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Mechanisms of gastroprotective effects of *Anabasis articulata* (Forssk.) Moq. decoction against ethanol-induced gastric mucosal injury in rats

Yasmina Makhlouf[1,*], Amel Bouaziz[1], Chahinez Hasnaoui[2], Lazhar Zourgui[3], Houcine Dab[3], Boutheina Yahia[3], Nabil Benazi[4], Nihed Barghout[1], Assia Bentahar[1], Saliha Djidel[1], Seddik Khennouf[1] and Saliha Dahamna[1]

[1]*Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Ferhat Abbas Setif 1*, Setif, Algeria

[2]*Department of Molecular and Cellular Biology, Faculty of Natural and Life Sciences, University Abbas Laghrour*, Khenchela, Algeria

[3]*Laboratory of Biodiversity, Molecules, Applications, (LR22ES02) Higher Institute of Applied Biology of Medenine, University of Gabes*, Medenine, Tunisia

[4]*Institut Pasteur Algeria, Antenna M'sila*, M'sila, Algeria

***Corresponding author**: yasmina.makhlouf@univ-setif.dz; yasmin.mak28@gmail.com

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Abstract: *Anabasis articulata*, commonly known as 'ajrem' or 'eshnan', is widely used in traditional medicine across the Arab world to alleviate ailments such as eczema, fever, and inflammatory diseases. Despite its extensive use, no prior studies have investigated the gastroprotective properties of the decocted extract of *A. articulata* (DEAA) or explored its mechanisms of action. This study is the first to evaluate the gastroprotective effects of DEAA in ethanol-induced gastric ulcers in rats and to elucidate its mechanism of action through three major protective pathways. Rats received DEAA *per os* (p.o.) at doses of 50, 100, and 200 mg/kg. Mechanistic investigations included pretreatments with glibenclamide (a potassium ATP-channel blocker), indomethacin (a cyclooxygenase (COX) inhibitor), and N-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor). DEAA at 200 mg/kg demonstrated significant gastroprotective activity in the acute ulcer model. The gastroprotective effects of DEAA were not affected by these pharmacological inhibitors, confirming that its action is independent of the ATP-sensitive potassium channel (KATP channel), prostaglandin synthesis, and nitric oxide (NO) production. Further analysis revealed that DEAA protects the gastric mucosa by reducing basal gastric juice secretion, enhancing mucus secretion, and increasing the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD). Additionally, DEAA mitigated inflammation by reducing neutrophil infiltration, as evidenced by decreased myeloperoxidase activity. These findings provide the first scientific validation of the gastroprotective effects of DEAA, independent of the three classical protective pathways. This study highlights the potential of *A. articulata* as a multi-targeted therapeutic agent for preventing and managing gastric ulcers.

Keywords: *Anabasis articulata*, gastric ulcer, glibenclamide, indomethacin, L-NAME

INTRODUCTION

Oxygen is essential for our survival, development, and adaptability, but it also carries risks of toxicity, acidity, degradation, and degeneration. Dysregulation of oxygen metabolism results in oxidative stress [1], which stimulates the formation of partially reduced, highly toxic, free radicals or reactive oxygen species (ROS) and reactive nitrogen species (RNS). These molecules, both radical or non-radical, are continuously generated by several cell types in aerobic organisms [2]. Oxidative stress is responsible for the inability to counteract ROS-induced damage [3]. It has garnered substantial attention within the medical community due to its pivotal role in the pathophysiology of chronic diseases, including atherosclerosis, cancer, diabetes, rheumatoid arthritis, cardiovascular disease, and aging [4]. Many disorders are linked to inflammatory

processes, and a significant number of individuals globally suffer from chronic conditions such as gastric ulcers [5]. Gastric ulcers are among the most prevalent digestive disorders, affecting individuals of all ages worldwide. Their detrimental effects stem from a disruption of natural defenses, such as the mucosal bicarbonate barrier, prostaglandin E_2, cell regeneration, antioxidant markers, and endogenous factors such as increased hydrochloric acid, ROS production, and pepsin disruption [6]. The disturbed equilibrium primarily results from excessive exposure of the gastric mucosa to non-steroid anti-inflammatory drugs (NSAIDs), smoking, nutritional deficiencies, stress, and alcohol consumption [7].

Medicinal plants, abundant in phenolic compounds, with diverse biochemical and pharmacological properties, including anti-inflammatory and antioxidant effects, are recognized as significant therapeutic resources and a natural pharmacy for human health preservation [8]. Flavonoids demonstrate gastroprotective, antisecretory, and antioxidant properties, while alkaloids and terpenoids exhibit gastroprotective qualities by influencing pH levels and ulcer indices. Tannins and saponins, present in numerous plants, possess an anti-ulcerogenic potential through the modulation of acid secretion [9].

Polyphenols, notably flavonoids and tannins, act in the gastrointestinal tract as antiulcer, antisecretory, and antioxidant agents [10]. Flavonoids help prevent ulcers primarily through their antioxidant action. This includes reducing free radicals, chelating ions and metals, inhibiting oxidative enzymes, enhancing antioxidants, and lowering lipid peroxidation [11]. Tannins are used in medicine mainly for their astringent properties; they interact with proteins in tissues, precipitating at ulcer sites to form a protective layer (tannin-protein/tannin polysaccharide complex) that prevents the absorption of toxic substances and enhances resistance to proteolytic enzyme activity, thus inhibiting stomach proteases such as pepsin [12].

Anabasis articulata, a Saharan plant belonging to the Chenopodiaceae family, has been utilized in traditional medicine by indigenous populations to treat fever, diarrhea, diabetes, asthma, rheumatism, and cancer. Plants in the Chenopodiaceae family are renowned for their wealth of bioactive substances. The *Anabasis* genus thrives in stony, sandy valleys frequented by camels and goats [13]. The continued reliance on traditional medicine is attributable not only to cultural factors and poverty but also to the lack of effectiveness of many existing drugs [14]. There is considerable interest in the biologically active compounds in plants and herbs for their safety and efficacy in preventing and treating human diseases [15].

In Algeria, Syria, Egypt, and Iraq, *Anabasis articulata* is widely used in traditional medicine to treat asthma, fever, eczema, and kidney infections [16] and has also been applied as a plaster for scabies treatment [17]. The species serves as a remedy for diabetes [18] and also has cholinergic properties [19]. The aerial parts are used in decoction and as a poultice to treat dermatoses, skin diseases (eczema), headaches, and fever [20]. No scientific investigations have explored the antidiabetic and antiulcerogenic effects of the ethanolic extract of the leaf and flower mixture of *A. articulata* from Algeria. This study investigated the *in vitro* antioxidant and *in vivo* antiulcerogenic activities. The primary goal was to evaluate the gastroprotective effects of DEAA and to investigate its potential mechanisms of action using an ethanol-induced gastric ulcer model in rats. This objective was successfully achieved, as the results show that DEAA, particularly at a dose of 200 mg/kg, significantly reduces ethanol-induced gastric damage. Rats were pretreated with distilled water, omeprazole, or DEAA (50, 100, and 200 mg/kg) before ethanol administration. Additional tests involved pretreatments with L-NAME, glibenclamide, and indomethacin. Gastric acidity, mucus adherence, and stomach tissues were analyzed to assess lesions, histological changes, and antioxidant enzyme activities.

MATERIALS AND METHODS

Ethics statement

All experimental studies were approved by the Committee of the Algerian Association of Sciences in Animal Experimentation (http://aasea.asso.dz/articles/) No. 8808/1988, associated with veterinary medical activities and protection of animal health (No. JORA 004/1988).

Nomenclature

The medicinal plant used was *Anabasis articulata* (Forssk.) Moq., locally known as "El Ajrem", belonging to the Chenopodiaceae family. The plant was identified by Professor B. Oujhih, an expert taxonomist at the Institute of Nutrition and Agronomy, University of Batna, Algeria. A voucher specimen was deposited in the laboratory under SNV 0045-2020.

Chemicals and instruments

All analytical-grade chemicals used in this study were procured from E. Merck, Germany, including absolute ethanol (EtOH), L-NAME (N-G-nitro-L-arginine), indomethacin and Alcian blue, sucrose, magnesium chloride, sodium acetate, and sodium hydroxide.

Plant material

A. articulata was harvested in October-November 2020 at the El Mergueb Natural Reserve of M'sila province (North Algeria) at 35° 42' N latitude and 4° 32' E longitude. The flowers and leaves of *A. articulata* were air-dried in the shade, crushed, and then powdered using an electric grinder.

Extraction procedure

Preparation of decocted extract

The decoction extract of the flowers and leaves of *A. articulata* (DEAA) was prepared according to Ferreira et al. [21]. Briefly, 100 g of powder was boiled for 10 min in 1000 mL of distilled water. The mixture was filtered through muslin and then Whatman filter paper. The filtrate was poured into glass plates and dried in the oven (37°C).

Animal material

Albino rats weighing 120-200 g were maintained in polycarbonate cages for 7 days under laboratory conditions (12/12 h light/dark cycle, 23±2°C) with *ad libitum* access to food and water before the beginning of the experimentation.

Antiulcerogenic *in vivo* investigations

Ethanol-induced gastric ulcer

The antiulcer activity of the DEAA was determined following the method used by Abdulla et al. [22]. Ulcers were induced by administering ethanol. All the animals (rats 5/group) were deprived of food for 24 h before the experiment but had free access to drinking water up to 1 h before the experiment. Group I: the ulcer control group was orally administered distilled water (5 mL/kg); Group II was treated with omeprazole (20 mg/kg) as a reference drug; Groups III, IV, and V were treated with 50, 100, and 200 mg/kg DEAA, respectively. One h after this pretreatment, all groups of rats received a dose of absolute ethanol (2.5 mL/kg) orally. The animals were killed by cervical dislocation 30 min after administration of a necrotizing agent. According to Bouaziz et al. [23], the evaluation of macroscopic gastric lesions using the percentage of ulceration was calculated using the following formula:

$$\% \text{ ulceration} = (\text{total ulcerated area} / \text{total mucosal area}) \times 100.$$

The total area of the lesions and the total area of the stomach were measured using Image J software.

Gastric ulcer induced by ethanol in animals pretreated with L-NAME, glibenclamide and indomethacin

Separate experiments were conducted to examine the roles of a non-selective inhibitor of nitric oxide synthase, L-NAME, the ATP-sensitive potassium channel (KATP) blocker glibenclamide, and the NSAID indomethacin, which inhibits the PGE_2 synthesis. According to Ribeiro et al. [24], rats that fasted for 18 h and were provided water *ad libitum* 1 h before the experiment were distributed into 8 groups of 5 animals each. Three groups were pretreated with L-NAME (10 mg/kg intraperitoneally, i.p.), glibenclamide (10 mg/kg i.p.), or indomethacin (10 mg/kg i.p) 30 min before receiving 200 mg/kg DEAA by gavage. Two additional groups were included, one received only 200 mg/kg DEAA, and one received only the vehicle (10 mL/kg, p.o.). Forty-five min later, all animals received absolute ethanol (4 mL/kg, p.o.) to induce ulcers. One h after ethanol treatment, the animals were killed.

Collection of the gastric juice and measurement of gastric acidity

After the animals were killed, their stomachs were removed. The stomach contents were collected, and centrifuged at 4°C at 55.9 ×g, and the pH was determined using a digital pH meter [25].

Macroscopic gastric lesion evaluation

After collecting the gastric juice, the stomachs of each animal were removed, opened along the greater curvature, washed with cold saline, and flattened stomach samples were photographed. The total area of the lesions and the total area of the stomach was measured using Image J software [23].

The percentage of ulceration was calculated according to the following formula:

$$\% \text{ ulceration} = (\text{total ulcerated area} / \text{total mucosal area}) \times 100.$$

Determination of adhered mucus to gastric wall

A modified method of Corne [26] was utilized for determining gastric mucus. A segment of the glandular region of the stomach was weighed and transferred to a test tube containing 7 mL of 0.1% Alcian blue in 0.16 M sucrose, 0.05 M sodium acetate, pH 5.8. After two consecutive rinses with 5 mL 0.25 M sucrose, 5 mL of 0.5 M $MgCl_2$ was added to each test tube for the extraction of mucus content with the dye. The glandular segment remained in this solution for 2 h, with intermittent agitation. Subsequently, 4 mL of the resultant blue solution was agitated vigorously with 4 mL of ethyl ether until the formation of an emulsion and was centrifuged at 724.46 ×g for 10 min. The absorbance of the supernatant was measured at 598 nm using a spectrophotometer. The concentration of Alcian blue was calculated through a calibration curve and the results were expressed in mg of Alcian blue/g of glandular tissue.

Histological evaluation of gastric lesions

For histopathological examination, the tissues were fixed in 10% formalin solution. The formalin-fixed stomach specimens were embedded in paraffin wax, and serially sectioned (3-5 μm), and stained with hematoxylin and eosin. The stained tissues were observed for pathological changes using light microscopy [27].

In vivo antioxidant activity

Preparation of the homogenate

Gastric tissue from treated and control rats were homogenized in lysis buffer (0.25 M sucrose, 0.05 M Tris-HCL, 1 mM EDTA, pH 7.4). After 24 h of incubation at -20°C, samples were centrifuged at 894.4 ×g for 15 min). The supernatant was collected and stored at -60°C until use [28].

Biochemical Procedures

Total protein was determined using the Bradford method [29], measuring absorbance at 595 nm.

SOD activity was assessed according to the Misra and Fridovich protocol [30] by monitoring the inhibition of adrenochrome formation at 480 nm, with one unit of SOD defined as the amount inhibiting 50% of the reaction.

Catalase (CAT) is a heme enzyme that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen. CAT activity was measured by tracking the decomposition of H_2O_2 at 240 nm and expressed activity in μmol/s/μg of total protein. [31]

Measurement of lipid peroxidation (MDA)

MDA levels were quantified using the double heating method described by Draper and Hadley [32]. Briefly, 500 μg of total protein was mixed with a reaction solution containing 8.1% sodium dodecyl sulfate (SDS), 20% acetate buffer (pH 3.5), and 0.8% thiobarbituric acid (TBA) for 3 min, followed by incubation at 95°C for 60 min. After cooling, the TBA-reactive substances were extracted with 1 mL of H_2O_2 and 2.5 mL of an n-butanol:pyridine mixture (15:1, v/v). The organic phase containing MDA was collected, and absorbance was measured at 532 nm. MDA concentration was calculated using the molar extinction coefficient (ε MDA-TBA) and expressed as nmol of MDA per gram of total protein.

Statistical analysis

Statistical comparisons were performed using Graph Pad Prism (version 7.00 for Windows). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were used. The statistics are presented as the mean±standard error of the mean (SEM) with n=5. When the P value was less than 0.05, a difference was considered significant.

RESULTS

Ethanol-induced gastric ulcer

Intragastric administration of absolute ethanol to the untreated group of rats (negative control) produced large band-like hemorrhagic erosions in the glandular stomach. Pretreatment with the DEAA at the tested doses (50,100 and 200 mg/kg p.o.) and omeprazole (20 mg/kg) offered varying degrees of protection to the mucosa against ethanol-induced damage (Fig. 1). Oral administration of the DEAA resulted in gastric ulceration rates of 37.75±6.28%, 16.034±1.308%, and 5.16±0.33%, respectively, compared to the negative control (45.92±1.87%), and to the group pretreated with omeprazole (positive control) with 4.54±2.22% ulceration.

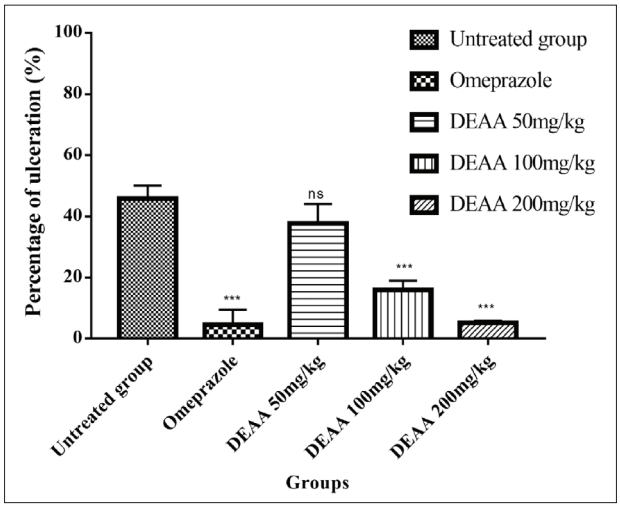

Fig. 1. Ethanol-induced gastric ulcer: percentage of inhibition of ulceration pretreated with the DEAA; ns – no significant difference, *P<0.01 and ***P<0.001 were considered significant when compared to omeprazole.

Macroscopic examination

The assay revealed the effect of ethanol on gastric tissues in the presence of the DEAA at different doses, as shown in Fig. 2. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in the untreated group (the vehicle) (Fig. 2B). The positive

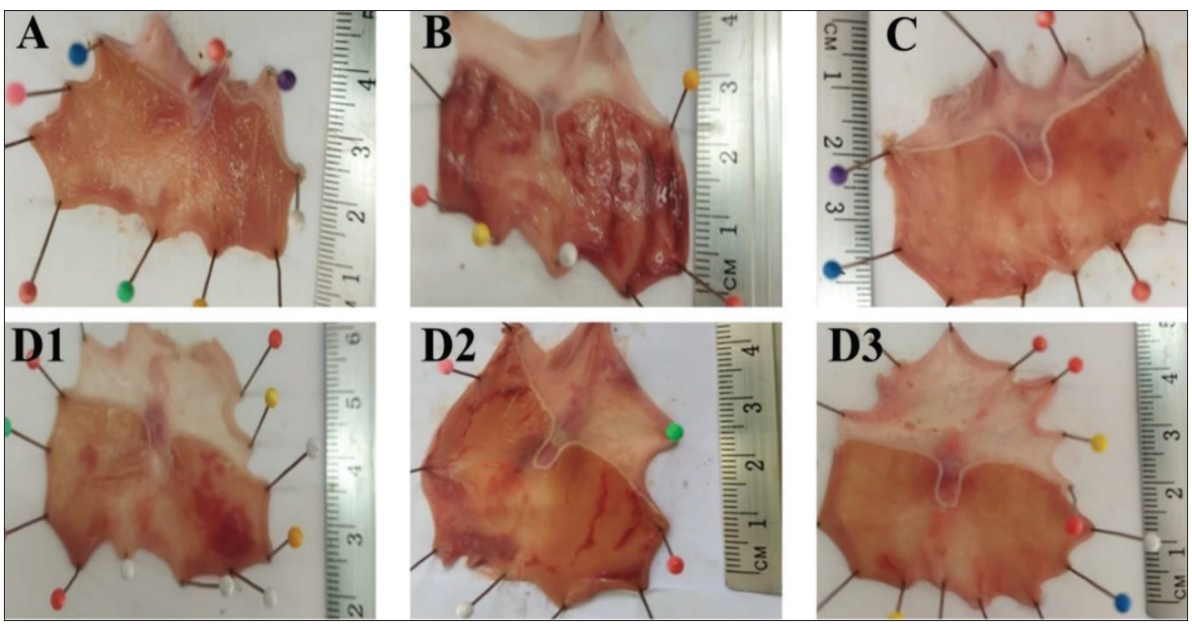

Fig. 2. Macroscopic examination of the stomach after gastric ulcer induction by ethanol. **A** – Control group; **B** – pretreated with the vehicle as the negative control; **C** – pretreated with omeprazole (20 mg/kg) as the positive control. **D1**, **D2** and **D3** – Groups pretreated with DEAA 50, 100, and 200 mg/kg, respectively.

control group treated with 20 mg/kg of omeprazole showed milder injuries to the gastric mucosa (Fig. 2C) compared to the untreated group. Oral administration of the DEAA (Fig.2D) reversed the ethanol-induced gastric injury in a dose-dependent manner, significantly reducing the gastric ulcer area. The protective properties of the DEAA at 200 mg/kg was similar to that of the positive group (Fig. 2C).

Mechanism of the gastroprotective effect

In a series of experiments, the mechanisms of gastroprotective effect of the DEAA were evaluated at a dose of 200 mg/kg to rats pretreated with L-NAME, glibenclamide, and indomethacin.

Measurement of gastric acidity

Fig. 3 shows the effect of administering *A. articulata* extract (200 mg/kg DEAA), omeprazole (20 mg/kg p.o.), vehicle, and groups pretreated with L-NAME, glibenclamide, and indomethacin with and without 200 mg/kg DEAA on gastric juice acidity in rats.

Oral administration of the DEAA in animals pretreated with L-NAME, glibenclamide, and indomethacin significantly reduced the acidity of the gastric juice (pH=3.85±0.35, 3.64±0.26, 4.09±0.33, respectively). The acidity in the normal group (4.89±0.08) was similar to that of the omeprazole group (pH=4.66±0.21; no significant difference). The pH of the gastric juice was acidic for the groups pretreated with L-NAME, glibenclamide, and indomethacin without the DEAA (2.25±0.36, 2.11±0.18, 2.97±0.29, respectively).

Macroscopic gastric lesion evaluation of mechanism

A macroscopic examination of the effect of 200 mg/kg DEAA in the absence or presence of different pharmacological substances (L-NAME, glibenclamide, and indomethacin) on ethanol-induced gastric mucosa damage is shown in Fig. 4. The protective properties of the DEAA (Fig. 4D) were similar to those of the positive group (Fig. 4B) when compared to the negative control (Fig. 4C). L-NAME caused severe damage to the gastric mucosa when administered without the DEAA (Fig. 4E1). However, in the presence of the DEAA (Fig. 4E2), the extent of damage was reduced,

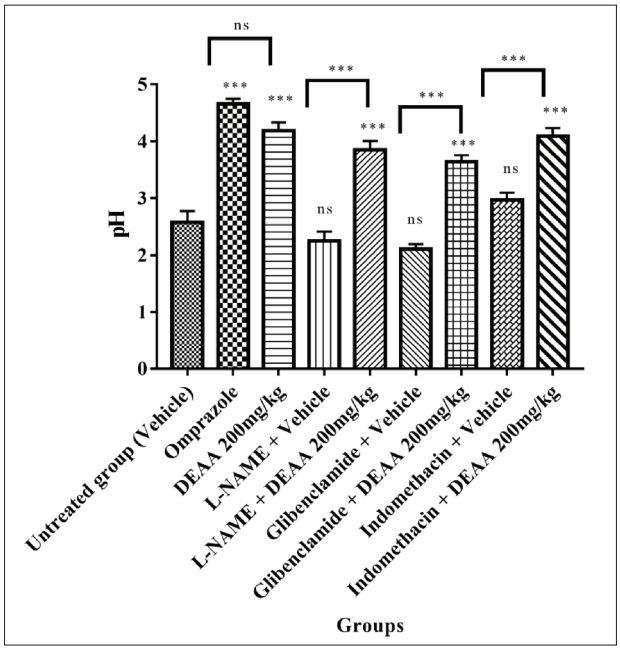

Fig. 3. Gastric acidity (pH) in the presence and absence of 200 mg/kg DEAA in rats pretreated with L-NAME, glibenclamide and indomethacin. The results are expressed as means±SEM; ns – no significant difference, n=5, ***P<0.001.

resulting in moderate lesions compared to the negative control. Glibenclamide caused significant damage to the stomach mucosa (Fig. 4F1), in comparison to the group without ulcers induced by ethanol. Pretreatment with both glibenclamide and the DEAA provided significant protection (Fig. 4F2), as observed in the control positive group (omeprazole) (Fig. 4F2). The administration of indomethacin resulted in moderate to severe gastric injury in response to ethanol-induced ulcers (Fig. 4G1). In contrast, pretreatment with both indomethacin and the DEAA had a milder effect (Fig. 4G2) compared to indomethacin alone. The protection provided by the DEAA against ethanol-induced ulcers in the presence of indomethacin was similar to the positive control group (omeprazole) and DEAA alone.

Histological evaluation of gastric lesions

Ethanol administration induced macroscopic lesions in gastric tissue, such as petechiae, hemorrhaging, and edema. These lesions are likely linked to mucus depletion from the gastric mucosa veins and arteries, leading to hemorrhage, inflammation, and tissue damage. To confirm the antiulcer results, the stomachs were also examined histopathologically (Fig. 5).

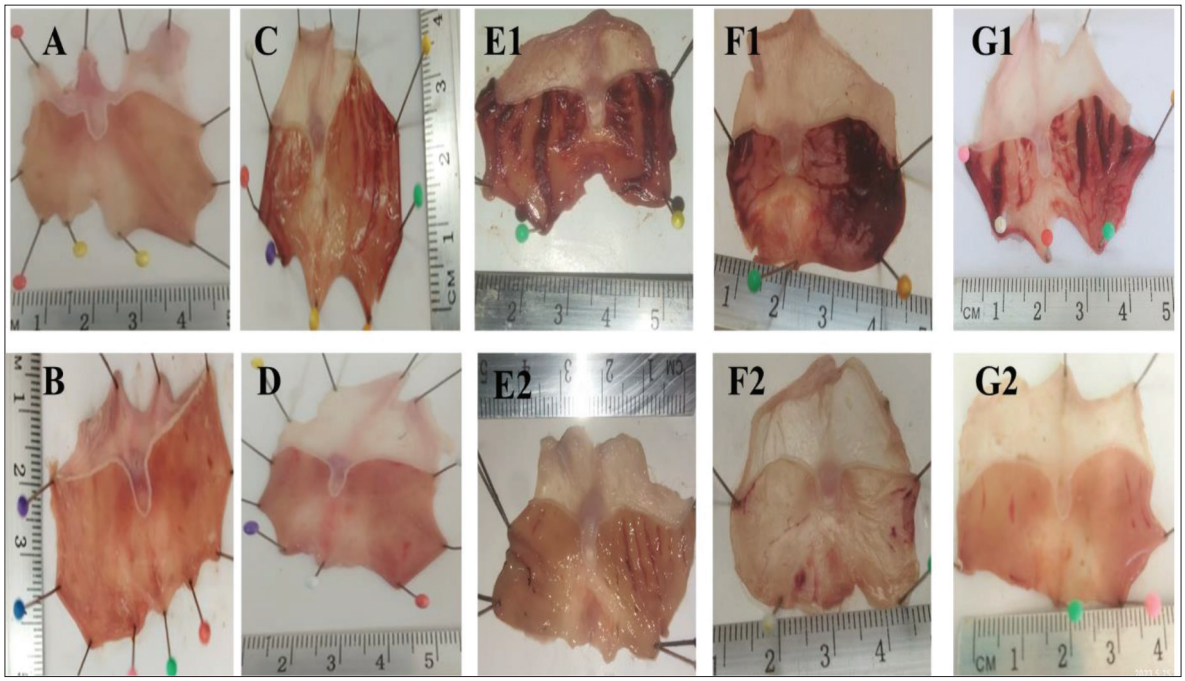

Fig. 4 : Gastric ulcer induced by oral administration of absolute ethanol (2.5 mL/kg) in rats pretreated with L-NAME, glibenclamide, and indomethacin. **A** – Normal group; **B** – pretreated with 20 mg/kg omeprazole (positive control); **C** – pretreated with vehicle (negative control); **D** – pretreated with 200 mg/kg DEAA. **E1**, **F1**, **G1** – pretreated with L-NAME, glibenclamide, and indomethacin, respectively in the absence of 200 mg/kg DEAA. **E2**, **F2**, **G2** – pretreated with L-NAME, glibenclamide, and indomethacin, respectively, in the presence of 200 mg/kg DEAA.

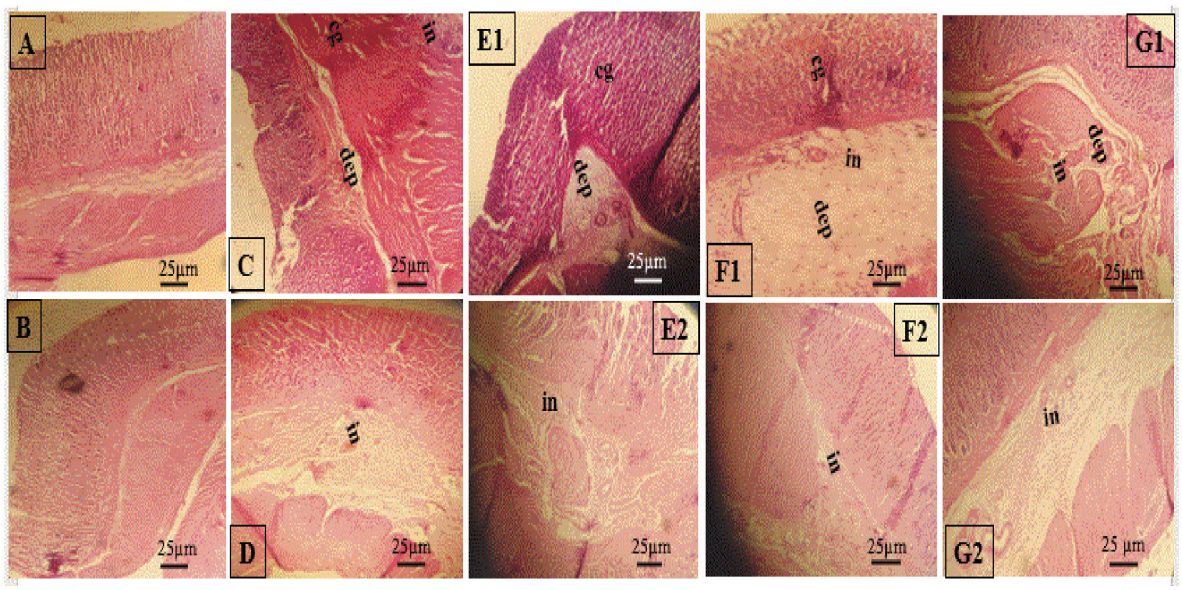

Fig. 5. Histological evaluation of gastric lesions in ethanol-induced gastric ulcers in animals pretreated with L-NAME, glibenclamide, and indomethacin. **A** – normal group; **B** – pretreated with 20 mg/kg omeprazole (positive control); **C** – pretreated with the vehicle (negative control); **D** – pretreated with 200 mg/kg DEAA. **E1**, **F1**, **G1** – pretreated with L-NAME, glibenclamide, and indomethacin, respectively, in the absence of 200 mg/kg DEAA. **E2**, **F2**, **G2** – pretreated with L-NAME, glibenclamide, and indomethacin, respectively, in the presence of 200 mg/kg DEAA; dep – detachment of the surface epithelium, infiltration of inflammatory cells; cg – congestion.

Histological observation revealed that the stomachs of healthy animals showed no damage and a normal histological structure in the mucosa, muscularis mucosa, and submucosa (Fig. 5A). However, 30 min after ethanol exposure, rats presented microscopic-level damage to gastric tissue. Ethanol-induced histopathological injury was caused by severe detachment of the surface epithelium, edema, formation of hemorrhagic and gastric lesions, and an inflammatory process marked by neutrophil infiltration (Fig. 5C). The stomach of rats in the positive control group pretreated with omeprazole (Fig. 5B) maintained an intact histological mucosal structure, with mild edema, inflammatory cell infiltration, and mildly dilated blood vessels in the submucosa compared to ulcer control rats. Rats pretreated with DEAA (Fig. 5D) exhibited significant protection, marked by reduced edema and leukocyte infiltration.

Histological observation confirms the ability of 200 mg/kg DEAA to reduce ethanol-induced gastric damage to the superficial layer of the gastric mucosa and reduce edema and leukocyte infiltration in the submucosa. There was severe submucosal edema and leukocyte infiltration in rats pretreated with L-NAME (Fig. 5E1) alone, and in rats pretreated with both L-NAME and the DEAA (Fig. 5E2), compared to the negative control (Fig. 5C). In rats pretreated with glibenclamide (Fig. 5F1), there was a moderate disruption of the surface epithelium and significant leukocyte infiltration and congestion of blood vessels in the submucosal layer compared to the vehicle-treated negative control rats. Glibenclamide in the presence of the DEAA (Fig. 5F2) significantly reduced edema, leukocyte infiltration, and blood vessel congestion in the submucosa compared to glibenclamide alone. Indomethacin administration in the presence of the DEAA (Fig. 5G2) significantly reduced leukocyte infiltration and blood vessel congestion in the submucosa compared to indomethacin alone (Fig. 5G1).

Evaluation of the percentage of ulceration

Fig. 6 shows the mechanisms of the gastroprotective effect of the DEAA. Pretreatment with the DEAA in the presence of L-NAME, glibenclamide, and indomethacin significantly reduced the ethanol-induced ulcerative injuries (9.16±1.12, 11.16±1.29, 5.46±1.93%, respectively) compared to the groups treated with L-NAME, glibenclamide, and indomethacin without DEAA

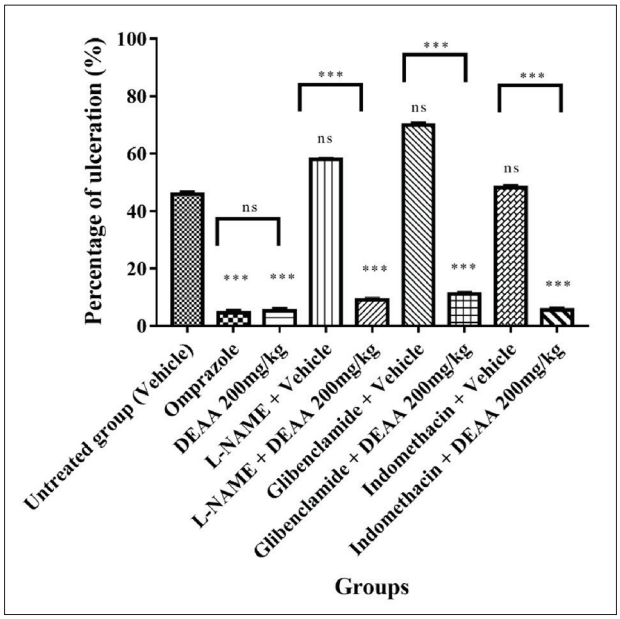

Fig. 6. Gastroprotective effect of 200 mg/kg DEAA in the presence of L-NAME, glibenclamide, and indomethacin. The results are expressed as the means±SEM; ns – no significant difference, n=5. ***P<0.001.

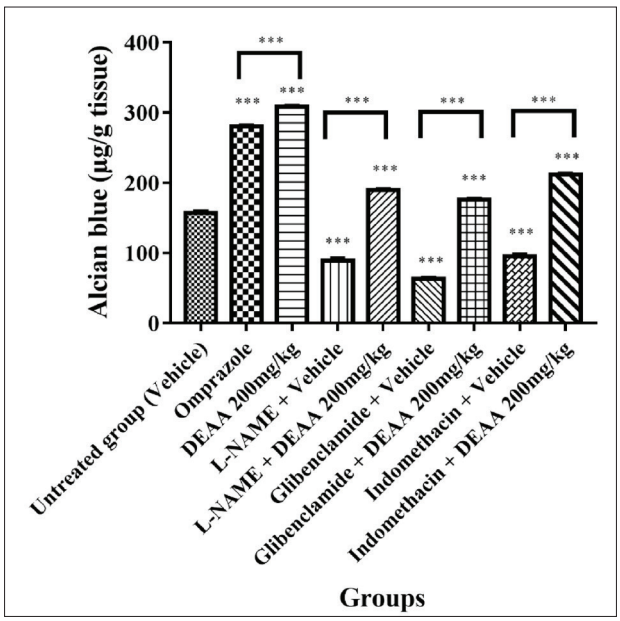

Fig. 7. Evaluation of gastric wall mucus adhesion with and without 200 mg/kg DEAA in rats pretreated with L-NAME, glibenclamide, and indomethacin. The results are expressed as the means±SEM; n=5, ***P<0.001

pretreatment (57.92±1.17, 69.92±2.17, 48.16±1.82%, respectively). There was, however, a significant difference between the gastroprotective effects of groups pretreated with L-NAME, glibenclamide, and indomethacin in the absence of DEAA compared to the omeprazole group and the group that received DEAA.

Determination of adhered mucus to gastric wall

The effects of DEAA on the gastric mucus content are shown in Fig. 7. When DEAA was combined with L-NAME, glibenclamide, and indomethacin, the production of gastric mucus content was increased (190±2.01, 176±2.11, 212±2.61 μg/g, respectively) compared to the vehicle of each group (89.43±4.12, 63.12±2.54, 95.65±3.12 μg/g tissue). The elevated gastric mucus content exhibited a substantial protective effect against ulceration.

Evaluation of *in vivo* antioxidant activity

Table 8 shows the impact of the DEAA on CAT and SOD activities, as well as MDA levels in the stomach tissue of rats pretreated with various substances for ethanol-induced gastric mucosal lesions. In the assessment of superoxide dismutase (SOD) activity, statistical analysis showed that all groups treated with the DEAA exhibited a significant increase in SOD activity (1.309±0.11 IU/min/μg of total protein for the DEAA, 1.204±0.32 IU/min/μg of total protein for indomethacin+DEAA, 1.138±1.38 IU/min/μg of total protein for glibenclamide+DEAA, and 1.008±1.01 IU/min/μg of total protein for L-NAME+DEAA) compared to the vehicle-treated animal groups (0.821±0.78; 0.712±0.27; 0.613±0.54; 0.454±1.43 IU/min/μg of total protein for indomethacin+vehicle; untreated group; glibenclamide+vehicle; L-NAME+vehicle, respectively.

Analysis of CAT activity revealed significant decreases in all DEAA untreated groups as follows: 1.12±0.89, 1.25±1.33, 1.43±0.67, and 1.98±0.65 μmol/s/μg of total protein in L-NAME+vehicle; glibenclamide+vehicle; indomethacin+vehicle; untreated group, respectively. L-NAME+DEAA; glibenclamide+DEAA; indomethacin+DEAA; DEAA, and omeprazole (20 mg/kg) significantly reversed the ethanol-induced decrease in CAT activity, with values of 2.08±2.13, 2.31±1.35, 2.65±0.07, 2.74±0.29, and 2.92±0.21 μmol/s/μg of total protein, respectively.

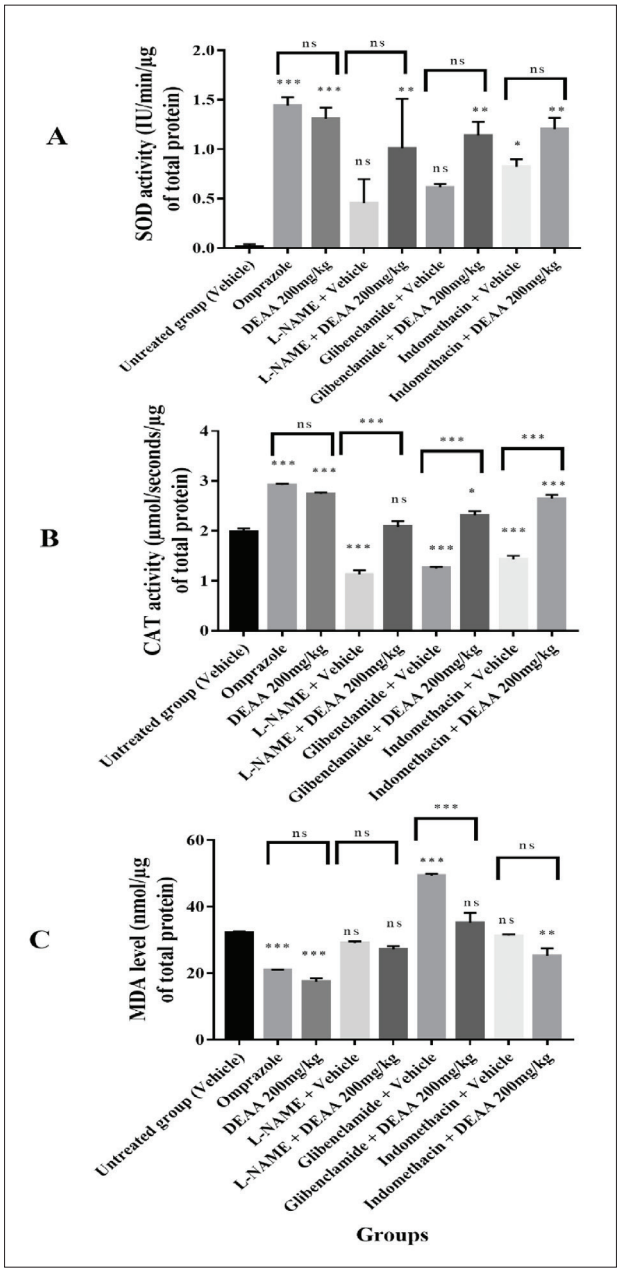

Fig. 8. Evaluation of the absence and presence of 200 mg/kg DEAA in rats pretreated rats with different pharmacological substances on **A**, **B**, **C** – SOD, CAT activities, and MDA levels, respectively.

Measurement of lipid peroxidation

The ethanol groups in the absence of the DEAA showed an increase in MDA levels: 49.32±0.53 nmol/μg of total protein for glibenclamide+vehicle, 32.16±0.41 nmol/μg of total protein for the untreated group, and 31.28±0.33 nmol/μg of total protein for the indomethacin+vehicle group. The groups that received the DEAA and the

positive control attenuated the ethanol-induced damage. The DEAA prevented the increase in lipid peroxidation more effectively (17.45±0.96 nmol/μg of total protein) than was observed in the positive control (omeprazole at 20 mg/kg, 21.03±0.09 nmol/μg of total protein).

DISCUSSION

This study aimed to explore the gastroprotective effects of the decocted extract of *Anabasis articulata* (DEAA) and its potential mechanisms of action in ethanol-induced gastric ulcers in rats. To our knowledge, there have been no previous studies investigating the gastroprotective properties of the DEAA or its interaction with pharmacological inhibitors. Our results showed three important findings: (i) the DEAA exhibited significant gastroprotective effects at a dose of 200 mg/kg, effectively reducing gastric ulceration; (ii) the gastroprotective effects of the DEAA were independent of KATP channels, COX pathways, and NO synthesis, as demonstrated by pharmacological inhibition tests; (iii) the DEAA's mechanism of action involved enhancing antioxidant enzyme activity, increasing mucus secretion, and reducing inflammation by inhibiting neutrophil infiltration, thereby providing substantial protection to the gastric mucosa. In this study, we utilized a rat model of ethanol-induced gastric ulcer to evaluate the potential antiulcer effects of the tested compounds. Ethanol is known to be a major cause of gastric ulcers in humans, and this model is frequently used in research to study gastroprotective agents. Gastric ulceration, a benign lesion of the mucosal epithelium caused by excessive acid secretion, is a key feature of this model, which serves as a practical tool for investigating the effectiveness of antiulcer drugs [33].

In our study, ulceration percentages were observed at 5.16±0.33% following administration of 200 mg/kg of the DEAA. In contrast, the negative control group showed a much higher ulceration rate of 45.92±1.87%, while the omeprazole-pretreated group exhibited only 4.54±2.22%. These results suggest that the DEAA provides significant protection against ethanol-induced gastric lesions. Ethanol induces mucosal microcirculatory disruptions and ischemia, generates free radicals, and releases endothelial factors, which contribute to its ulcerogenic effects [34]. The increase in ulcer indices observed in rats treated with ethanol may result from free radical production or suppression of prostaglandin synthesis [35]. Flavonoids are known to enhance mucosal prostaglandin levels, which are critical for gastroprotection. Lowered prostaglandin levels lead to decreased protection and increased gastric output, facilitating ulcer formation [36].

The complex etiology of gastric ulcers necessitates pharmacological agents targeting multiple pathways to understand their mechanisms and develop new treatments. Rats pretreated with L-NAME, glibenclamide, and indomethacin were used to investigate the effects of a DEAA on ethanol-induced gastric lesions. Ethanol directly contacting stomach mucosa can result in damage due to its irritant properties, causing hemorrhagic lesions in the gastric lining as reported in previous studies [24]. The gastric mucosa defends itself by maintaining blood flow, secreting mucus, and promoting cell growth to preserve integrity during exposure [37,38]. Endogenous prostaglandins enhance mucosal protection by stimulating the production of NO, hydrogen sulfide, and carbon monoxide, all of which are involved in maintaining mucosal integrity and promoting gastroprotection [39].

NO plays a crucial role in the gastrointestinal tract by regulating blood flow, mucus secretion, mast cell activity, and smooth muscle tone [40]. Our findings suggest that the DEAA (200 mg/kg) prevents the inhibition of NO production caused by L-NAME treatment, highlighting the importance of NO in the gastroprotection provided by the DEAA. Furthermore, the DEAA offered protection against the effects of glibenclamide, a KATP channel blocker that inhibits prostaglandin generation. Prostaglandins are essential for regulating mucosal blood flow and enhancing epithelial resistance to cytotoxic injury [41]. As an NSAID, indomethacin primarily induces gastric injury by inhibiting COX1 isozymes, thereby reducing prostaglandin production [42]. The results indicated that the DEAA (200 mg/kg) significantly protected the stomach from lesions caused by ethanol and indomethacin. This protective effect was not hindered by pharmacological pretreatments with glibenclamide, indomethacin, or L-NAME.

Ethanol-induced gastric lesions are characterized by inflammation, oxidative stress, free radical production, and apoptosis [43]. Ulcer indices, such as pH and lesion counts, serve as indicators of gastric

injury caused by toxins. An increase in gastric acidity, indicated by a lower pH, is associated with greater gastric damage [44]. Our findings are consistent with this, as both the pH and lesion numbers decreased following DEAA treatment, suggesting a protective effect against ethanol-induced gastric damage. The imbalance between free radical production and scavenging abilities may contribute to the observed decrease in gastric acidity [45].

The connection between ROS and ethanol-induced gastric mucosal damage has been well established. Increased ROS contribute to oxidative injury, cell death, and epithelial damage [46]. ROS also reduce prostaglandin synthesis, impair mucus formation, and increase gastric acid output, all of which exacerbate ulcer formation [47]. Gastric mucus, composed of mucin glycoproteins, is the first line of defense against gastric acid [48]. Our study showed that ethanol treatment significantly reduced gastric mucus content, but DEAA administration (200 mg/kg) enhanced mucus production, contributing to its gastroprotective effects. Increased mucus secretion helps maintain the gastric mucosa's integrity by reducing the back diffusion of hydrogen ions, improving buffering capacity, and minimizing friction during peristalsis [49].

Histological examination confirmed that ethanol treatment caused significant hemorrhagic lesions, including epithelium disintegration, necrosis, edema, and blood vessel congestion. In contrast, DEAA treatment (200 mg/kg) reversed these changes, significantly reducing the gastric ulcer area compared to the negative control. These findings align with previous reports indicating that ethanol induces gastric mucosal damage by disrupting cellular membranes and damaging microvessels, leading to bleeding and necrosis [44, 50].

Our results indicate that the gastroprotective effect of the DEAA may be attributed to its high content of phenolic compounds and flavonoids. These compounds enhance prostaglandin levels, which are crucial for maintaining gastric mucosal integrity and reducing histamine release from mast cells [10]. Ethanol induces oxidative stress by generating ROS, leading to lipid peroxidation and protein oxidation, both of which contribute to ulcer formation [51]. The generation of free radicals plays a key role in gastric damage, while inflammatory mediators further contribute to tissue disruption [45]. Gastric lesions, hemorrhage, and necrosis arise due to blood flow stasis and microvascular disruption, subsequently leading to neutrophil infiltration [52].

Previous studies have shown a connection between ethanol-induced gastric damage and ROS generation [53], with inflammatory mediators playing a central role in the damage process. Our findings are consistent with these studies, showing that ROS-induced oxidative stress contributes to both acute and chronic ethanol-induced gastric ulcers. Scavenging free radicals is a key mechanism in ulcer healing, with enzymes such as SOD, CAT, and GPx playing vital roles in protecting gastric tissue [54]. Excessive ROS generation depletes these antioxidants, leading to lipid peroxidation and further damage. MDA, a product of lipid peroxidation, serves as a key indicator of oxidative stress and tissue damage [55].

Concurrent treatment with L-arginine mitigated the damaging effects of ethanol and pharmacological agents (L-NAME, glibenclamide, and indomethacin), which resulted in increased MDA levels and decreased antioxidant enzyme activity. However, DEAA (200 mg/kg) treatment resulted in a reduction in gastric mucosal lesions, lowered MDA levels, and improved antioxidant enzyme activity, consistent with previous reports [56]. The use of NOS inhibitors, such as L-NAME, exacerbates gastric mucosal damage and impairs blood flow [57].

Several studies have described the role of KATP channels in gastric protection [58], and the findings indicate that KATP channels may contribute to the gastroprotective effect of the DEAA. Pretreatment with the KATP channel blocker glibenclamide diminished the gastroprotective effect of the DEAA, indicating that KATP channels are likely involved in its protective mechanism. NSAIDs, which inhibit prostaglandin production, are known to cause gastric ulcers [59]. Our study confirmed that prostaglandins play a crucial role in the gastroprotective activity of the DEAA, as evidenced by the inhibition of ulceration following pretreatment with L-NAME, glibenclamide, and indomethacin.

CONCLUSION

This study is the first to comprehensively evaluate the gastroprotective effects of a DEAA in ethanol-induced gastric ulcers in rats, focusing on three key mechanisms: KATP channels, cyclooxygenase pathways, and nitric oxide synthesis. The DEAA significantly reduced the severity of gastric lesions, enhanced gastric mucus production, and improved tissue integrity without affecting gastric acidity. Mechanistically, the protective effects of the DEAA were not mediated by nitric oxide, ATP-sensitive potassium channels, or prostaglandins, as confirmed by pharmacological inhibition using glibenclamide, indomethacin, and L-NAME. Additionally, the DEAA demonstrated antioxidant properties by increasing SOD and CAT activities, reducing MDA levels, and inhibiting neutrophil infiltration, as evidenced by decreased myeloperoxidase activity. These findings suggest that DEAA could be a cost-effective, safer natural alternative for preventing and treating gastric ulcers compared to conventional treatments. This study reveals the multi-targeted mechanisms of DEAA, suggesting its potential uses in functional foods and pharmaceuticals.

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SUPPLEMENTARY MATERIAL

RESEARCH DATASET

The raw data underlying this article is available as an online supplementary research dataset:
https://www.serbiosoc.org.rs/NewUploads/Uploads/Makhlouf%20et%20al_Research%20Dataset.pdf