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Original Article

Chrysanthemum coronarium L: Chemical Composition and Gastroprotective Potential of Methanolic Leaf Extract in Ethanol-induced Gastric Ulcers in Male Wistar Rats

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ABSTRACT

Background: Our study evaluated the effect of *Chrysanthemum coronarium* L. (CC) leaves on ethanol-induced acute gastric ulcers in Wistar rats. **Materials and Methods:** The organic extract of CC was obtained by Soxhlet extraction with methanol and then divided into two dose groups: 250 mg/kg and 500 mg/kg. Omeprazole was used as a positive control at 20 mg/kg. Our extract was subjected to the separation of bioactive compounds by High-Performance Liquid Chromatography (HPLC). Lethality tests (LD_{50}) were carried out using standard procedures. Gastric protection was assessed by measuring gastric juice volume, total acidity, and free acidity. Gastric mucosal damage was assessed by histopathological examination. **Results:** Chromatographic analysis of the Methanolic Extract of *Chrysanthemum coronarium* L. (MECC) identified the presence of 19 phenolic compounds, representing 46.47% of the total sample. The dominant components were o-coumaric acid (9.55%), chlorogenic acid (6%), myricetin (4.19%), and benzoic acid (2.87%). Oral LD_{50} value was more than 5000 mg/kg in rat. In the present study, the methanolic extract of CC decreased total and free gastric acidity (53.80±7.038 and 17.8±2.375 respectively) for the 500 mg/kg dose and (60.40±4.490 and 24.8±1.855 respectively) for the 250 mg/kg dose. Omeprazole also decreased free and total gastric acidity (54.40±3.092 and 20±2.449 respectively), compared with the ethanol groups (19.40±2.909 and 4.4±0.678 respectively). In the histological study, we found that the gastric mucosal barrier could be significantly strengthened when the rats were pretreated with 500 mg/kg of the MECC and showed almost normal histology compared with the ethanol-ulcerated groups. **Conclusion:** Based on the present results, we can conclude that CC leaves could be a promising food for the protection of the gastric mucosa against ethanol-induced lesions.

Keywords: *Chrysanthemum coronarium* L., Hplc, LD_{50}, Ethanol, Gastric ulcer, Rat.

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INTRODUCTION

Considered a benign lesion, a gastric ulcer is a localized loss of substance from the stomach wall. It has become a public health problem because of its high prevalence in the world population and high rate of morbidity and mortality.[1] Gastric ulcers are one of the most common gastrointestinal diseases of the 21st century, affecting people of all ages worldwide. Lifetime prevalence is estimated at 5-10% in the general population, with an annual incidence of 0.1-0.3%. It is a disease with a complex pathophysiology resulting from a disturbance in the balance between protective factors such as the secretion of hydrochloric acid, bicarbonate, and mucus, the biosynthesis of prostaglandins, and aggressive factors.[2] Multifactorial risks, such as smoking, helicobacter infection, psychological stress, and excessive consumption of non-steroidal anti-inflammatory drugs or alcohol, are linked to the development of stomach ulcers. Among these factors, alcohol consumption is an important contributor to gastrointestinal hemorrhage.[3] Given that alcoholic beverages

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come into contact with the gastric mucosa and can therefore cause direct damage to the mucosa, alcohol-related gastric disorders develop as early as 30 min after consumption, and this damage to the gastric mucosa can begin to manifest itself and reach its peak after around 60 min.[4] The mechanisms underlying alcohol-induced stomach ulcers are not yet fully understood.The lesions are due to increased permeability of the gastric mucosa, leakage of hydrogen ions from the lumen, and diffusion of hydrochloric acid into the subluminal mucosa and submucosal layer. Ethanol-induced microvascular injury by reducing blood flow increases cyclooxygenase enzymes, cytokines, free radicals, and signaling molecules following gastric mucosal injury, leading to inflammation in addition to the ongoing production of free radicals that damage mucosal cell DNA due to intracellular oxidative stress.[5] This stimulation by ethanol is accompanied by a sharp increase in the levels of pro-inflammatory factors in gastric tissue, congestion and edema of the gastric mucosa, epithelial cell death, and tissue necrosis and degeneration.[6] The animal model of acute gastric ulcer induced by ethanol is one of the most widely used experimental models for the preclinical evaluation of molecules with potential gastroprotective activity, as it has many of the same characteristics as an acute peptic ulcer in humans.[7] As the rat stomach is anatomically and functionally similar to the human stomach and can be divided into two parts, the upper non-glandular and non-secretory part, and the lower glandular and secretory part, rats have been chosen as the model of choice for the induction of ulcers.[8] For the treatment of ethanol-induced gastric ulcers, antacids, demulcents, histamine H2-receptor antagonists, and anticholinergics are generally used. However, these drugs cause an increase in acidity after a short period of treatment, resulting in a relapse of the ulcer.[9] According to,[10] kidney damage, hip fractures, pneumonia, and gastric cancer have been observed after administration of proton pump inhibitors. An effective and inexpensive plant-based anti-ulcer drug, therefore, remains a medical challenge.[11] The use of phytotherapy is a fundamental element of Algerian culture, and the population has been using medicinal plants for centuries to treat various illnesses.[12] Due to the diversity of its climatic conditions and its geographical position in North Africa, Algeria is known for its very rich and diverse flora.[13] With 3,139 different species of wild plants, Algeria is one of the richest Arab countries in terms of plant diversity.[14] According to WHO data, traditional medicine is the main method of healthcare for almost 80% of people in Africa. The recent growth in the use of medicinal plants is probably due to their regional abundance, cultural importance, and low cost of acquisition.[15] Although many of these plant species have been used traditionally to treat various diseases, most of them have not been studied scientifically.[16] This makes the use of medicinal plants for the pharmaceutical industry a virgin field in Algeria.[17] Among these aromatic plants, *Chrysanthemum coronarium* L. (CC) is an annual flowering plant belonging to the Asteraceae family widely distributed in the Mediterranean region.[18] Commonly known as gihouana, it is used in traditional medicine to treat digestive disorders.[19] Studies have shown that *Chrysanthemum coronarium* L is capable of reducing inflammation[20] and improving antioxidant defense,[21] with numerous food,[22] antibacterial, and anticancer uses.[23] In Egypt, this plant is considered a leafy vegetable because it contains abundant nutrients.[24] It is also a popular vegetable in Japan and Korea, as it is rich in beta-carotene, iron, calcium, potassium, and dietary fiber.[21] In addition, medicinal uses of *Chrysanthemum coronarium* extracts have been reported in Jordan and Italy, suggesting that they could be useful for the prevention of infectious and allergic diseases.[25] Studies on the chemical components of *Chrysanthemum coronarium* and their associated biological activities conducted by[26] showed that the plant mainly comprises active components such as flavonoids and polyphenols.

The present study was undertaken to investigate the gastroprotective effect of *Chrysanthemum coronarium* L. leaves growing in the Sidi Bel Abbès region (Northwest Algeria) on ethanol-induced acute gastric ulcers.

MATERIALS AND METHODS

Animals

The present study was conducted on male Wistar rats weighing 150-200 g, obtained from the animal house of the Pasteur Institute of Algeria. One-week acclimatization was performed with a temperature of 24°C±1°C and a 12 hr light/dark cycle. Food and water were provided *ad libitum*.

Plant material and preparation of the extract

The leaves of CC were collected in the locality of Sidi Bel Abbés (northwest Algeria). The identification was carried out by Professor Terras Mohamed, a botanist, and biologist at the Department of Biology at the University of Saida. The leaves were dried at room temperature and protected from light, ground to powder using a pestle and mortar, sieved, and then stored in a sealed glass bottle protected from light. The extraction was performed by a Soxhlet apparatus according to the method described by Boubekeur.[27] Twenty (20) g of CC leaves were exhausted in a Soxhlet in 250 mL of methanol for 10 hr at 80°C. All components were then filtered through Whatman Paper (n°1). The organic extract was placed under rotary evaporation at 40°C for 2 hr until the solvent had completely evaporated, and the solubilized active ingredients are well preserved in the cold.

Quantitative analysis by HPLC

High-Performance Liquid Chromatography (HPLC) was used under the following conditions: a Knauer Eurospher II column [100 A° pore size, 10 µm particle size, C_{18}, 250 mm×4mm], a flow rate of 1 mL min-1; the mobile phase was composed of (A) water+1% acetic acid and (B) methanol. The HPLC run conditions include a 5% B gradient from 0 to 55 min, 95% B at

55 min, and 5% B and 95% A at 56 min; a temperature of 25°C was controlled for the column. With the detection wavelength set at 254 nm, a 20 µL aliquot of the sample was injected, and UV spectral data for each peak were accumulated over the wavelength range of 240 to 400 nm. The retention times and UV spectra of the chromatographic peaks from the analysis are compared to those of reference standards.[28]

Acute toxicity tests

Initially, a batch of nine rats was used. The animals were randomly divided into three batches of three rats each. The median lethal dose (LD_{50}) of CC methanolic extract was determined according to the protocol described in.[29,30] According to Table 1, methanolic extract of CC was administered orally to rats at doses ranging from 10 to 5000 mg/kg body weight. The rats were given the extract only once, and within 24 hr we observed signs of toxicity and mortality.

Experimental design and induction of gastric ulcers by ethanol

The ethanol-induced gastric ulcer model was performed as described by El-Din.[3] Food and water were removed 24 hr and 2 hr, respectively, before the start of each experiment.[31] Rats were randomly divided into five groups (n=5), namely:

1. Distilled water group: 1 mL of water;

2. Ethanol group: 1 mL of water and 1 hr later, 8 mL/kg of absolute ethanol.

3. Dose 01 group: 500 mg/kg methanolic extract of CC and 1 hr later, 8 mL/kg absolute ethanol.

4. Dose 02 group: 250 mg/kg methanolic extract of CC and 1 hr later, 8 mL/kg absolute ethanol.

5. Omeprazole (OMP) group: 20 mg/kg OMP and 1 hr later, 8 mL/kg absolute ethanol.

The rats were then euthanized by cervical dislocation; their stomachs were excised and opened along the greater curvature; and the gastric tissues were isolated.

Titration of the acidity of gastric juice

In a conical flask, 1.00 mL of the gastric juice was centrifuged at 1000 rpm for 10 min and filtered. Titration was performed with a 0.01N NaOH solution with 1% phenolphthalein for total acidity and Topfer's reagent for free acidity as an indicator. An orange (free acidity) or permanent pink (total acidity) color was observed.[32] The acidity is expressed in meq/l as follows: Total/free

$$\text{acidity}=n\times0.01\times36.45\times1000$$

n=volume of NaOH consumed, 0.01=normality of NaOH, 36.45=molecular weight of NaOH, and 1000=factor represents in liters.

Histological analysis

Small 1-2 cm slices of each stomach glandular epithelium were fixed immediately in 10% buffered formalin solution at room temperature for 24 hr, followed by dehydration in 70°, 95°, and 100° alcohols, clarification with xylene, impregnation, and embedding in kerosene. After deposition on cold plates, 5 µm-thick tissue sections were made using a Leica RM2235 microtome. The sections stained with a routine Hematoxylin-Eosin-Saffron (HES) stain were then made for histopathological examination under a light microscope.[33,34]

Statistical analysis

All data were expressed as the mean±standard error of the mean. Multiple comparisons were performed using one-way ANOVA followed by Tukey's HSD test for *post hoc* analysis. A value of $p<0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 25.0 software.

Table 1: Acute toxicity test data.

Substances	Administration route	First stage of the investigation		LD_{50}	Second stage of the investigation		LD_{50}
		Dose mg/kg	Number of rats	Monitor 24 hr a day mortality. If no deaths are observed, the next stage is passed.	Dose mg/kg	Number of rats	Behavioral changes and deaths were observed for 24 hr.
Methanolic extract of CC	Orally	10	3		1500	3	
		100	3		2500	3	
		1000	3		5000	3	

RESULTS

The gastroprotective activity of a methanolic extract of CC was assessed by inducing an experimentally induced ulceration model *in vivo* using pure ethanol.

Acute toxicity test for the methanolic extract of CC

When the acute toxicity test was performed continuously for the first 2 hr and then observed for up to 24 hr, no detectable mortality or behavioral changes were observed in rats treated with 10, 100, 1000, 1500, 2500, and 5000 mg/kg of methanolic extract of CC. These results, therefore, indicate that the methanolic extract of CC has a low toxicity profile and that the 50% lethal dose (LD_{50}) value of the methanolic extract of CC is greater than 5000 mg/kg.

Chemical composition of the methanolic extract of CC

To analyze the compounds, present in the methanolic extract of CC, we used the HPLC method. Figure 1 shows the phenolic profile of the methanolic extract of CC.

Chromatographic analysis of the methanolic extract of CC identified the presence of 19 phenolic compounds, including o-coumaric acid, chlorogenic acid, myrecitin, benzoic acid, propylparaben (IS), 3-hydroxyflavone, ferulic acid, linoleic acid, vanillic acid, ascorbic acid, protocatechic acid, quercetin, catechin hydrate, cis, trans-abscissic acid, caffeic acid, p-coumaric acid, epicatechin, and p-hydroxybenzoic acid, representing 46.47% of the total sample. The dominant components were o-coumaric acid (9.55%), chlorogenic acid (6%), myricetin (4.19%), and benzoic acid (2.87%), as shown in Table 2.

Acidity titration of gastric juice

Table 3 shows the effects of CC leaf and Omeprazole on the total and free acidity of gastric juice in ethanol-induced gastric ulceration in rats. Administration of methanolic extract of CC resulted in a significant decrease in total acidity ($p<0.0001$) and free acidity ($p<0.0001$) of gastric juice in the omeprazole, methanolic extract of CC (500 mg/kg), and methanolic extract of CC (250 mg/kg) groups compared to the ethanol groups (Table 3). Ethanol significantly increased free and total gastric acidity. For the ethanol group, the values were 19.40±2.909 (total acidity) and 4.4±0.678 (free acidity). Methanolic extract of CC reduced total and free gastric acidity (53.80±7.038 and 17.8±2.375 respectively) for the 500 mg/kg dose and (60.40±4.490 and 24.8±1.855 respectively) for the 250 mg/kg dose. Omeprazole also decreased free and total gastric acidity in ethanol-induced gastric ulcers.

Histological evaluation of gastric lesions

Ethanol administration induced striking histopathological changes, such as exfoliation and necrosis of the superficial gastric epithelium. In addition, ethanol induced massive ulcerations in the glandular part of the rat stomach. The incidence of ulceration

Table 2: Chemical components of the methanolic extract of CC identified by HPLC.

N°	Compounds	Retention time (min)	Percentage of total
1	o-Coumaric acid	28	9.5525
2	Chlorogenic acid	18.9	6.0011
3	Myrecitine	30.9	4.1926
4	Benzoic acid	27.3	2.8732
5	Propylparaben (IS)	41.3	2.7784
6	3-hydroxyFlavone	47.3	2.356
7	Ferulic acid	26.3	2.2257
8	Linoleic acid	52.3	2.1305
9	Vanillic acid	20.7	1.7401
10	Ascorbic acide	2.91	1.7276
11	Protocatechuic acid	11	1.5623
12	Quercetin	34.3	1.5366
13	Catechine hydrate	14.6	1.4129
14	Cis, trans-Abscisic acid	32.6	1.3936
15	Caffeic acid	21.1	1.3154
16	p-Coumaric acid	23.7	1.144
17	Epicatechin	23.1	1.108
18	p-Hydroxy benzoic acid	17.2	0.8305
19	Catechin	17.9	0.5935

Table 3: Gastric-free and total acidity in an ethanol-induced gastric ulcer.

Treatment	Dose (mg/kg)	Total acidity (mmol/h)	ANOVA		Free acidity (mmol/h)	ANOVA	
			F	Sig		F	Sig
Ethanol	8 mL/kg	19.40±2.909			4.4±0.678		
Omeprazole	20 mg/kg	54.40±3.092*	15.843	0.000	20±2.449*	19.647	0.000
Dose 01	500 mg/kg	53.80±7.038*			17.8±2.375*		
Dose 02	250 mg/kg	60.40±4.490*			24.8±1.855*		

Results are presented as mean±sem (n=5), analyzed by 1-way ANOVA followed by Tukey's *post hoc* test. *Significantly different from the control group (p<0.05).

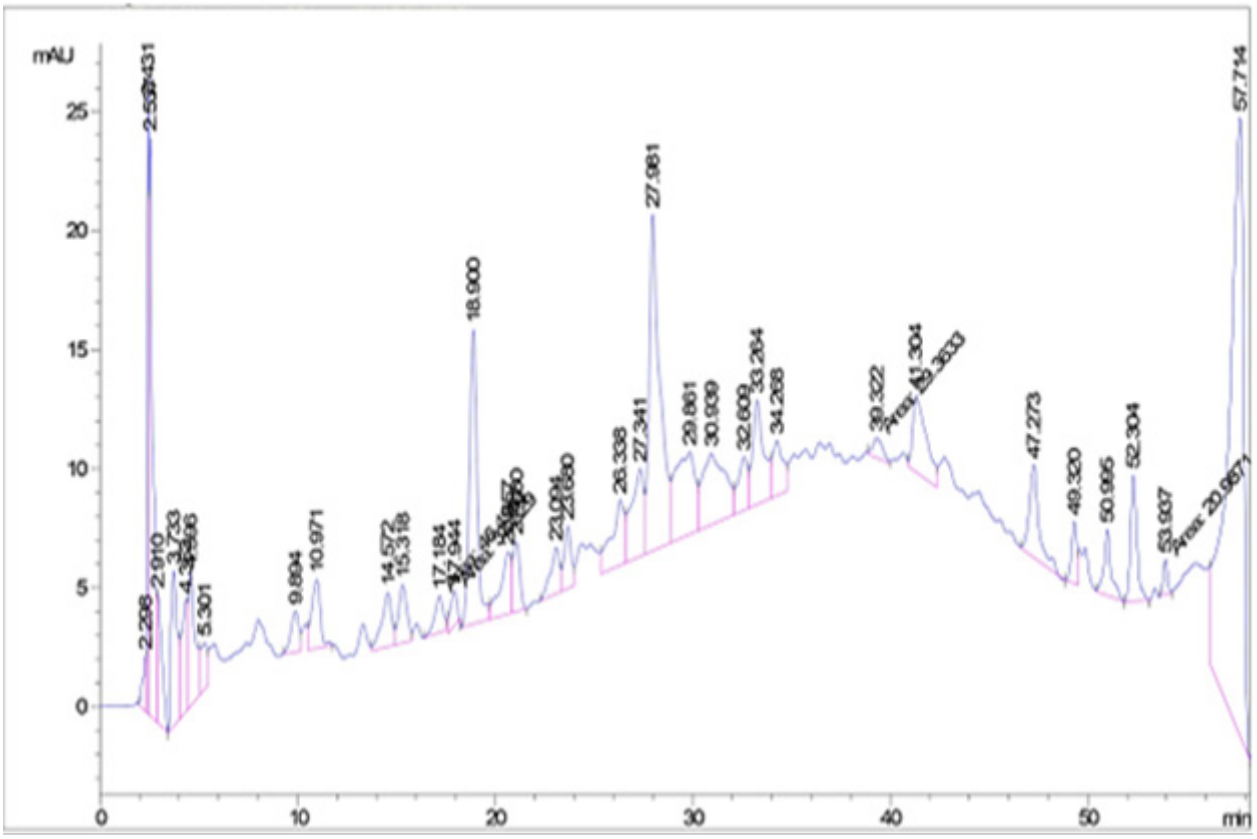

Figure 1: The phenolic profile of MECC.

was 100%. Acute dilatation, severe hemorrhage, and hyperaemia were observed, as well as perforation of the stomach (Figure 2).

We then studied the histological changes associated with ethanol-induced gastric ulceration. The group pretreated with Omeprazole, the standard reference drug, showed powerful protection of the gastric mucosa.Animals pretreated with a methanolic extract of CC showed a marked dose-dependent attenuation of ethanol-induced gastric histopathological changes. In the histological study, it is interesting to note that the gastric mucosal barrier could be significantly strengthened when rats were pretreated with 500 mg/kg of methanolic extract of CC and showed a superficial abrasion of the mucosa, demonstrating the gastroprotective effect of 500 mg/kg of methanolic extract of CC in attenuating ethanol-induced mucosal ulcers.Pretreatment with our second dose of 250 mg/kg methanolic extract of CC attenuated the damage to the gastric mucosa and showed less alteration; histological samples showed mucosal dehiscence (Figure 3). In Figure 4, the evaluation of the histological response after administration of ethanol without pretreatment with a methanolic extract of CC showed ulceration and erosion of the superficial epithelium, resulting in the disappearance of mucosal cells. In addition, these histological changes were associated with destructive mucosal edema.

DISCUSSION

Alcohol consumption can cause damage to the epithelial structure of the gastric mucosa, leading to erosions and ulcers.[35,36] With late treatment of the latter, it can progress to gastritis and even gastric cancer and be life-threatening.[37] However, a high recurrence rate and multiple side effects of drugs prescribed for the treatment of

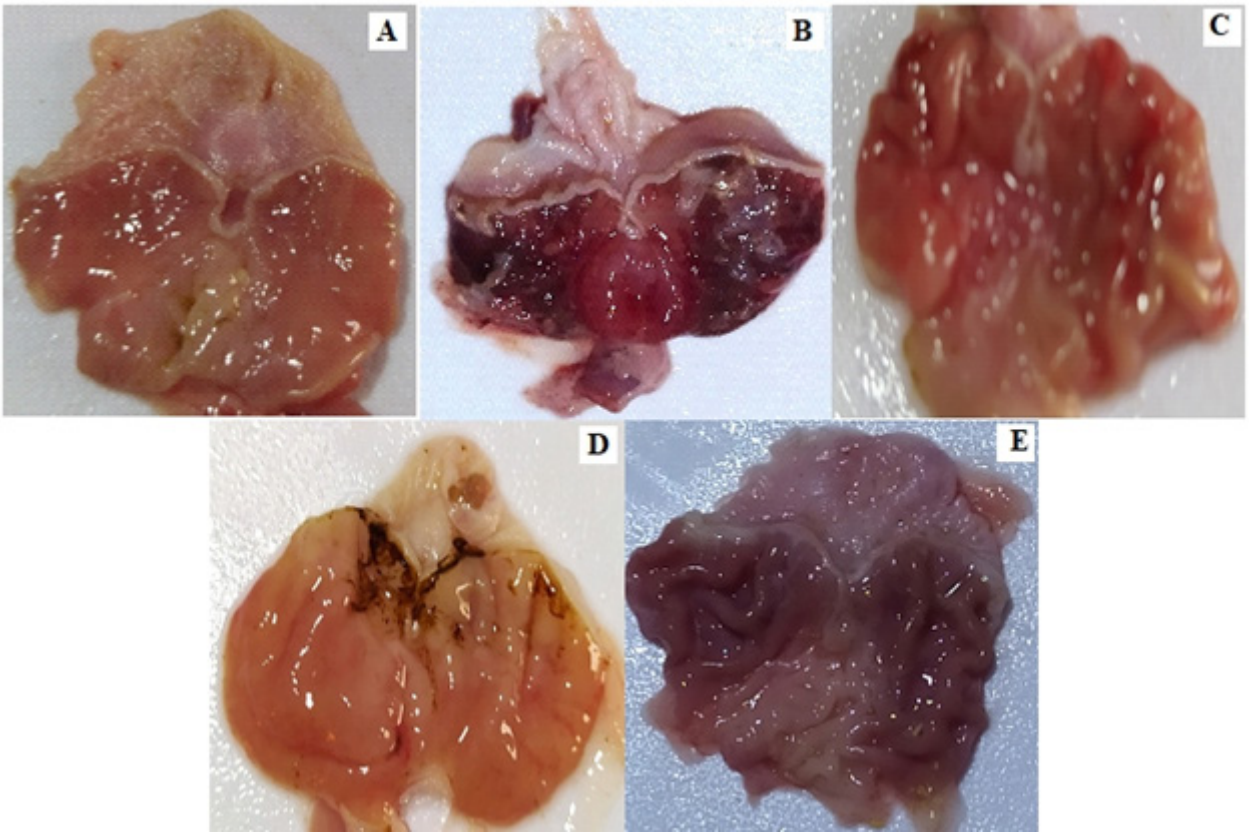

Figure 2: Macroscopic examination of the gastric mucosa.

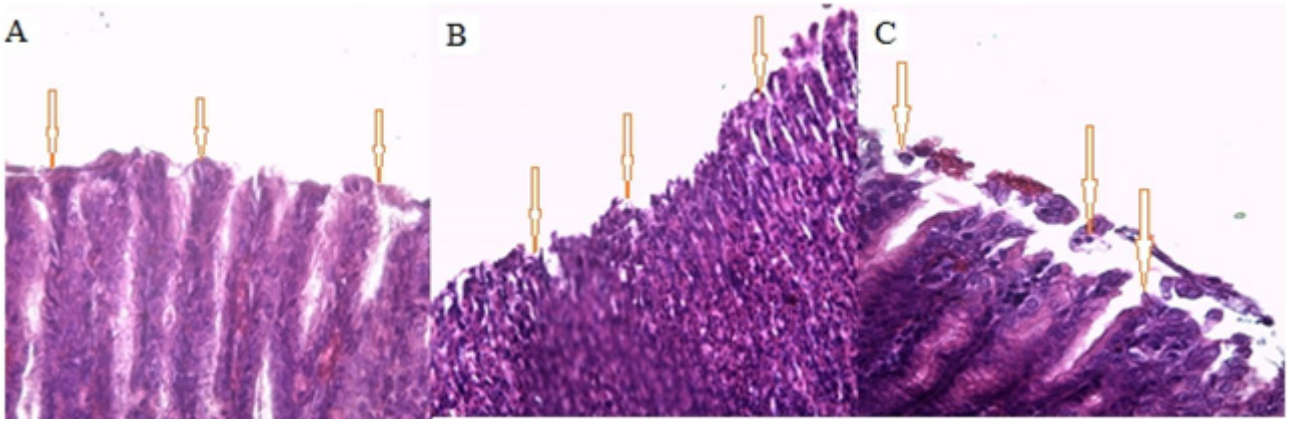

Figure 3: Effect of omeprazole and EMCC pretreatment on histological structures of the gastric mucosa against ethanol-induced damage.

gastric ulcers have increased the need for new bioactive molecules extracted from natural substances.[38] Among them is a plant native to Mediterranean regions, CC, an annual common in ruderal vegetation, field margins, roadsides, and urban wasteland.[39] The medicinal use of the infusion of their flowers has been reported to relieve gastric disorders and treat inflammation.[40] In general, the location of the ulcer and symptoms of bleeding and swelling are used to assess the degree of damage to the gastric mucosa.[11,41] To our knowledge, the anti-ulcer property of the methanolic extract of CC has not yet been studied. At the administered doses (10-5000 mg/kg), none of the rats treated with our extract showed visible signs of toxicity, morbidity, or mortality. This confirms its use as an edible and non-toxic plant.[24,42]

Phenolic acids, such as caffeic, gallic, p-coumaric, vanillic, ferulic, and protocatechic acids, are natural substances found in the plant kingdom. They have structural similarities and contain carboxyl groups.[43] According to,[44] the chemical composition of *Chrysanthemum* sp. cultivars varies according to geographical origin, environment, and analytical procedures. In the present study, HPLC identified the presence of 19 phenolic compounds, with o-coumaric acid (9.55%), chlorogenic acid (6%), myricetin (4.19%), and benzoic acid (2.87%) predominating. It should be noted that there is little information on the chemical

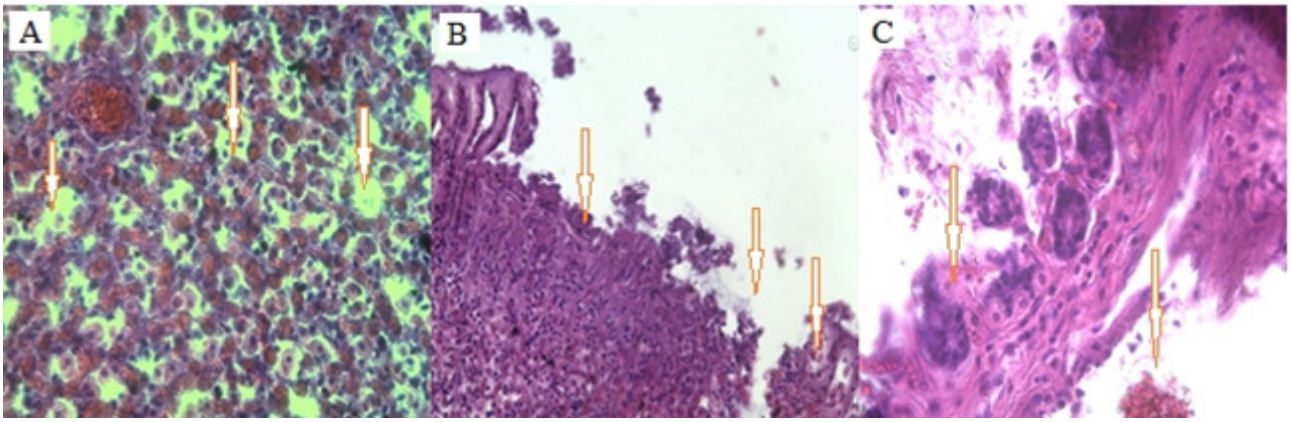

Figure 4: Histological structures of the gastric mucosa in untreated subjects after ethanol-induced lesions.

characterization of this plant in our country or on its traditional uses in the treatment of stomach ulcers. In the study conducted by Ivashchenko[45] in Ukraine, the methanolic extract of CC revealed the presence of isochlorogenic acid (35.48%), caffeic acid (10.25%), caffeoylquinic acid (17.18%), and luteolin-7-glycoside (7.85%). In another study, chlorogenic acid, di-caffeoylquinic acid isomers, rutin, luteolin, luteolin-7-O-glucoside, myricetin-3-O-galactoside, and tricin were all identified by HPLC-PDA-MS in CC flowers growing in Zaghouan province in Tunisia by Hosni K 2013.[46] In the pathogenesis of ulcers, damage to the gastric mucosa is caused by the generation of pro-inflammatory cytokines and various reactive oxygen species.[47]

According to Joshi *et al,* 2023,[48] caffeic, gallic, p-coumaric, vanillic, ferulic, and protocatechic acids possess good anti-ulcer activity. The anti-ulcer effect of phenolic acids may be due to their antioxidant activity.[43] Quercetin[49] and myricetin[50] have antioxidant activity, while p-coumaric and caffeic acids and chlorogenic acids have anti-inflammatory and antioxidant effects.[51]

Furthermore, our findings demonstrated that pretreatment with the methanolic extract of CC had effects on gastric pH that were equivalent to those of the reference medication, Omeprazole, which has a potent ability to reduce gastric acid production and neutralize the acidic environment in the stomach. A review of the literature showed that little is known about the anti-ulcer properties of CC. In the present study, the total acidity (p<0.0001) and free acidity (p<0.0001) of gastric juice were remarkably decreased in the Omeprazole, methanolic extract of CC (500 mg/kg), and methanolic extract of CC (250 mg/kg) groups compared to the ethanol groups. This suggests an anti-secretory mechanism by our plant, as already cited in the literature.[52] Previous studies have shown that increasing hydrogen ion concentration is an aggressive factor that facilitates gastric lesions.[53] Myricetin is a member of the flavonoid family. The gastroprotective role of flavonoids was reported in the study by[54] by increasing the pH value of gastric juice. Moreover, this can be justified by the reduction of the aggression factors of the gastric mucosa following the inhibition by our extract of the interaction between histamine, gastrin, and acetylcholine with their receptors, activators of the proton pump.[55] The biologically active constituents of our extract, such as chlorogenic acid, caffeic acid, and quercetin, are known to decrease gastric secretion.[56]

According to Youssef 2020,[57] the flowers of several chrysanthemums were frequently used to treat ulcerative colitis. Similarly,[58] report this activity in *Chrysanthemum morifolium*. The development of the ulcer results from the decrease in the amount of mucus that protects the epithelial cells from ethanol; this decrease causes the digestion of the gastric mucosa by hydrochloric acid.[59] In our study, oral administration of absolute ethanol in rats destroyed stomach tissues, causing submucosal edema, hemorrhage, and desquamation of epithelial cells. The same results were reported by Mousa *et al*, 2019,[60] who justified these lesions by the rapid and easy penetration of ethanol into the gastric mucosa and said that these lesions are characteristic and typical of alcohol-induced lesions in humans. Furthermore,[61] report that microvascular lesions and disruption of the vascular endothelium, leading to increased vascular permeability, edema formation, and epithelial lifting, are the first signs of ethanol-induced damage to the gastrointestinal mucosa. Previous studies have demonstrated that these gastric lesions are experimentally induced by ethanol in rats.[62,63]

The histological studies, therefore, provided further evidence of the gastroprotective effect of the methanolic extract of CC and supported the studies of the chemical constituents. The preservation of the integrity of the gastric epithelium resulting from the administration of our extract could be due to the protective layer produced, similar to gastric mucus, which inhibits contact with ethanol; this effect has already been reported by the work of [64] in the region of Jijel, eastern Algeria, in 2018. In addition, a protective effect against gastric ulcers due to the presence of phenolic acids and flavonoids in various plant extracts has been reported by different studies.[52,65] To this end, we suggest

future research based on current results for further exploration of these bioactive compounds.

CONCLUSION

The traditional usage of *Chrysanthemum coronarium* L. for protection against gastric ulcers has a scientific foundation for the first time, which is demonstrated by this study. Based on the present results, we concluded that the oral pre-administration of a methanolic extract of CC leaves effectively protects the gastric mucosal barrier against ethanol-induced injury by decreasing total and free gastric acidity and producing a protective layer similar to gastric mucus. The anti-ulcer effect could also be attributed to the synergistic pharmacological activities of the biological substances of CC, such as anti-secretory (chlorogenic acid, caffeic acid, quercetin), antioxidant (quercetin, myricetin), and anti-inflammatory (p-coumaric acid, caffeic acid, chlorogenic acid) activity. Further studies are needed to better understand the mode of action of CC phenolic compounds and to explore this plant as a new natural antiulcer agent. Although we are well aware that all drugs have their own limitations, particularly in the case of ulcers, which are a complex pathology.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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