



Integrating network pharmacology and *in vivo* experimentation to investigate the antiulcer mechanism of *Coix lacryma-jobi*

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Abstract

Coix lacryma-jobi L., Poaceae, is traditionally used for the treatment of gastrointestinal disorders, yet its gastroprotective and ulcer-healing properties remain poorly explored. This study, integrating network pharmacology, *in vivo* assays, and molecular docking, demonstrates that the hydroalcoholic extract of *C. lacryma jobi* exerts its effects through convergent antioxidant, anti-inflammatory, and cytoprotective pathways. In ethanol-induced acute gastric injury, extract of *C. lacryma jobi* (100 mg/kg) prevented the depletion of reduced glutathione levels and decreased myeloperoxidase activity. Histological and histochemical analyses confirmed preservation of gastric mucosal structure. In addition, the reversal of extract of *C. lacryma jobi* protective effects by pretreatment with *N*-ethylmaleimide, indomethacin, and *N*ω-nitro-L-arginine methyl ester (L-NAME) indicates the involvement of sulfhydryl groups, prostaglandins, and nitric oxide pathways in its gastroprotective mechanism. In chronic acetic acid-induced ulcer, extract of *C. lacryma jobi* not only reduced the ulcerated area but also produced clear histological improvement, characterized by re-epithelialization, reorganization of mucosal layers, and partial restoration of glandular architecture. These activities are likely related to decreased levels of reduced glutathione, lipid hydroperoxides, and diminished myeloperoxidase activity. Network pharmacology analyses indicated that the bioactive constituents of extract of *C. lacryma jobi* primarily exert their effects by modulating inflammatory signaling pathways. Mass spectrometry identified polyphenols, among which quercetin and kaempferol exhibited the highest binding affinity to myeloperoxidase and to IL-1β in docking simulations. Furthermore, no evidence of toxicity was observed *in vivo* and *in silico* assays for extract of *C. lacryma jobi* and its bioactive compounds. Collectively, these findings demonstrate that extract of *C. lacryma jobi* exerts multi-target gastroprotective and healing effects, supporting its potential as a natural antiulcer agent.

Keywords Gastroprotective · Ulcer healing · Molecular docking · Bioactive compounds · Polyphenols

Introduction

Despite the widespread use of proton pump inhibitors (PPIs) since their clinical introduction over three decades ago, peptic ulcers remain a persistent therapeutic challenge (Joshi et al. 2024). Gastric ulceration is classically attributed to a disruption in the balance between mucosal defense and aggressive luminal factors. Protective mechanisms, including bicarbonate secretion, mucosal mucus integrity, antioxidant systems, and adequate mucosal perfusion, are often compromised. Conversely, the upregulation of deleterious elements, including gastric acid, pepsin activity, and oxidative stress, contributes to mucosal injury and ulcerogenesis (Périco et al. 2020). Moreover, well-established etiological factors encompass *Helicobacter pylori* infection, excessive

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alcohol consumption, prolonged use of non-steroidal anti-inflammatory drugs, sedentary behavior, and psychosocial stressors (Sostres et al. 2014).

In addition, numerous studies have highlighted the negative impacts associated with the prolonged use of antisecretory therapies, particularly PPIs, including adverse effects such as impaired absorption of essential nutrients, alterations in the gut microbiota, and an increased risk of gastrointestinal infections (Lehault and Hughes 2017), reduced intestinal absorption of vitamin B12, magnesium, calcium, and iron, which may lead to clinically significant deficiencies (Ito and Jensen 2010; Lombardo et al. 2021). Moreover, chronic acid suppression alters the gastrointestinal environment, disrupting gut microbiota composition and promoting dysbiosis, thereby predisposing individuals to systemic inflammation and metabolic disturbances (Imhann et al. 2016). Additionally, evidence suggests that discontinuation of PPIs may trigger rebound acid hypersecretion, increasing the likelihood of gastric ulcer recurrence and complicating disease management (Somensi et al. 2022).

Natural plant extracts have demonstrated significant anti-inflammatory and antiulcer properties, positioning them as promising candidates for developing novel therapeutic agents. Numerous studies have shown that bioactive compounds, including flavonoids, alkaloids, tannins, and terpenoids, exert protective effects on the gastric mucosa by modulating key inflammatory pathways and enhancing antioxidant defenses (Boakye-Yiadom et al. 2021; Figueiredo 2023).

Coix lacryma-jobi L., is a grass belonging to the Poaceae family, with a genus comprising approximately 30 species. Native to Asia and widely distributed elsewhere, *C. lacryma-jobi* is known in different regions as “lágrimas-de-nossa-senhora” or “capim-miçanga”. The traditional use indicates the preparations of the plant for the treatment of non-alcoholic fatty liver disease (Chiang et al. 2020; Ma et al. 2022), type 2 diabetes mellitus, metabolic syndrome (Ha et al. 2010), polycystic ovarian syndrome (Wu et al. 2014), liver fibrosis (Wang et al. 2022), and gastrointestinal disturbances (Acharya et al. 2024). Pharmacological investigations indicate that the therapeutic potential of *C. lacryma-jobi* is closely linked to its major bioactive constituents, including coixenolide, coixol, flavonoids, phenolic acids, and polysaccharides. Coixol contributes to anti-inflammatory activity by modulating key signaling pathways and downregulating cytokines such as TNF- α and IL-6 (Zhang et al. 2019).

However, elucidating the effects of phytoconstituents is difficult due to their multiple targets, pathways, and modes of action. The aforementioned features have severely constrained the internationalization and modernization of herbal medicines (Gan et al. 2019). In this context, network pharmacology has emerged as an effective approach for observing the interactions and effects of different compounds on

disease networks (Li and Zhang 2013; Hao and Xiao 2014). Therefore, in this study, we investigated the gastroprotective and gastric-healing effects of *C. lacryma-jobi* in rodent models and complemented these findings with *in silico* analyses to better understand the molecular interactions and biological pathways potentially associated with these effects. By integrating *in vivo* pharmacological evaluation with computational analyses, this study provides a broader and more comprehensive investigation of the therapeutic potential of *C. lacryma-jobi*, contributing not only to the understanding of its possible mechanisms of action but also to strengthening the scientific evidence supporting the use of this species as a promising source of bioactive compounds for the management of gastric disorders.

Materials and methods

Plant material

The botanical material of *Coix lacryma-jobi* L., Poaceae, was collected in March 2024 in the municipality of Fraiburgo, Santa Catarina (SC), Brazil (27.0236° S; 50.9223° W), and identified by Nádia Lucas Antunes. A specimen of the plant was deposited in the Herbarium of the State University of Santa Catarina (Lages, SC), under registration number #12179.

Extract production

The leaves from *C. lacryma-jobi* (100 g) were dehydrated (20 ± 5 °C), ground in a knife mill, and extracted by maceration (7 days) in 70% ethanol (1000 ml). The extract thus obtained was filtered (Büchner), evaporated under reduced pressure at 40 °C, lyophilized, weighed (15.72 g; yield 15.72%), and identified as hydroalcoholic extract of *C. lacryma-jobi* (HECL) (Farmacopeia Brasileira 2024).

Phytochemical study

HPLC and Mass spectrometry analysis

The identification and quantitative analysis of polyphenols in HECL were performed using ESI-MS, following the procedure reported by Arruda et al. (2018). The determination and quantitative assessment of the standard phenolic compounds were performed on a Shimadzu LC-MS/MS-2020 system fitted with a Shim-pack GISS C18 column (4.6 mm $\times$ 150 mm, 3 μ m). A gradient elution system was applied, consisting of 0.3% formic acid in water (solvent A) and methanol (solvent B). The program initiated at 5% B at 1 min, rose to 60% at 4 min, progressed to 70% at 7 min, and then reached 100% at 10 min, where it was maintained

through 15 min. The gradient was then decreased to 5% B at min 17.5 and maintained until min 21, with a constant flow rate of 0.4 ml/min. The ESI source temperature was set to 350 °C, and the nebulizing gas flow was adjusted to 1.5 l/min. Prior to sample analysis, calibration curves were generated for each of the four phenolic standards, all of which exhibited correlation coefficients (*r*) exceeding 0.99.

Network pharmacology studies

Prediction of molecular targets

Target prediction for HECL compounds was performed using SuperPred (<http://prediction.charite.de/>) (Nickel et al. 2014) and SwissTargetPrediction (<http://www.swisstargetprediction.ch/>), with *Homo sapiens* selected as the target species. Predicted targets were integrated and standardized using the UniProt database (<https://www.uniprot.org/>) (Gan et al. 2019). Gastric ulcer-related target genes were retrieved from the GeneCards database (<https://www.genecards.org/>). Common targets between HECL constituents and gastric ulcer were identified using the Venn Diagram Online Tool v2.1.0 (<https://bioinfo.cnb.csic.es/tools/venny/>) (Omoboyede et al. 2023).

Construction of protein–protein interaction network

The STRING database (<https://string-db.org/>) was used to construct the protein–protein interaction (PPI) network for shared targets between HECL compounds and gastric ulcer, applying a medium-confidence threshold of 0.4. The resulting network was subsequently visualized in Cytoscape v3.9.1, and network parameters were analyzed using the CytoHubba plugin (Kohl et al. 2011).

Enrichment analysis

ShinyGo v0.76 (<https://bioinformatics.sdstate.edu/go/>) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for the key targets. In this study, the top 20 enriched KEGG pathways were presented, and only those with an FDR < 0.05 were retained for further analysis. In addition, Cytoscape v3.9.1 was used to construct a compound–target–pathway network.

In vivo study

Animals

Female Wistar rats (*Rattus norvegicus*, 200–250 g) and female Swiss mice (*Mus musculus*, 25–30 g) were obtained from the Central Animal House of Unochapecó and housed

in polypropylene cages under controlled conditions (22 ± 2 °C, 12 h light/dark cycle), with ad libitum access to standard chow and water. Animals were fasted for 12 h prior to experiments. Previous evidence indicates that estrous cycle-related hormonal variations do not affect antiulcer outcomes in female rodents (Oliveira et al. 2022; Miorando et al. 2025). All procedures complied with the International Principles for Biomedical Research Involving Animals (National Research Council 2011) and were approved by the Institutional Animal Ethics Committee of Unochapecó (protocol no. 006/CEUA/2024).

Evaluation of acute toxicity in vivo

Acute oral toxicity of HECL was evaluated in female mice according to OECD Guideline 423 (Gothe et al. 2023). Animals (*n* = 3/group) received a single oral dose of vehicle (distilled water) or HECL (2000 mg/kg) and were observed for 14 days for clinical or behavioral signs of toxicity. At study termination, animals were euthanized, and the spleen, heart, kidneys, liver, lungs, and uterus were excised, weighed, and expressed as relative organ weights using the formula (organ weight/body weight) × 100, as previously described (Da Silva et al. 2015).

Evaluation of gastroprotective activity

Ethanol-induced acute ulcers

The rats were randomly assigned to different groups (*n* = 6) and treated orally (gavage) with vehicle (water, 1 ml/kg), omeprazole (20 mg/kg, as a positive control), or HECL (3, 30, or 100 mg/kg), in addition to a naive group. The selected HECL doses were defined using a logarithmic dose-progression strategy commonly employed in experimental pharmacology studies involving medicinal plant extracts and gastric injury models, allowing evaluation of low, intermediate, and high exposure levels and identification of possible dose-dependent effects (Pineda-Peña et al. 2023). Given the limited information available on the biological activity of *C. lacryma-jobi* in gastric disorders, this dose range was deemed appropriate for a comprehensive preclinical evaluation of its gastroprotective and gastric-healing potential. One hour after treatment administration, gastric ulcers were induced orally with ethanol (1 ml/kg), as described by Takeuchi (2012). The naive group was not subjected to ulcer induction and received only vehicle (water, 1 ml/kg). The animals were euthanized 1 h after lesion induction, and the stomachs were removed, opened along the greater curvature, washed, and the gastric lesions quantified using EARP® software.

Investigation of the gastroprotective mechanism associated with the HECL

a) Evaluation of acute gastric ulcers induced by acidified ethanol in mice pretreated with inhibitors of nitric oxide (L-NAME) or sulfhydryl groups (NEM).

Mice were allocated into groups ($n = 12$) and pretreated subcutaneously with L-NAME (70 mg/kg), NEM (10 mg/kg), or saline (10 ml/kg). After 30 min, animals received vehicle (water, 1 ml/kg) or HECL (100 mg/kg, minimum effective dose) orally. Gastric lesions were induced 1 h later by acidified ethanol (60% ethanol in 0.3 M HCl; 10 ml/kg, *p.o.*) (Arrieta et al. 2003; Leite et al. 2009). One hour after ulcer induction, animals were euthanized, stomachs were excised, opened along the greater curvature, and ulcerated areas were quantified as described previously.

b) Pyloric ligation: evaluation of pH, volume, acidity, and peptic activity.

Fasted female rats were allocated into four groups ($n = 6$) and subjected to pylorus ligation under isoflurane anesthesia according to the method of Shay (1945). Animals received intraduodenal saline, omeprazole (20 mg/kg), or HECL (100 mg/kg). After 4 h, stomachs were excised, gastric juice was collected and centrifuged, and volume, pH, and total acidity were determined by titration. Pepsin activity was assessed using the Anson (1938) method and expressed as micromoles of tyrosine released per milliliter over 4 h ($\mu\text{M}/\text{ml}/4\text{ h}$).

Evaluation of gastric healing activities

Female rats were anesthetized with isoflurane (3%) and subjected to laparotomy for gastric exposure. Chronic gastric ulcers were induced by topical application of 80% acetic acid (500 μl) to the serosa using a 6-mm plastic cylinder, according to Okabe et al. (1971). After 1 min, the acid was removed, the area rinsed with saline, and the abdomen sutured. Following recovery, animals were randomized into groups ($n = 6$). Oral treatments with vehicle (water, 1 ml/kg), omeprazole (20 mg/kg), or HECL (100 mg/kg) were initiated on day 2 post-induction and administered twice daily for 7 days; a naive group received vehicle only. At the end of treatment, animals were euthanized, stomachs excised, and ulcerated areas quantified using a graduated scale.

Histological and histochemical evaluation

Gastric tissues were fixed for 24 h in a solution containing ethanol (85%), formaldehyde (10%), and acetic acid (5%), then paraffin-embedded, sectioned at 5 μm , and processed through graded alcohol and xylene. Sections were stained with hematoxylin and eosin, and glycoproteins (mucins)

were visualized by periodic acid–Schiff (PAS) staining and quantified using ImageJ software (Pereira et al. 2013).

Quantification of inflammatory and oxidative stress parameters

Subcellular fraction preparation and total protein measurement

Gastric mucosa from acetic acid-induced lesions was washed and homogenized in 200 mM potassium phosphate buffer (pH 6.5), as described by Da Silva et al. (2015). The homogenate was used to determine reduced glutathione (GSH) and lipoperoxides (LOOH). After centrifugation ($9000 \times g$, 20 min), the supernatant was used to assess glutathione *S*-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activities, while the pellet was reserved for myeloperoxidase (MPO) analysis. Total protein content was determined by the Bradford method using bovine serum albumin as the standard.

Determination of reduced glutathione and lipoperoxides levels

Reduced glutathione levels were determined as described by Sedlak et al. (1968). Tissue homogenates were deproteinized with trichloroacetic acid, centrifuged, and the supernatant reacted with 5,5'-dithiobis(2-nitrobenzoic acid) in Tris buffer (pH 8.9). Absorbance was measured at 415 nm, and GSH concentrations were calculated from a standard curve (1.25–10 $\mu\text{g}/\text{ml}$) and expressed as $\mu\text{g}/\text{mg}$ tissue. Lipoperoxides were quantified according to Jiang et al. (1991), with minor modifications. Methanol-treated homogenates were centrifuged, and the supernatant reacted with a xylene orange-based reagent containing butylated hydroxytoluene, ferrous sulfate, and sulfuric acid. After incubation, absorbance was measured at 560 nm, and LOOH levels were calculated using an extinction coefficient of $46.6\text{ }\mu\text{M}^{-1}\text{ cm}^{-1}$ and expressed as mmol/mg tissue.

Determination of SOD, CAT, and GST enzyme activities

Superoxide dismutase activity was determined according to Marklund and Marklund (1974) by monitoring pyrogallol autooxidation at 405 nm, and results were expressed as units per milligram of protein. Catalase activity was assessed following Aebi (1984) by measuring the decomposition of hydrogen peroxide at 240 nm and expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein. Reduced glutathione activity was quantified according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as substrate, with absorbance monitored at 340 nm and results expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein.

Activity of myeloperoxidase

Myeloperoxidase activity was determined from the pellet fraction as described previously, following the methods of Bradley et al. (1982) and Young et al. (1989). Samples were resuspended in potassium phosphate buffer (80 mM, pH 5.4) containing hexadecyltrimethylammonium bromide

and centrifuged. Myeloperoxidase activity was quantified by monitoring 3,3',5,5'-tetramethylbenzidine oxidation in the presence of hydrogen peroxide at 620 nm, and results were expressed as mO.D./mg protein.

in silico assays

Molecular docking simulations

Molecular docking was performed to investigate the binding of HECL phytoconstituents to interleukin-1 β (IL-1 β) and myeloperoxidase (MPO). Protein structures were obtained from the Protein Data Bank (PDB IDs: 1ITB and 1DNU), and protonation states were assigned at pH 7.4 using APBS-PDB-2PQR (Jurrus et al. 2018). Proteins were prepared with AutoDockTools, while ligands retrieved from PubChem were protonated (pH 7.4), energy-minimized using the MMFF94 force field in Avogadro and converted to PDBQT format via Open Babel. Docking simulations were conducted using AutoDock Vina 2.4, with grid boxes defined in PyMOL 2.4.0. Binding interactions were analyzed by residue proximity, and docking accuracy was validated by redocking of co-crystallized ligands (RMSD $\leq$ 2.0 Å). The best-scoring poses were selected and visualized in PyMOL (Steffler et al. 2026).

Drug-likeness evaluation

Drug-likeness and oral bioavailability of HECL constituents were predicted based on Lipinski's Rule of Five, using thresholds for log P, molecular weight, and hydrogen bonding capacity to estimate pharmacokinetic suitability.

ADMET prediction

Pharmacokinetic and toxicological properties of HECL compounds were predicted using the PreADMET platform, including assessments of intestinal absorption, blood–brain barrier permeability, plasma protein binding, cytochrome P450 inhibition, and mutagenic potential.

Statistical analysis

Results are expressed using GraphPad Prism 8.0.1 software (GraphPad Software, San Diego, CA, USA). The Kolmogorov–Smirnov normality test was used, and the data were represented as the mean $\pm$ standard error of the mean. Differences between means were assessed using one- or two-way analysis of variance (ANOVA) followed by Tukey's test. A p -value < 0.05 was considered significant in all experiments.

Results

Chemical analysis of the hydroethanolic extract of *Coix lacryma-jobi*

Chromatographic and spectrometric analysis (HPLC–MS) of HECL revealed the presence of four compounds:

quercetin, kaempferol, caffeic acid, and *p*-coumaric acid (Figure S1). Structural elucidation was performed by comparing the retention times and mass fragmentation patterns of the compounds with those of authentic standards and published data. The annotated polyphenolic constituents are markers of *C. lacryma-jobi* and have been reported in related phytochemical studies (Zhang et al. 2019; Huang et al. 2021).

Network pharmacology analyses

The compounds quercetin, kaempferol, caffeic acid, and *p*-coumaric acid present on HECL were combined using a Venn diagram, which revealed 169 targets shared among the polyphenols. Conversely, the set of disease-associated targets comprised 5.824 entries, among which 113 were shared with polyphenols (Figure S2A). Using the STRING platform, a PPI network was constructed, and topological analysis identified IL-6, IL-1 β , ESR1, NFKB1, GSK3B, MAPK3, STAT1, MTOR, CXCR4, and CDK1 as key nodes in the network (Figure S2B). Enrichment analyses indicated that these genes are associated primarily with inflammatory signaling, gastrointestinal disorders, and cancer pathways (Figure S3A–B). A pharmacological network linking the four compounds, 10 relevant targets, and 20 predicted pathways were constructed based on enriched pathways (Figure S3C).

Toxicity *in vivo* of hydroethanolic extract

No clinical manifestations indicative of systemic toxicity or behavioral changes were observed in animals receiving the hydroalcoholic extract of *C. lacryma-jobi* at 2000 mg/kg throughout the 14-day observation period. In agreement with these findings, macroscopic examination of major organs revealed no meaningful differences in relative organ weights when compared with the vehicle-treated controls. The relative masses of the brain, heart, lungs, liver, kidneys, spleen, and uterus were comparable across groups. Collectively, these results demonstrate that oral administration of HECL did not elicit detectable systemic toxicity or disrupt organ homeostasis under the experimental conditions employed (Table S1).

Hydroethanolic extract of *Coix lacryma-jobi* reduces ethanol-induced gastric ulcers

The animals in the naive group, which were not exposed to ethanol and received no pharmacological intervention, maintained gastric mucosal integrity. Ethanol administration induced pronounced ulcerogenic effects, resulting in gastric

mucosal lesions measuring $119.9 \pm 13.85 \text{ mm}^2$ in the vehicle-treated group (Fig. 1A). In contrast, animals pretreated with omeprazole (20 mg/kg, *p.o.*), used as a positive control, showed a significant reduction in lesion area of 54.86%. No significant gastroprotection was observed after administration of HECL at doses of 3 or 30 mg/kg. However, animals pretreated with the hydroalcoholic extract of *C. lacryma-jobi* (100 mg/kg, *p.o.*) showed an even greater reduction in gastric lesions (61.2%; $p < 0.001$) than the ulcerated group, reinforcing its gastroprotective potential. These findings were further supported by macroscopic observations (Fig. 1B).

Pharmacological mechanisms involved in the gastroprotection

Preservation of mucin-type glycoproteins

Histochemical analysis performed with periodic acid–Schiff staining revealed a significant preservation of mucin-like glycoproteins in the gastric mucosa of animals treated with HECL (100 mg/kg), corresponding to an approximately twofold increase relative to the vehicle-treated ulcerated

group ($1.69 \pm 0.34 \text{ pixels} \times 10^6/\text{field}$; $p < 0.01$) (Fig. 2A). Although the omeprazole-treated group (20 mg/kg) showed a tendency toward increased staining intensity, no statistically significant difference was observed compared with the vehicle group using Tukey's multiple comparisons test ($p = 0.1503$). Representative photomicrographs illustrating the distribution and intensity of mucin-like glycoproteins across the treatment groups are presented in Fig. 2B.

Gastroprotective effects in mice treated with L-NAME, NEM, or indomethacin in the ethanol/HCl-induced ulcer model

The gastroprotective effect of HECL was abolished entirely in all three pretreatment conditions. Administration of indomethacin (Fig. 3A), L-NAME (Fig. 3B), and NEM (Fig. 3C) significantly increased the ulcerated area by 274.98, 294.22, and 500.81%, respectively, in comparison to the group pretreated with saline alone ($p < 0.05$ and $p < 0.01$, respectively). These findings suggest that the protective activity of HECL involves the synthesis of nitric oxide (NO), the integrity of sulfhydryl groups, and prostaglandin-mediated pathways, highlighting a multifactorial mechanism of mucosal

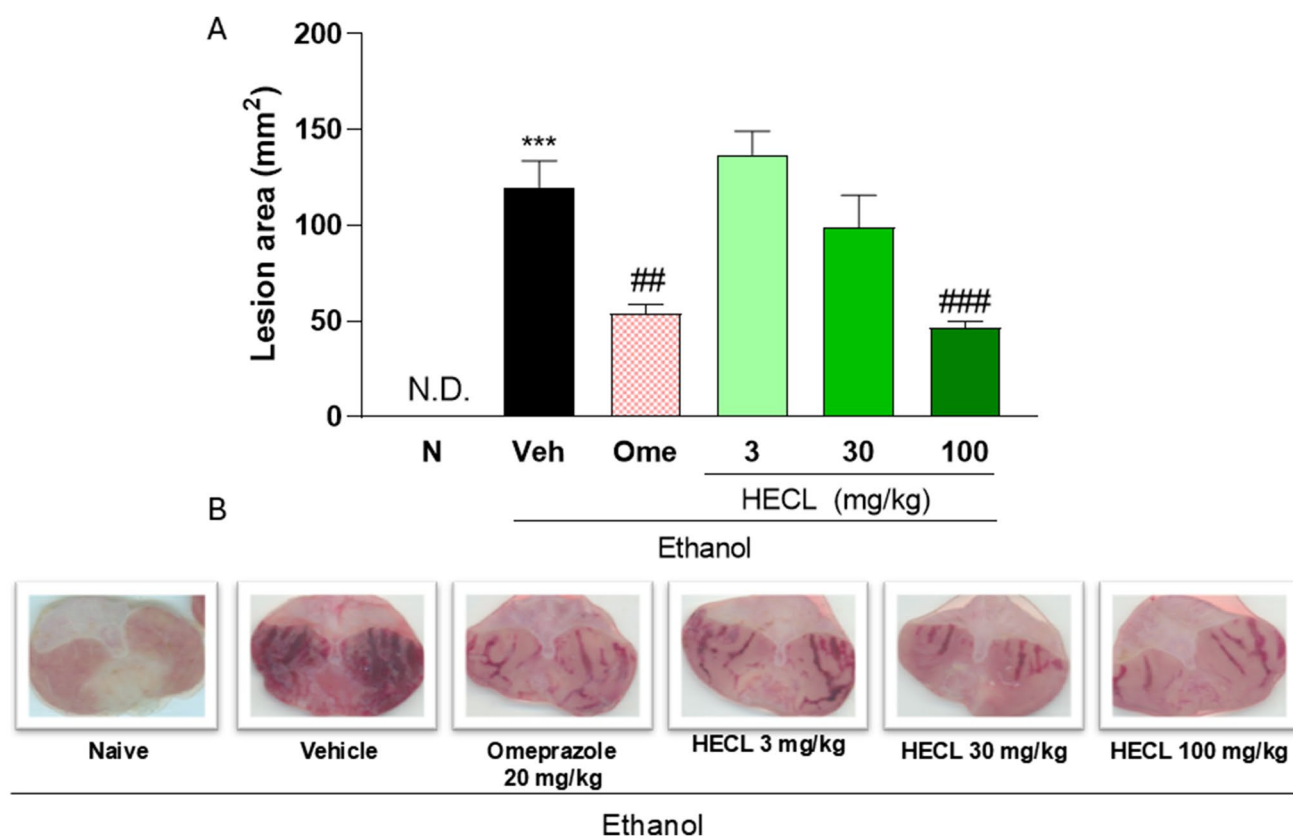
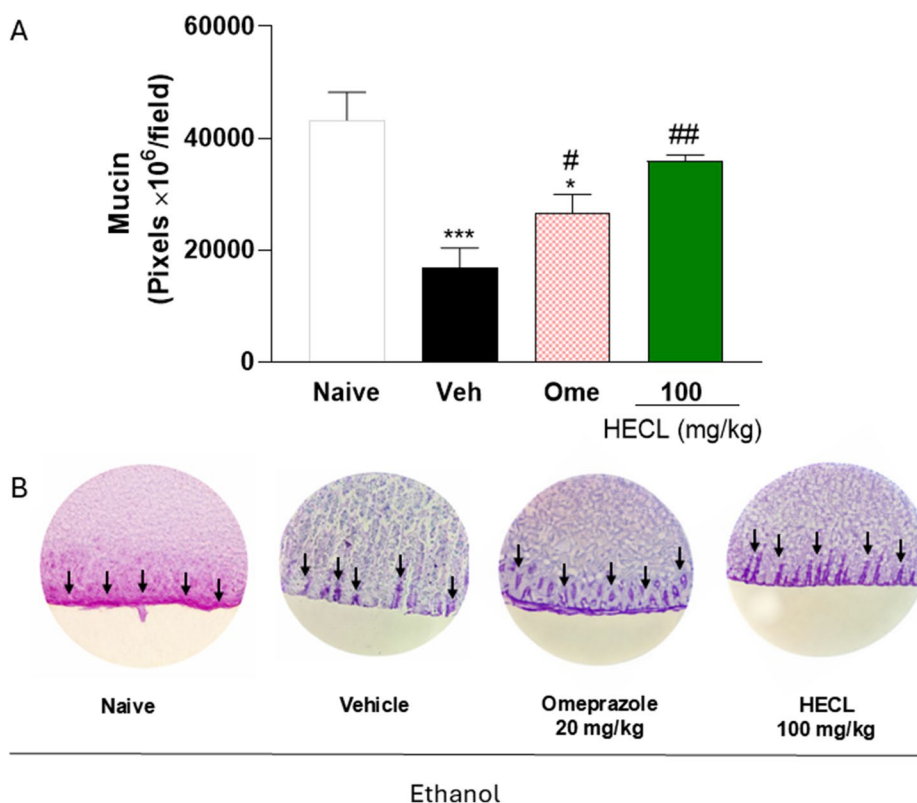


Fig. 1 (A) Effects of hydroalcoholic extract of *Coix lacryma-jobi* (3, 30, or 100 mg/kg) in ethanol-induced ulcers. (B) Representative macroscopic images of each group. One-way ANOVA followed by the Tukey test (mean $\pm$ S.E.M.; $n = 6$). *** $p < 0.001$ compared to the naive

group. ## $p < 0.01$ and ### $p < 0.001$ compared to vehicle group. N.D.: Not detected; N: Naive (not induced); Veh: Vehicle (distilled water); Ome: Omeprazole (20 mg/kg); HECL: hydroalcoholic extract of *Coix lacryma-jobi*

Fig. 2 (A) Effects of hydroalcoholic extract of *Coix lacryma-jobi* (100 mg/kg) in periodic acid-Schiff-staining for mucin-like glycoproteins in ethanol-induced ulcers. (B) Representative microscopic images of naive, vehicle, omeprazole (20 mg/kg), and HECL. Black arrows indicate mucin-like glycoproteins, magnification = 400×. One-way ANOVA followed by Tukey test (mean ± S.E.M.; n = 6). *** $p < 0.001$ compared to naive group; # $p < 0.05$, ## $p < 0.01$ compared to vehicle group. N: Naive (not induced); Veh: Vehicle (distilled water); Ome: Omeprazole (20 mg/kg); HECL: hydroalcoholic extract of *Coix lacryma-jobi*



defense that is likely dependent on endogenous cytoprotective mediators.

Hydroethanolic extract did not change gastric secretion parameters

In the vehicle-treated group, gastric juice volume was 8.03 ± 0.3 ml, with a pH of 4.0 ± 0.83 , total acidity of 23.1 ± 1 Eq[H⁺]/ml/4 h, and peptic activity of 5.3 ± 0.9 μ M tyrosine/4 h. Administration of hydroethanolic extract of *C. lacryma-jobi* (100 mg/kg i.d) did not result in significant changes in any of these parameters compared to the control.

Conversely, omeprazole (20 mg/kg) significantly reduced gastric secretion volume by 61.5% and increased pH to 6.8 (Table S2).

Effects on oxidative stress parameters and inflammatory markers

As shown in Table 1, animals from the naive group exhibited basal antioxidant and inflammatory parameters, with low MPO activity (197.26 ± 3.71 m.D.O/mg protein), preserved GSH levels (71.26 ± 3.71 μ g/mg of tissue), and physiological activities of SOD (0.69 ± 0.01 U/mg of protein),

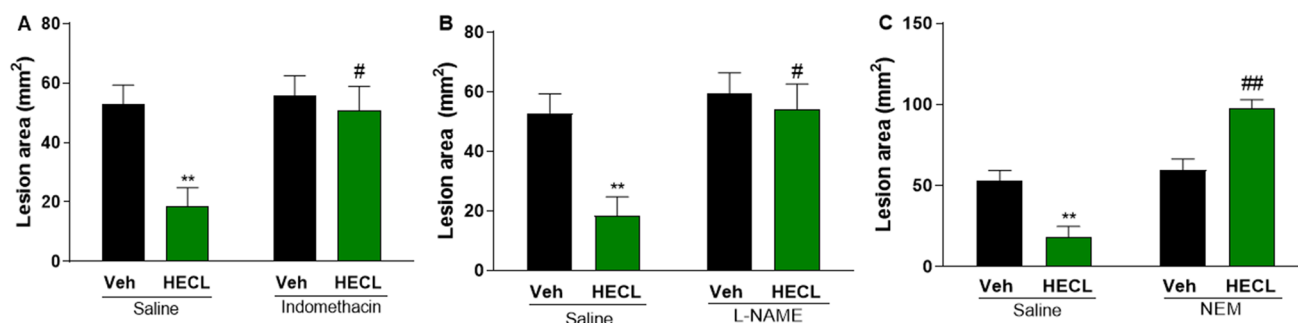


Fig. 3 Effects of hydroalcoholic extract of *Coix lacryma-jobi* (100 mg/kg) in pretreatment with (A) indomethacin, (B) L-NAME, or (C) NEM. Results are expressed as mean ± S.E.M. (n = 6). Two-way

ANOVA followed by Tukey test. ** $p < 0.01$ vs. vehicle-saline group. # $p < 0.05$ and ## $p < 0.01$ vs. HECL treated group. HECL: hydroalcoholic extract of *Coix lacryma-jobi*

Table 1 Effects of the hydroalcoholic extract of *Coix lacryma-jobi* (100 mg/kg) on biochemical parameters in ethanol-induced ulcers. Naive (not induced); vehicle (water); Omeprazole (20 mg/kg)

Treatments	MPO	GSH	SOD	CAT	GST
Naive	197.26 ± 3.71	71.26 ± 3.71	0.69 ± 0.01	661.4 ± 102.4	235.8 ± 27.64
Vehicle (1 ml/kg <i>p.o.</i>)	648.81 ± 54.50 ^{***}	48.11 ± 2.56 ^{***}	1.83 ± 0.23	1160.01 ± 306.1	167.3 ± 9.86
Omeprazole (20 mg/kg, <i>p.o.</i>)	521.03 ± 41.33	42.36 ± 0.74	1.11 ± 0.03	1115.02 ± 248.1	176.9 ± 14.41
HECL (100 mg/kg, <i>p.o.</i>)	410.7 ± 22.37 ^{###}	59.47 ± 1.92 ^{###}	1.18 ± 0.08	605.5 ± 194.1	204.8 ± 14.08

Myeloperoxidase (MPO, mD.O/mg protein); reduced glutathione (GSH, µg/mg of tissue); superoxide dismutase (SOD, U/mg of protein); catalase (CAT, µmol/min/mg of protein) and glutathione S-transferase (GST, mmol/min/mg of protein). Values are expressed as mean ± S.E.M. (n=6). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. ****p* < 0.001 when compared to the Naive group. ###*p* < 0.001 when compared to the vehicle-treated group

CAT (661.4 ± 102.4 µmol/min/mg of protein), and GST (235.8 ± 27.64 µmol/min/mg of protein), indicating intact gastric redox homeostasis. Ethanol-induced gastric injury markedly reduced GSH levels in the vehicle-treated group (48.11 ± 2.56; *p* < 0.001 vs. naive), whereas HECL (100 mg/kg) significantly avoided the depletion of this oxidative stress parameter (59.47 ± 1.92; *p* < 0.001 vs. vehicle). In contrast, omeprazole (20 mg/kg) did not modify GSH levels (42.36 ± 0.74).

Hydroethanolic extract of *C. lacryma-jobi* (100 mg/kg) did not significantly alter SOD, CAT, or GST activities when compared to the naive group, indicating minimal modulation

of these enzymatic defenses. Additionally, HECL markedly reduced MPO activity by 36.78% relative to the vehicle group (410.7 ± 22.37 vs. 648.81 ± 54.50; *p* < 0.001), indicating pronounced suppression of neutrophil infiltration and underscoring its substantial anti-inflammatory contribution to gastric mucosal protection.

Hydroethanolic extract promotes gastric mucosal healing in rats

As represented in Fig. 4A, repeated administration of HECL (100 mg/kg) or omeprazole (20 mg/kg) twice daily for

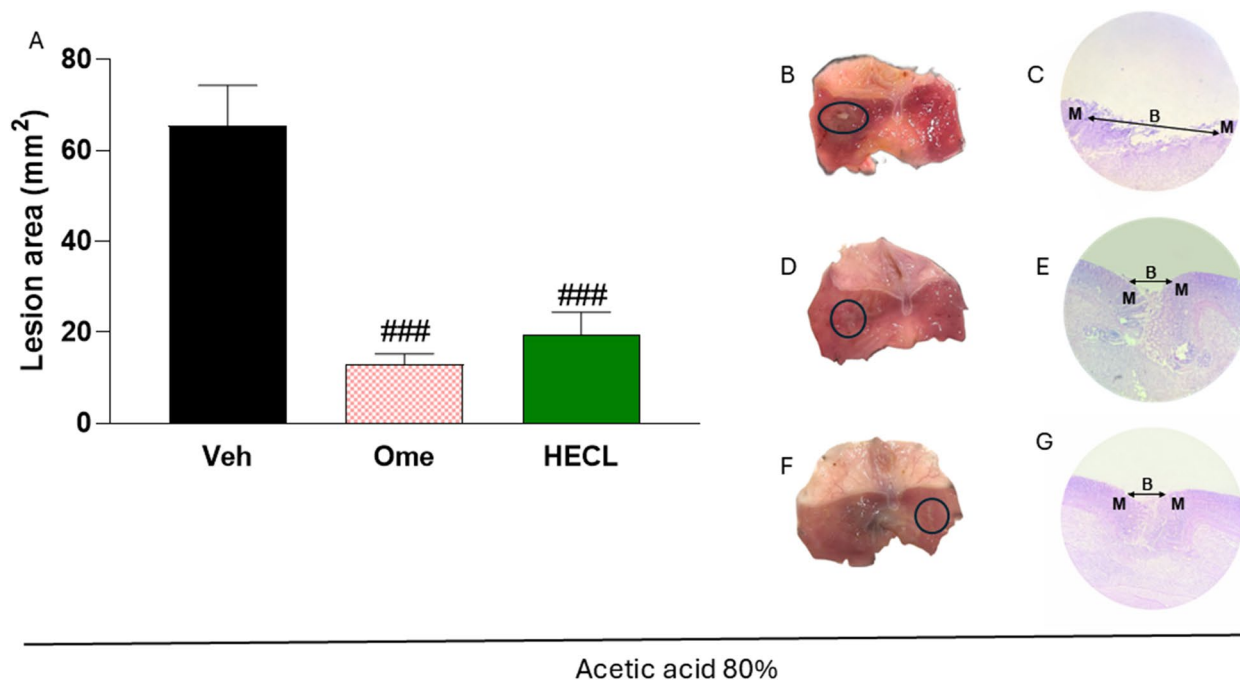


Fig. 4 (A) Effects of hydroalcoholic extract of *Coix lacryma-jobi* (100 mg/kg) (7 days) in 80% acetic acid-induced ulcers. (B) Representative macroscopic and microscopic images of (B–C) vehicle, (D–E) Omeprazole (20 mg/kg) and (F–G) HECL. Black circles indicate the location of the ulcer; M indicates the edge of the ulcer, and

B indicates the base of the ulcer. One-way ANOVA, followed by the Tukey test (mean ± S.E.M.; n=6). ###*p* < 0.001 compared to the vehicle group. Veh: Vehicle (distilled water); Ome: Omeprazole; HECL: hydroalcoholic extract of *Coix lacryma-jobi*

seven days markedly reduced the lesion area in the acetic acid-induced gastric ulcer model by 70 and 80%, respectively, compared with the vehicle treated group ($65.50 \pm 8.77 \text{ mm}^2$; $p < 0.001$). These macroscopic findings were corroborated by histological analysis of the ulcer site. The vehicle-treated ulcerated group exhibited severe tissue damage, characterized by an extensive ulcer base and minimal epithelial regeneration at both the base and margins of the lesion (Fig. 4B–C). In contrast, animals treated with either omeprazole (Fig. 4D–E) or HECL (Fig. 4F–G) showed marked improvements, including reduced ulcer depth and enhanced re-epithelialization at the ulcer margins, indicating a significant acceleration of the healing process compared with the vehicle-treated group.

Pharmacological mechanisms involved in gastric mucosal healing

Hydroethanolic extract preserves mucin in acetic acid-induced gastric ulcers

Histochemical analysis using PAS staining revealed a marked increase in mucin-like glycoprotein content following treatment with HECL (100 mg/kg, orally), with values reaching 13.890 ± 1.31 , compared with the vehicle-treated ulcerated group (4.747 ± 0.36), corresponding to an approximately threefold increase ($p < 0.001$). Omeprazole (20 mg/kg, orally) also significantly enhanced mucin

levels (9.649 ± 0.74), representing an approximately two-fold increase ($p < 0.05$) and restoring glycoprotein content to levels comparable to the naive group (9.822 ± 0.22). Notably, HECL treatment resulted in mucin levels 41.41% higher than the naive group, further underscoring its strong mucus-preserving effect (Fig. 5A). This robust enhancement of mucosal glycoproteins highlights the prominent role of HECL in reinforcing the gastric mucus barrier, a key component of mucosal defense and ulcer healing. Representative photomicrographs illustrating these differences across treatment groups are shown in Figs. 5B–E.

Effects of hydroethanolic extract on parameters of inflammatory and oxidative stress

In gastric ulcers induced by acetic acid, oxidative stress was evidenced by a marked reduction in GSH levels in the vehicle-treated group ($3121 \pm 335.2 \text{ } \mu\text{g/mg}$ tissue) when compared to non-ulcerated (naive) animals, which exhibited preserved baseline values ($4922 \pm 254.9 \text{ } \mu\text{g/mg}$ tissue). Pre-treatment with HECL (100 mg/kg, *p.o.*) significantly attenuated this depletion, restoring GSH levels to $4931 \pm 612.6 \text{ } \mu\text{g/mg}$ tissue ($p < 0.05$ vs. vehicle), indicating a substantial recovery of the gastric antioxidant defense system.

In parallel, LOOH levels were significantly higher in the ulcerated group treated with vehicle ($0.6170 \pm 0.0568 \text{ mmol/mg}$ tissue) than in naive animals, which had basal LOOH concentrations ($0.4514 \pm 0.0177 \text{ mmol/mg}$ tissue). Notably, treatment with HECL significantly reduced LOOH levels to $0.4239 \pm 0.0162 \text{ mmol/mg}$ tissue ($p < 0.01$ vs. vehicle),

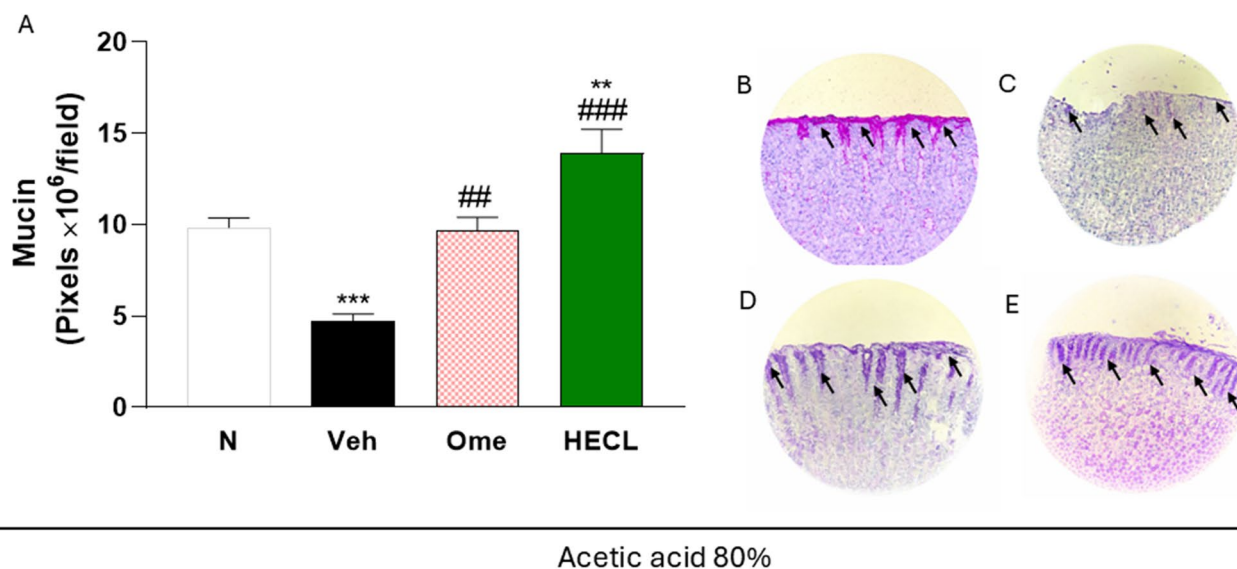


Fig. 5 (A) Effects of hydroalcoholic extract of *Coix lacryma-jobi* (HECL; 100 mg/kg) (7 days) in periodic acid-Schiff-staining for mucin-like glycoproteins in 80% acetic acid-induced ulcers. Representative microscopic images of (B) naive, (C) vehicle, (D) omeprazole (20 mg/kg), and (E) HECL. Black arrows indicate mucin-like glycoproteins, magnification = 400 $\times$. One-way ANOVA followed by

Tukey test (means $\pm$ S.E.M.; $n = 6$). ** $p < 0.01$ and *** $p < 0.001$ compared to naive group. ## $p < 0.01$ and ### $p < 0.001$ compared to vehicle group. N: Naive (not induced); Veh: Vehicle (distilled water); Ome: Omeprazole (20 mg/kg); HECL: hydroalcoholic extract of *Coix lacryma-jobi*

demonstrating effective attenuation of lipid peroxidation and reinforcing the antioxidant component of its gastroprotective action.

Hydroethanolic extract (100 mg/kg, *p.o.*) did not significantly affect SOD, CAT, or GST activities when compared to the vehicle-treated group, indicating that its protective effect is not primarily mediated by the modulation of these enzymatic antioxidant pathways.

Myeloperoxidase activity, an important marker of neutrophil infiltration, was markedly increased in the ulcerated group treated with vehicle (469.5 ± 43.37 mD.O./mg protein), representing a 178.44% increase compared to naive animals, which exhibited basal MPO activity (263.1 ± 34.85 mD.O./mg protein). Treatment with HECL (100 mg/kg, *p.o.*) significantly attenuated this inflammatory response, reducing MPO activity to 270.1 ± 42.46 mD.O./mg protein (47.5% reduction compared to the vehicle; $p < 0.05$). These findings demonstrate a pronounced anti-inflammatory effect of HECL, associated with reduced neutrophil accumulation and attenuation of gastric mucosal inflammation (Table 2).

Molecular docking analyses

Docking analyses indicated that quercetin and kaempferol exhibited the highest binding affinities toward both MPO (PDB: 1DNW; -8.3 and -8.0 kcal/mol, respectively) and IL-1 β (PDB: 1ITB; -8.4 and -8.1 kcal/mol, respectively), surpassing caffeic and *p*-coumaric acids (Tables S3 and S4). Their predicted interactions with key MPO catalytic residues (Thr100, Glu102, Arg239, Arg333) (Fig. 6) and IL-1 β residues (Lys103, Gln236, Asn204, His301) (Fig. 7) support their identification as major contributors to MPO inhibition and IL-1 β modulation, in line with the anti-inflammatory effects observed *in vivo*.

Drug-likeness assays

To explore the drug-likeness potential of the compounds identified from *C. lacryma-jobi*, the Lipinski Rule of Five was applied (Lipinski et al. 2001). None of the evaluated molecules violated the established parameters, indicating physicochemical properties consistent with orally viable drug candidates (Saritha et al. 2024) (Table S5). Subsequently, pharmacokinetic parameters were estimated, and computational safety assessments were carried out to anticipate relevant risks, including mutagenicity, cytotoxicity, and potential cardiovascular effects (Rodrigues et al. 2023). Overall, the results suggested favorable absorption and metabolism (Table S6), while the toxicological evaluation indicated a low risk of critical adverse outcomes (Table S7), supporting these compounds as promising candidates for further pharmacological investigation.

Discussion

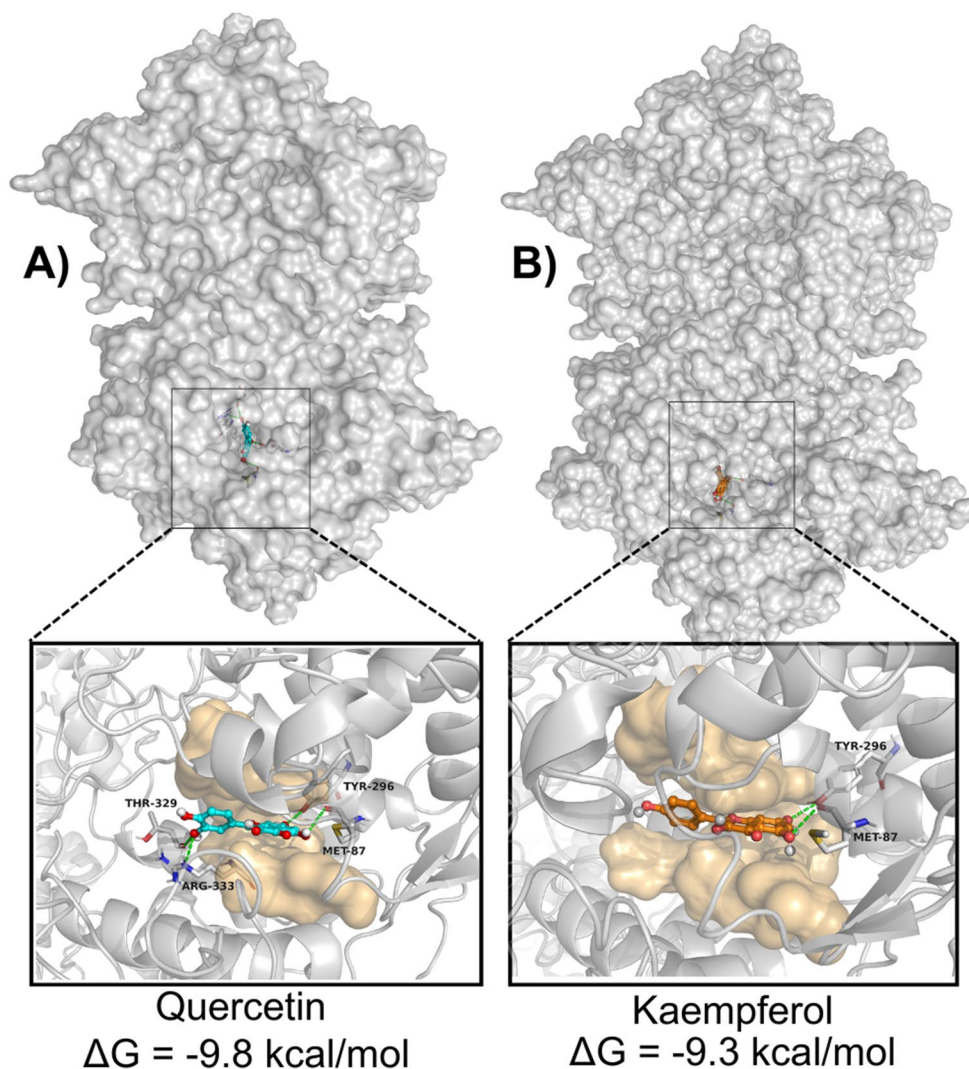
The use of medicinal plants in the treatment of gastric ulcers remains a promising area of research in experimental and clinical pharmacology, particularly given the therapeutic limitations associated with prolonged use of proton pump inhibitors. Although effective in inhibiting acid secretion, PPIs are associated with side effects, including hypomagnesemia, malabsorption of iron and vitamin B12, intestinal dysbiosis, and an increased risk of gastrointestinal infections (Imhann et al. 2016; Kavitt et al. 2019). In this context, natural substances have been shown to be effective in protecting and restoring the gastric mucosa, particularly flavonoids, saponins, alkaloids, and phenolic acids, which act through multifactorial mechanisms, including increased endogenous prostaglandins,

Table 2 Effect of hydroalcoholic extract of *Coix lacryma-jobi* (100 mg/kg) on biochemical parameters in 80% acetic acid-induced ulcers. Naive (not induced); vehicle (water); Omeprazole (20 mg/kg)

Treatments	MPO	LOOH	GSH	SOD	CAT	GST
Naive	263.1 ± 34.85	0.4514 ± 0.0177	4922 ± 254.9	1.06 ± 0.44	400.8 ± 13.80	263.7 ± 23.41
Vehicle (1 ml/kg, <i>p.o.</i>)	$469.5 \pm 43.37^*$	$0.6170 \pm 0.0568^*$	$3121 \pm 335.2^*$	1.25 ± 0.39	1532 ± 481.3	213.1 ± 25.59
Omeprazole (20 mg/kg, <i>p.o.</i>)	362.7 ± 58.48	0.5456 ± 0.0408	2391 ± 360.7	1.28 ± 0.81	700.3 ± 247.5	194.6 ± 40.11
HECL (100 mg/kg, <i>p.o.</i>)	$270.1 \pm 42.46^{\#}$	$0.4239 \pm 0.0162^{\#\#}$	$4931 \pm 612.6^{\#}$	1.20 ± 0.51	1115 ± 265.3	320.4 ± 18.04

Myeloperoxidase (MPO, mD.O./mg of protein); lipid hydroperoxides (LOOH, mmol/mg of tissue); reduced glutathione (GSH, μ g/mg of tissue); superoxide dismutase (SOD, U/mg of protein); catalase (CAT, μ mol/min/mg of protein) and glutathione *S*-transferase (GST, mmol/min/mg of protein). Values are expressed as mean $\pm$ S.E.M (n=6). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. $^*p < 0.05$ when compared to the naive group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ when compared to the vehicle-treated group

Fig. 6 Docking poses and interaction profiles of the compounds: (A) quercetin and (B) kaempferol from the hydroalcoholic extract of *Coix lacryma-jobi* docked with human myeloperoxidase (MPO; PDB ID: 1DNW). Green dashed lines indicate hydrogen-bond interactions. Highlighted in gold are hydrophobic interactions between amino acid residues and compounds



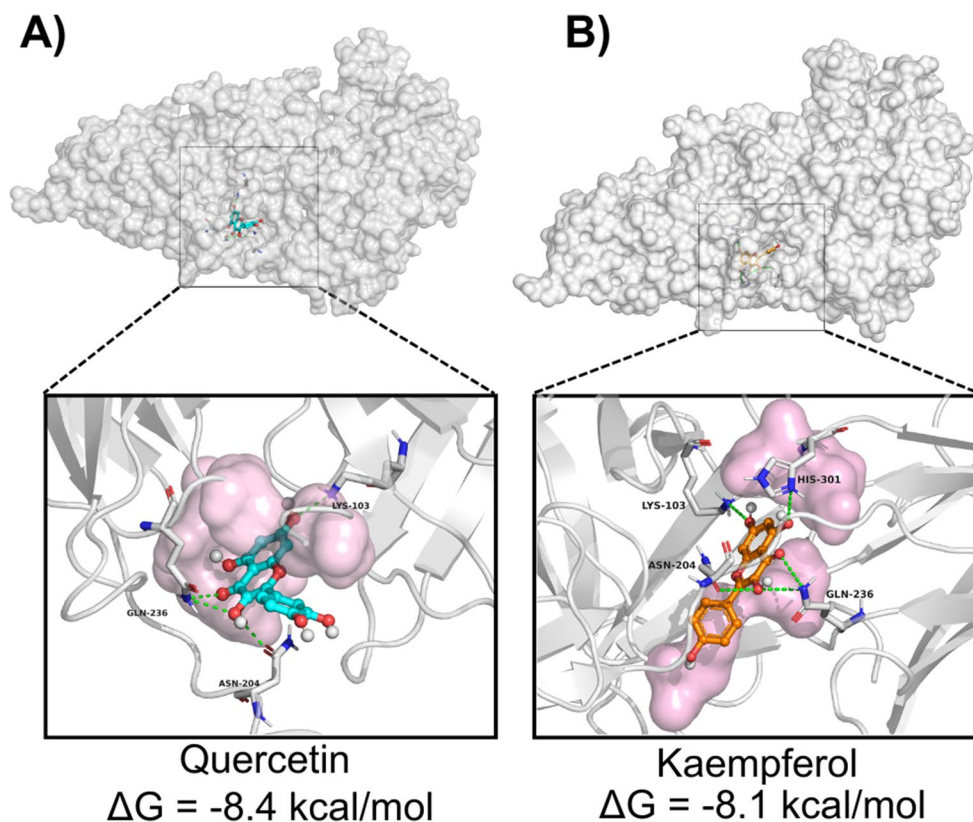
nitric oxide (NO), sulfhydryl groups (GSH), and regulation of oxidative stress (Kuna et al. 2019; Boakye-Yiadom et al. 2021).

The present study demonstrated that the hydroalcoholic extract of *Coix lacryma-jobi* exhibits significant gastroprotective and healing activity in experimental models of acute and chronic gastric injury, supported by cytoprotective, antioxidant, and anti-inflammatory mechanisms. Gastric protection was evident in the acute model, as demonstrated by histochemical and histological analyses showing preserved mucus production and maintenance of mucosal architecture. Hydroalcoholic extract of *C. lacryma-jobi* did not alter gastric secretion or volume, indicating that its protective action is not mediated by antisecretory mechanisms. Instead, the hydroalcoholic extract modulated key anti-inflammatory and oxidative stress parameters, with more pronounced effects on GSH levels and myeloperoxidase activity. During the gastric healing phase, protective effects on the mucosa were once again evident, accompanied by consistent antioxidant

and anti-inflammatory responses. These included reductions in GSH and lipid peroxidation levels, along with a marked decrease in MPO activity. These biochemical findings were further supported by molecular docking analyses, which revealed favorable binding energies between HECL-identified compounds and crystallized MPO and IL-1 β proteins, reinforcing the potential mechanistic basis for the extract's gastroprotective effects.

The phytochemical characterization of HECL by HPLC-MS identified quercetin, kaempferol, caffeic acid, and *p*-coumaric acid, bioactive phenolics widely reported in *C. lacryma-jobi* and known for their antioxidant and anti-inflammatory activities (Chen et al. 2011; Zeng et al. 2022). These flavonoids and phenolic acids have been consistently associated with gastroprotective effects in preclinical models, in which they preserve gastric mucosal integrity by modulating oxidative stress, attenuating inflammatory responses, and enhancing endogenous defense mechanisms (Hu et al. 2015; Serafim et al. 2020).

Fig. 7 Docking poses and interaction profiles of (A) quercetin and (B) kaempferol from the hydroalcoholic extract of *Coix lacryma-jobi* bound to interleukin-1 β (IL-1 β ; PDB ID: 1DNW). Green dashed lines denote hydrogen bonds, and hydrophobic interactions are highlighted in gold



In the ethanol-induced acute gastric injury model, HECL at 100 mg/kg produced a marked reduction in the ulcerated area, with an effect that was percentage-wise comparable to, or greater than, that of omeprazole. Ethanol causes rapid and direct disruption of the gastric mucosa, increasing epithelial permeability, promoting excessive generation of reactive oxygen species, and depleting cytoprotective factors such as mucin and reduced glutathione (Liu et al. 2017; Tsermpini et al. 2022). Within this context, the observed efficacy of HECL suggests a protective effect linked to preservation of the mucosal barrier and attenuation of oxidative injury, consistent with previous findings for plant extracts rich in phenolic constituents (Chen et al. 2011).

Interestingly, HECL markedly increased glycoprotein-type mucin, as demonstrated by PAS staining, in both the acute and chronic models. Mucin is a key structural component of the gastric barrier and represents the first line of defense against chemical and mechanical injury. The preservation and stimulation of mucin production are recognized as central mechanisms of gastroprotection and ulcer healing, particularly in therapeutic approaches that do not directly target acid secretion. Polyphenols have been reported to enhance mucus integrity and promote gastric repair in experimental ulcer models (Wallace and Miller 2000; Wallace 2008; Boeig et al. 2021).

Corroborating this cytoprotective profile, HECL did not induce significant changes in gastric secretion parameters, including gastric juice volume, pH, total acidity, or peptic activity. This finding reinforces that its gastroprotective effect is not mediated by acid suppression but rather by the modulation of mucosal defense mechanisms. Such a profile represents a pharmacological advantage over proton pump inhibitors, who's long-term use has been associated with clinically relevant risks (Imhann et al. 2016; Chaudhry et al. 2025). Acid-independent gastroprotection has been widely documented for medicinal plants and natural compounds (Kuna et al. 2019).

Hydroalcoholic extract of *C. lacryma-jobi* also demonstrated the ability to modulate oxidative and inflammatory pathways, reinforcing its cytoprotective profile. Treatment markedly restored GSH, a central endogenous antioxidant, and consistently decreased MPO activity, indicating diminished neutrophil infiltration and a blunted inflammatory response. In the chronic model, the significant reduction in LOOH further supports a decrease in lipid peroxidation associated with ulcerative injury. Notably, this coordinated antioxidant and anti-inflammatory response suggests that HECL acts upstream in the injury cascade, preserving redox homeostasis before irreversible mucosal damage occurs. Although similar effects have been reported for isolated phenolic acids such as caffeic and *p*-coumaric acids (Kolgazi

et al. 2021; Boeing et al. 2021), the magnitude and consistency of the response observed here indicate a potential synergistic interaction among HECL constituents, highlighting a pharmacological profile that extends beyond the activity of individual compounds.

The correspondence between the phytochemical profile of HECL and its pharmacological effects reinforces the mechanistic plausibility of its gastroprotective action. Although flavonoids and phenolic acids are known modulators of oxidative and inflammatory pathways, the integrated response observed in this study, encompassing mucin preservation, redox balance, and reduced neutrophil-driven inflammation, suggests a synergistic phytocomplex that strengthens mucosal defense through multiple convergent mechanisms (Kuna et al. 2019; Zeng et al. 2022).

In the chronic acetic acid-induced ulcer model, HECL not only reduced lesion size but also promoted marked histological recovery, including re-epithelialization, mucosal reorganization, and partial restoration of glandular architecture. This structural improvement was accompanied by a robust increase in gastric mucin, with levels surpassing those of naive animals. Comparable barrier-reinforcing effects have been documented for other Poaceae species, such as *Cymbopogon citratus* (DC.) Stapf. (Carbajal et al. 1989; Sagra-das et al. 2015; Venzon et al. 2018) and *Oryza sativa* L. (Tonchaiyaphum et al. 2021), whose extracts or constituents enhance mucin staining and support healing in experimental ulcer models. Collectively, these findings indicate that suprabasal mucin enhancement is a characteristic response within Poaceae-derived treatments, consistent with the structural and functional changes observed following HECL treatment. In the context, the combination of structural regeneration with sustained modulation of oxidative and inflammatory pathways positions HECL as a phytocomplex capable of promoting coordinated mucosal recovery, a profile consistent with natural agents that exhibit multifactorial gastroprotective actions (Wallace and Miller 2000; Wallace 2008; Chen et al. 2011).

Additionally, the integrated network pharmacology and docking analyses suggest that HECL may exert its antiulcer and anti-inflammatory effects by modulating key inflammatory pathways, particularly those centered on IL-1 β , IL-6, NF- κ B, and MAPK signaling, all of which contribute to gastric mucosal injury. Within this framework, quercetin and kaempferol emerged as the most pharmacologically relevant constituents, exhibiting the strongest predicted affinities for IL-1 β (−8.4 and −8.1 kcal/mol, respectively). Their docking poses revealed stable hydrogen-bond networks involving residues Lys103, Asn204, Gln236, and His301, interactions that are functionally implicated in IL-1 β receptor engagement and downstream signaling (Liu et al. 2023).

In addition, both compounds also displayed high predicted affinity for MPO, a key enzyme in neutrophil activation and gastric inflammatory damage. Docking simulations

revealed binding energies of −8.3 kcal/mol (quercetin) and −8 kcal/mol (kaempferol), markedly stronger than those of caffeic and *p*-coumaric acids. Their interactions with residues Thr100, Glu102, Arg239, and Arg333, critical for MPO catalytic activity, support the hypothesis that these polyphenols may contribute to MPO inhibition, thereby reducing neutrophil-driven oxidative stress and enhancing gastroprotection. Although these *in silico* findings strengthen the mechanistic plausibility of HECL's biological effects, they remain predictive and do not account for pharmacokinetic or metabolic constraints.

Taken together, the *in vivo* and *in silico* toxicity assessment safety predictions indicate that HECL and its major constituents exhibit a favorable toxicological profile. Oral administration of the extract at 2000 mg/kg produced no clinical signs of systemic toxicity or alterations in organ weights, supporting its short-term safety under experimental conditions. Consistently, the identified compounds complied with Lipinski's criteria, showed predicted pharmacokinetic properties compatible with oral bioavailability, and presented low risk for mutagenicity, cytotoxicity, or cardiotoxicity. These convergent findings reinforce the suitability of HECL constituents as promising candidates for further pharmacological development.

Hydroalcoholic extract of *C. lacryma-jobi* exhibits a pharmacological profile characterized by cytoprotection, antioxidant activity, and inflammatory modulation, without affecting acid secretion, positioning it as a relevant candidate for strengthening mucosal defenses. Unlike proton pump inhibitors, which primarily act by suppressing acid secretion, the extract targets multiple aspects of gastric pathophysiology, thereby supporting protection and tissue repair even when acidity is maintained. This multilevel activity highlights the potential of HECL as an alternative or adjunct to long-term acid suppression in situations where long-term acid suppression is limited or undesirable.

Despite the promising findings of the present study, several limitations should be considered. Although the experimental models employed provide relevant evidence regarding the gastroprotective and gastric-healing effects of HECL, the molecular mechanisms involved were inferred mainly from biochemical and *in silico* analyses, without direct assessment of intracellular signaling pathways or protein expression. Furthermore, long-term pharmacokinetic and toxicological evaluations were not performed and remain necessary for future translational investigations. In this context, future studies should focus on confirming the molecular targets and signaling pathways associated with HECL activity, particularly those related to oxidative stress and inflammatory modulation, and on investigating pharmacokinetic parameters, chronic safety, and possible synergistic interactions among its bioactive compounds. Nevertheless, integrating phytochemical characterization, experimental

pharmacology, and computational analyses provides a comprehensive overview of the therapeutic potential of *C. lacryma-jobi* in gastric disorders and supports its potential as a promising source of bioactive compounds for the development of novel gastroprotective therapies.

Conclusion

Collectively, the findings of this study demonstrate that the hydroalcoholic extract of *C. lacryma-jobi* exerts potent gastroprotective and healing effects in experimental models of gastric injury. Its multimechanistic profile, encompassing modulation of oxidative and inflammatory pathways, reinforcement of mucosal defenses, and promotion of tissue repair, highlights a therapeutic potential that extends beyond simple cytoprotection. By acting independently of acid suppression, HECL emerges as a promising natural candidate for the prevention and management of gastric ulcers, particularly in contexts where long-term acid-inhibitory therapy is limited or undesirable.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s43450-026-00771-y>.

Author's contributions LBS: experimental design, data analysis, and writing. DM: acute and chronic experiments, biochemical and histological analyses. AMS: acute and chronic experiments, biochemical and histological analyses. CADV: acute and chronic experiments, biochemical and histological analyses. CVF: acute and chronic experiments, biochemical and histological analyses. ALTB: acute and chronic experiments, biochemical and histological analyses. KF: acute and chronic experiments, biochemical and histological analyses. IRG: *in silico* analysis. MVB: *in silico* analysis. JCM: acute and chronic experiments, biochemical and histological analyses. APNM: acute and chronic experiments, biochemical and histological analyses. EG: data analysis and writing. WARJ: experimental design, data analysis, and writing.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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