by the method of Basile et al.1990.

by following this formula:

$$\text{Prevention index (\%)} = \frac{(\text{UI Control} - \text{UI Treated})}{\text{UI Control}} \times 100$$

UI Control = ulcer index in the control group

UI Treated = ulcer index in the treated group

Histological Assessment

Performed the histopathological analysis of the stomach of all animals was to examine the lesions microscopically. Stomachs from all groups were fixed in a buffered formalin solution (10 %) within 48 hours and are embedded in paraffin. Then, paraffin samples were cut at five µm were prepared using a manual rotary microtome to take sections, which are stained with Hematoxylin and Eosin. The tissue was observed using an optical microscopy.

Evaluation of Lipid Peroxidation Level

Lipid peroxidation in the gastric tissue was evaluated using the thiobarbituric acid (TBA) reaction method. After the homogenate preparation, added 1 mL of the homogenate supernatant in 1 mL of TBA reactive constitutes 0.67 % w/v of TBA, and 15% of TCA (Trichloroacetic acid) dissolved in 0.25 N of chloridric acid. Then, the reaction mixture was placed in a boiling water bath (100 °C) for 30 min and centrifuged at 4750 g for 5 min. The mixture placed in ic bath to stop the reaction. This reaction can form TBA-reactive substance (TBARS) from MDA present in the gastric tissue, which give a pink product that can be assayed spectrophotmetrically at 535 nm (El-Ashmawy et al. 2016). The calculation of MDA amounts was made using the extinction coefficient of 1.56 x 10^5 $M^{-1}.cm^{-1}$. The results are expressed in nanomoles of MDA per milligrams of the gastric tissue (nmol/mg) (H Draper and Hadley 1990 ; Iqbal et al. 1999).

Statistic Treatment

The results were expressed as the mean ± SEM. Moreover, the difference between the groups was calculated with a one-way analysis of variance (ANOVA) using GraphPad Prism software, version 5.01 (San Diego, California USA), followed by a post hoc Tukey test. The difference was significant when p is less than 5% ($p < 0.05$).

Results

Phytochemical Screening

The results obtained showed that the two extracts are rich in flavonoids and tannins. However, terpenes were present in the aqueous extract but not in the hydro-ethanolic extract. (Table 1).

Table 1: Qualitative Phytochemical screening of *Artemisia campestris* subsp. glutinosa *extracts*. (+): Detectable; (−): not detectable. (AEAc: aqueous extract), (EEAc: hydro-ethanolic extract).

Extract	Yield (% w/w)	Flavonoids	Tannins	Terpene
AEAc	**19%**	**+**	**++**	**+**
EEAc	**14%**	**+**	**++**	**-**

Acute Toxicity Studies in Mice.

The single oral dose of 4 g/kg and 8 g/kg of AEAc and EEAc administered to the mice did not produce any bodyweight modification, toxicity symptoms, or mortality during the 14 days of observations (Table 2).

Table 2: Mortality and toxic symptoms of acute toxicity of aqueous and hydro-ethanolic extracts of *Artemisia campestris* subsp. glutinosa and their effect on bodyweight change of mice. Data are expressed as mean ± SEM (n = 5). The comparison in the statistical analysis is between first and final weight. NS (Not significant).

Extract	Dose	Death/Total	Toxic symptoms	Bodyweight (g)		
				First weight (day 0)	Final weight (14th day)	Statistical analysis
D.W		0/5	None	30.04 ± 2.45	28.40 ± 1.50	NS
AEAc	4 g/kg	0/5	None	26.16 ± 1.60	26.50 ± 1.66	NS
AEAc	8 g/kg	0/5	None	27.85 ± 2.54	27.98 ± 2.47	NS
EEAc	4 g/kg	0/5	None	26.34 ± 0.76	26.84 ± 0.88	NS
EEAc	8 g/kg	0/5	None	28.42 ± 1.93	27.55 ± 1.68	NS

Ethanol-induced Gastric Ulcer in Mice

Determination of macroscopic gastric damage.

Oral administration of ethanol produced multiple mucosal lesions in the stomach of the mice independently from the acid secretion. The results we found showed that no macroscopic lesions were observed in the normal group [Figure. 1 (Aa)]. Compared to the normal group, oral administration of 80% ethanol-induced macroscopic morphological changes [Figure. 1 (Ab)], such as mucosal erosion, ulcers, necrosis, linear hemorrhages, and hyperemia with an estimated ulcer area of 52.66% in gastric ulcer control group mice [Figure. 1 (B)]. The mice pretreated with Omeprazole significantly prevent the ulcer area by 69.21% compared to the ulcer control group [Figure. 1 (B)]. There is also the absence of mucosal erosion, ulcers, necrosis, and linear hemorrhages [Figure. 1 (Ac)]. About our extracts, we observe a significant decrease in the gastric ulcer surface compared to the control group with a percentage of ulcer proportional to the growth dose of the AEAc; that is to say, the lowest concentration (100 mg/kg) caused the strongest protection (% ulcer area = 7.8%). For 200 mg/kg and 400 mg/kg, the percentage of the ulcer area estimated is 18.05%, 19.49%. For the

EEAc, the results we found are as follows; 15.72%, 15.18%, 11.38% of ulcer area, respectively, for the groups pretreated with 100, 200, 400 mg/kg [Figure. 1 (B)].

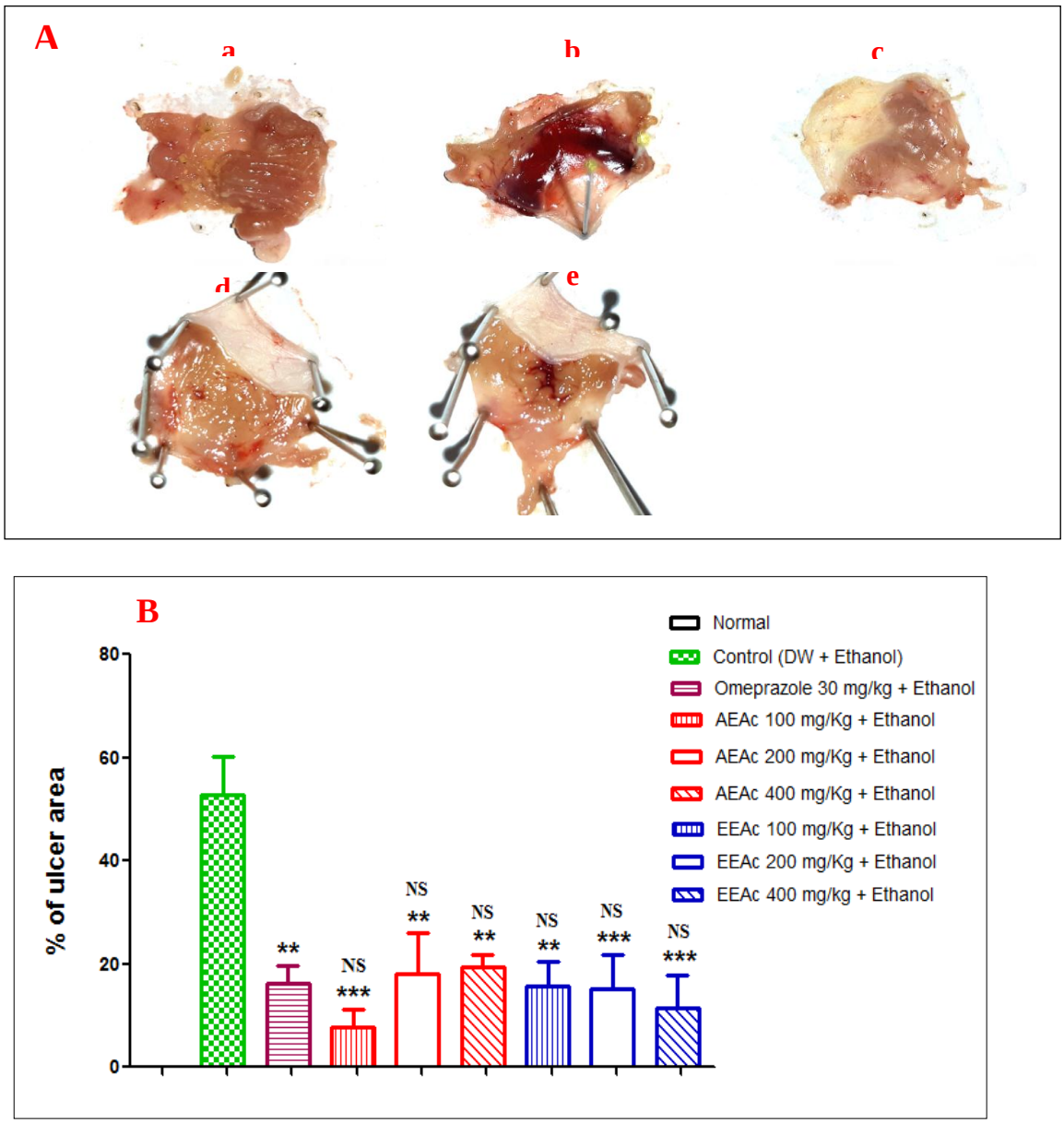

Figure 1: (A) Photographs showing ethanol induced gastric ulceration in mice in different groups. (a: Normal group/ b: Control (distilled water + Ethanol)/ c: Omeprazole 30 mg/kg + Ethanol/ d: AEAc 100 mg/kg + Ethanol/ e: EEAc 400 mg/kg + Ethanol). **(B)** Histogram of the Effect of different doses of aqueous (AEAc) and hydro-ethanolic (EEAc) extracts of *Artemisia campestris* subsp. glutinosa on the ulcer area in ethanol-induced gastric ulcer model in mice. NS, Not significant (Compared to the omeprazole group) and **, $p < 0.01$ and ***, $p < 0.001$. The difference is statistically significant at ulcer control (mean ± SEM, n = 5).

The ulcer index and preventive index are shown in Table 3. Omeprazole significantly reduced the ulcer index (0.75 ± 0.16) compares to the control group ($p < 0.01$). The latter, in which ulcer index estimated to be 5 ± 0.00; while the ulcer index of mice pretreated with 100, 200, 400 mg/kg of AEAc were 0.87 ± 0.19, 1.37 ± 0.61, and 1.87 ± 0.36 respectively, in the other hand the ulcer index of mice pretreated with 100, 200, 400 mg/kg of EEAc were 2.75 ± 1.00, 1.12 ± 0.08 and 1.37 ± 0.47 respectively. The preventive index of different doses of both extracts was almost similar to that of Omeprazole and not significant compared to it.

Table 3: Effect of different doses of aqueous and hydro-ethanolic extracts of *Artemisia campestris* subsp. glutinosa on the ulcer index in ethanol-induced gastric ulcer model in mice. * $p < 0.05$ and **, $p < 0.01$. The difference is statistically significant at control (mean ± SEM, n = 5).

Groups	Dose	Ulcer index	Preventive index (%)
Normal	-	0	
Control	-	5 ± 0	
Omeprazole	30 mg/kg	0.75 ± 0.16**	85
AEAc	100 mg/kg	0.87 ± 0.19**	82.5
AEAc	200 mg/kg	1.37 ± 0.61**	72.5
AEAc	400 mg/kg	1.87 ± 0.36**	62.5
EEAc	100 mg/kg	2.75 ± 1.00*	45
EEAc	200 mg/kg	1.12 ± 0.08**	77.5
EEAc	400 mg/kg	1.37 ± 0.47**	72.5

Histological Assessment

The mice of the control group were treated with 80% of ethanol, showing a decrease of mucosal thickness, disruption of the superficial region of the gastric gland with epithelial cell loss, edema, and intense hemorrhage [Figure. 2 (B)]. Animals treated with 80% ethanol and Omeprazole (30 mg/kg) showed healing of the ulcer and increased the thickness of the mucosa [Figure. 2 (C)]. Animals treated with 80% ethanol–AEAc (100 mg/kg) showed preservation of gastric mucosa [Figure. 2 (D)]. Animals treated with 80% ethanol–EEAc (400 mg/kg) showed a similar result to the AEAc groups [Figure. 2 (E)].

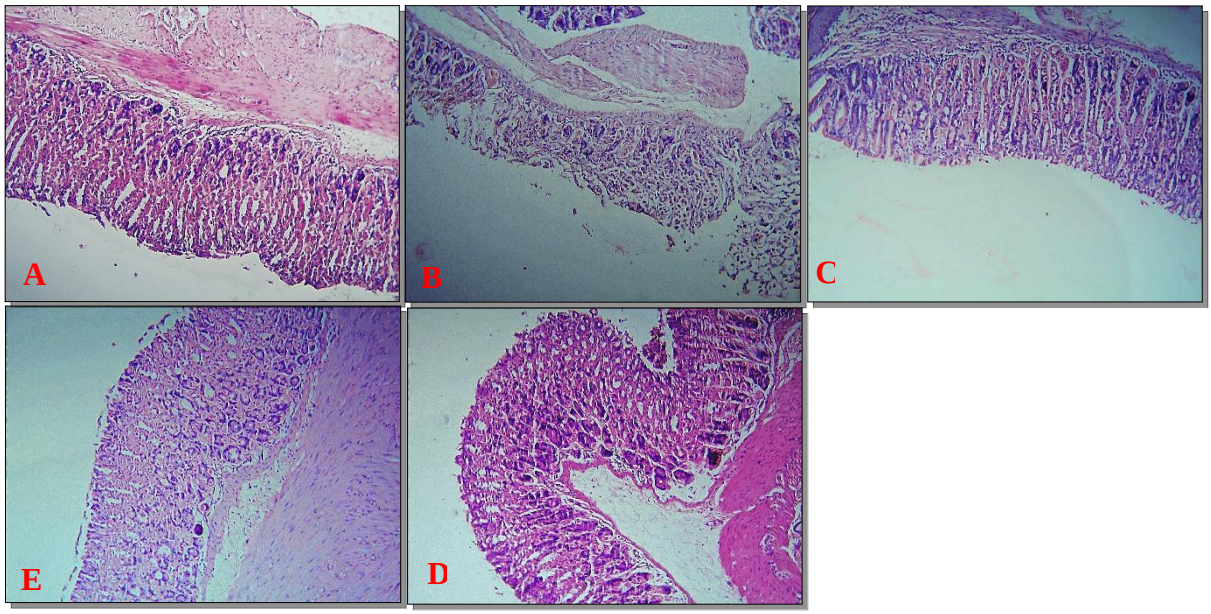

Figure 2: Photomicrographs Hematoxylin/Eosin staining of the gastric mucosa (100x). **(A)**: Normal group; **(B)**: Control group (distilled water + Ethanol); **(C)**: Omeprazole 30 mg/kg + Ethanol; **(D)**: AEAc 100 mg/kg + Ethanol; **(E)**: EEAc 400 mg/kg + Ethanol.

Evaluation of Lipid Peroxidation Level

Figure 3 shows the amount of TBARS expressed as nmol of malondialdehyde (MDA) per mg of sample (stomach) produced in all groups tested. The results show that the level of MDA in the ulcer control group increased by an amount of MDA equal a 2.37 ± 0.3 nmol/mg compared to the normal group (1.65 ± 0.6 nmol/mg). Regarding, the level of lipid peroxidation in the group that received omeprazole (30 mg/kg) was reduced (1.65 ± 0.5 nmol/mg) compared to the ulcer control group and was comparable to the normal group. In the group treated with 100, 200, 400 mg/kg of AEAc, the level of lipid peroxidation was reduced compared to the control group by an amount of MDA level equal a 1.73 ± 0.26, 1.83 ± 0.72 and 1.61 ± 0.21 nmol/mg. Concerning the group treated with different dose of EEAc, the MDA level was also reduced by 1.83 ± 0.61, 1.24 ± 0.25 and 1.13 ± 0.29 nmol/mg. All doses of the two extracts of *Artemisia campestris* L. subsp. glutinosa show non-significant results when compared with the group treated by the omeprazole, so both extracts have a comparable effect likened to the omeprazole. The same results have been found for the different concentrations of the two extracts of *Artemisia campestris* particularly by the two

concentrations 200 and 400 mg/Kg of EEAc when compared with control ulcer group, but not significantly.

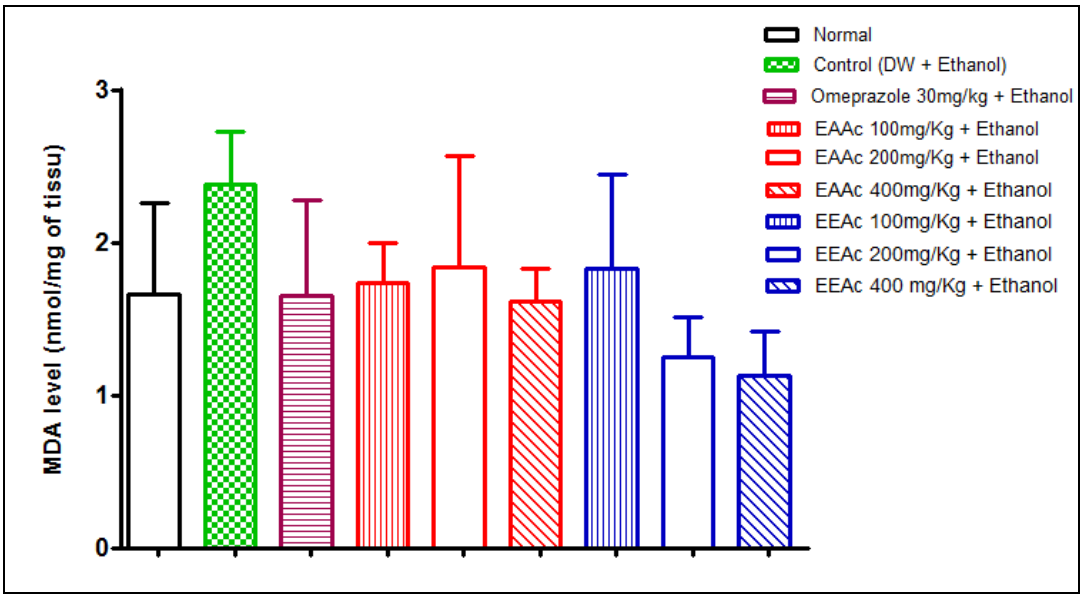

Figure 3: The effect of different doses of aqueous (AEAc) and hydro-ethanolic (EEAc) extracts of *Artemisia campestris* subsp. glutinosa in the lipid peroxidation level in ethanol-induced gastric ulcer model in mice. (Mean ± SEM, n = 5). All results are not significant.

Discussion

The present work investigated the antiulcer activity of the Moroccan *Artemisia campestris* L subsp. glutinosa against ethanol-induced gastric ulcers in mice. Oral administration of ethanol in mice induces gastric damage through contact of concentrated ethanol with the superficial epithelium, which causes necrosis and perturbation of superficial epithelial, endothelial, and mucosal mast cells. Dilation of arterioles and arteries rapidly follows, leading to marked congestive hyperemia, edema, and hemorrhage (Oates and Hakkinen 1988). Also, ethanol reduces the secretion of bicarbonate, nitric oxide, and gastric mucus and induces oxidative stress by increasing the production of malondialdehyde and reducing glutathione production (Karampour et al. 2019). Our study shows that the AEAc (100, 200, 400 mg/kg) and EEAc (100, 200, 400 mg/kg) significantly decrease the gastric ulcer surface compared to the control group. We also found that both extracts have a similar effect than the group treated with Omeprazole. It's the case in certain medicinal plant, which showed that the aqueous extracts had a gastric cytoprotective greater than the reference drug (Gonzales et al. 2000). The preventive index of different doses of both extracts was almost similar to that of Omeprazole. A previous studies showed that a pectic fraction isolated for another subspecies of *Artemisia*

campestris (subsp maritima) had a gastroprotective effect by reducing the gastric ulcer index and ameliorating the protection percentage of injury induced by ethanol and aspirin in rats (Corrêa-Ferreira et al. 2018 ; Sebai et al. 2014). Aspirin occurs a damage gastric not directly in the stomach but by creating an environment more vulnerable to exogenous and endogenous (H. pylori, acid, pepsin) factors and, therefore, more likely to develop a gastric ulcer (Cryer and Mahaffey 2014). However, ethanol-induced gastric ulceration directly by necrosis and perturbation of superficial epithelial has already been shown to be relieved by many plant extracts such as *Glycyrrhiza Glabra L* (Jalilzadeh-Amin et al. 2015), *Napoleona imperalis* (Etim et al. 2017), *Byrsonima serice*a (Rodrigues et al. 2012), *Foeniculum vulgare* (Birdane et al. 2007). According to histological observations, this effect may be due to an increase in the thickness of the mucosa and the gain of epithelial cell that protects gastric mucosa from ulcerogenic effects of ethanol.

Ingestion of ethanol predisposes to the formation of gastric ulcers with inflammatory responses accompanied by the production of reactive oxygen species (ROS), leading to the formation of lipid peroxides that are metabolized to malondialdehyde (MDA) (Zheng et al. 2016; Kwiecien et al. 2014). Pretreatment with AEAc (100, 200, 400 mg/kg) and EEAc (100, 200, 400 mg/kg) decrease the level of malondialdehyde in the gastric tissue but not significantly compared to the control ulcer group, mainly for EEAc (200 and 400 mg/kg). That is to say, the effect of our extracts could be due to the elimination of free radicals and the reduction of oxidative stress. The qualitative phytochemical analysis of the plant extracts revealed the presence of tannins and flavonoids. We have already performed the HPLC analysis with three major peaks: chlorogenic acid, vicenin-2 and 3, 5-Dicaffeoylquinic acid (Dib et al. 2017b). We also have performed the phytochemical profile of its essential oils (Dib et al. 2017c) which contained α and β pinene, spathulenol, and *p*-Cymene**.** Chlorogenic acid had gastroprotective effect against gastric ulcer through restoration of normal autophagy flux (Ahmed et al. 2021) and a protective effects against experimental reflux esophagitis (Kang and Lee 2014), Vicentin-2 had anticancer and antioxidant activities, α and β pinene, spathulenol, and *p*-Cymene showed antioxidant activities (Salehi et al. 2019**,** Nascimento et al. 2017, Wang et al. 2021). In a previous phytochemical study (Megdiche-ksouri et al. 2014), showed that *Artemisia campestris* L is rich in flavonoid such as: quercetin, **c**atechin, naringenin, apigenin, and rutin known for their antioxidant activities. This may lead to establishing a relationship between these compounds and the anti-ulcer effect obtained by our plant. That confirmed by the fact that these molecules are some of the most important

biochemical compounds with a protective activity on ethanol-induced ulcers (De la Lastra et al. 1994 ; Gonzales et al. 2000 ; Borrrelli and A.Izzo 2000). These molecules have been reported for their anti‑ulcerogenic activity with a good level of gastric protection (Sumbul et al. 2011)**.** Quercetin and naringenin showed a significant decrease in ulcer index and the percent of haemhorragic stomachs (Martin et al. 1993). The study performed by Coşkun et al. 2018 showed also that the quercetin significantly reduced ethanol-induced gastric damage due to the reduction in the lipid peroxidation, increased antioxidant enzyme activities and reduce the intragastric concentration of histamine (Martin et al. 1993). That is to say that this molecule prevents the release of histamine by gastric mast cells and inhibits the gastric proton pump H^+/K^+ which decreases the gastric acid secretion (De Lira Mota et al. 2009). In the other hand, the catechin had a significant protective effect against the gastric mucosal lesions and ethanol-induced gastric ulcer, by inhibited the release of gastrin, histamine and also by increasing in the gastric mucus action that maybe involved in the mechanism of action of this effect (Sato et al. 2002 ; Hamaishi et al. 2006). The flavonoids also had gastric cytopretective effect via the regulation of prostaglandin levels (Zhang et al. 2020)**.** Moreover, the flavonoids neutralized the generation of reactive oxygen species (ROS) (Kwiecien et al. 2014). *Artemisia campestris* has an anticholinergic effect (Marghich et al. 2021), which improves our results since acetylcholine is one of the gastrointestinal hormones regulating gastric acid secretions by stimulating the parietal cells to secrete acid (Zhang et al. 2020).

The highest dose of our extracts (4 and 8 g/kg) showed no acute toxicity when tested on the mice during the 14 days of observations. The same remark emerges from Dib et al. 2017b during oral administration in mice of doses below 4 g/kg of AEAc. In conclusion, these results indicate that *Artemisia campestris* is a safe way to eliminate gastric dysfunction.

Conclusion

Artemisia campestris L. subsp. glutinosa has a safe protective effect against ethanol-induced gastric lesions in mice by reducing the ulcer area and the ulcer index; this effect can be due to eliminating free radicals, reducing oxidative stress, and increasing the thickness of the mucosa.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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