Short Communication
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Preliminary gastroprotective effect of aqueous *Bixa orellana* leaf extract in an HCl/ethanol-induced gastric ulcer rat model

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ABSTRACT

Gastric ulceration develops when aggressive luminal factors overwhelm gastric mucosal defense. *Bixa orellana* leaves are used traditionally for gastrointestinal complaints, but direct evidence for aqueous *B. orellana* leaf extract (BOE) in chemically induced gastric ulcer models remains limited. This exploratory study evaluated the effects of aqueous BOE against HCl/ethanol-induced gastric ulceration in rats. Twenty-four male Sprague-Dawley rats were allocated to four groups (n = 6/group): untreated ulcer control, omeprazole 100 mg/kg, BOE 50 mg/kg, and BOE 150 mg/kg. Treatments were administered orally for 7 days. After 24 h fasting, ulcers were induced with 1.0 mL HCl/ethanol, and stomach tissues were collected for macroscopic ulcer-index scoring and hematoxylin and eosin histological assessment. No measured outcome reached statistical significance. The ulcer index showed a non-significant overall difference among groups (Kruskal-Wallis H = 6.57, p = 0.087). Histological scores were also non-significant for congestion (p = 0.458), edema (p = 0.158), inflammatory infiltration (p = 0.126), and necrosis (p = 0.089). Omeprazole and BOE 150 mg/kg showed numerically lower ulcer-index values than the untreated ulcer control, whereas BOE 50 mg/kg remained close to the ulcer control. BOE 150 mg/kg also showed numerically lower edema, inflammatory infiltration, and necrosis scores, but these differences were not statistically confirmed. The present study demonstrates biological trends rather than statistically confirmed gastroprotective efficacy. Aqueous BOE showed a possible dose-related gastroprotective trend, with 150 mg/kg producing the most favorable numerical pattern. Larger dose-response studies with extract standardization, blinded morphometric assessment, ulcer-area and mucus endpoints, and biochemical assays are required before efficacy or mechanistic claims can be made.

Keywords: *Bixa Orellana; gastric ulcer; HCl/ethanol-induced ulcer; histopathology; gastroprotection and medicinal plant*

INTRODUCTION

The Peptic ulcer disease remains an important gastrointestinal disorder because ulceration and ulcer-related complications continue to affect clinical care despite improvements in acid suppression, Helicobacter pylori eradication, and prevention of non-steroidal anti-inflammatory drug (NSAID)-induced injury (Almadi et al., 2024; Chey et al., 2024; Kamada et al., 2021). Despite improvements in prevention and treatment, peptic ulcer continues to impose a

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clinically relevant burden, with smoking remaining an important modifiable contributor. In parallel, gastrointestinal safety associated with non-steroidal anti-inflammatory drugs remains an active area of clinical and research attention, particularly because of their well-established association with gastric mucosal injury and ulceration (Wang et al., 2025; Alshadfan et al., 2025; Alhatim et al., 2026). Gastric mucosal injury reflects an imbalance between aggressive factors, including acid, pepsin, ethanol, NSAIDs, H. pylori infection, and reactive oxygen species, and endogenous protective factors such as mucus-bicarbonate secretion, epithelial restitution, microvascular blood flow, and prostaglandin-mediated cytoprotection (Mizui & Doteuchi, 1983; Robert, 1979).

The HCl/ethanol model is widely used for exploratory gastroprotection studies because it produces acute mucosal necrosis, congestion, edema, inflammatory infiltration, and hemorrhagic injury within a short period. This model is useful for screening cytoprotective and barrier-preserving effects, but it does not by itself identify the biochemical mechanism responsible for any observed protection (Badr et al., 2019; Mizui & Doteuchi, 1983). Therefore, findings from this model should be interpreted cautiously, particularly when only macroscopic and histological endpoints are measured. Experience with other plant-derived aqueous extracts also supports the use of rodent ulcer models as exploratory screening systems, although the biological responses and measured endpoints vary according to the plant preparation and experimental model. For example, aqueous botanical preparations have been investigated for effects on gastric secretion, gastric pH, mucus-related protection, and experimentally induced gastric lesions (Gamberini et al., 1991; Ahmed et al., 2022).

Bixa orellana L. is a medicinal plant traditionally used for several inflammatory and gastrointestinal complaints (de Araújo Vilar et al., 2014; Molina-Romani et al., 2023). *B. orellana* leaves contain variable phenolic, flavonoid, carotenoid, terpenoid, and related constituents, and previous studies have reported antioxidant, antimicrobial, wound-healing, anti-inflammatory, antihistamine, and vascular-permeability effects (Franklin et al., 2023; Sarkar et al., 2024; Yong et al., 2011, 2013a, 2013b, 2015, 2018). These properties provide biological plausibility for testing BOE in gastric mucosal injury models. More recent syntheses of the *B. orellana* literature further demonstrate the broad biochemical diversity and biomedical potential of the species. Bioprospecting studies have identified multiple classes of bioactive constituents associated with diverse biological activities, while systematic evidence also indicates expanding biomedical applications of annatto-derived extracts and compounds, including their incorporation into nanostructured delivery systems (Guerrero-Lagunes et al., 2025; da Silva et al., 2024).

However, the specific knowledge gap is that direct experimental evidence for aqueous BOE in HCl/ethanol-induced gastric ulceration remains limited. The present short communication therefore reports an exploratory rat experiment evaluating aqueous BOE at 50 and 150 mg/kg using ulcer-index and semi-quantitative histological outcomes. Because the study was small and did not include biochemical assays, the objective was to identify whether a biological trend existed rather than to establish definitive gastroprotective efficacy.

METHODOLOGY

Chemicals and reagents

Omeprazole and standard chemicals were obtained from Sigma-Aldrich, Malaysia. Distilled water was used as the extraction solvent and administration vehicle for the aqueous extract.

Plant material and extraction

B. orellana leaves were collected from Universiti Putra Malaysia, Serdang, Selangor, Malaysia, and authenticated at the Phytochemical Herbarium, Institute of Bioscience, Universiti Putra Malaysia (voucher number NL 16). The leaves were oven-dried at 60 °C for approximately 2 days, ground into powder, soaked in distilled water at a 1:20 ratio, heated in a water bath at 60 °C for 24 h, filtered, and freeze-dried at -80 °C (Somchit et al., 2014). The freeze-dried aqueous extract was reconstituted in distilled water immediately before oral administration.

Animals and ethical considerations

Twenty-four male albino Sprague-Dawley rats weighing approximately 150-200 g were housed under standard animal house conditions with free access to pellet diet and water during acclimatization. Animals were acclimatized for 1 week before the experiment. Ethical approval was obtained from the Animal Care and Use Committee, Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00495). Reporting was revised with reference to ARRIVE 2.0 principles for animal research transparency (Percie du Sert et al., 2020).

Humane endpoints included abnormal distress, severe lethargy, marked behavioral abnormality, inability to access food or water, or any unexpected sign requiring veterinary intervention. The 24 h fasting period was included to standardize gastric contents and improve reproducibility of the acute HCl/ethanol ulcer model, animals were monitored during the fasting and experimental period. Analgesia was not administered before ulcer induction because analgesic or anti-inflammatory drugs could confound gastric mucosal injury and inflammatory scoring, instead, animals were euthanized under anesthetic overdose shortly after ulcer induction according to the approved animal ethics protocol.

Experimental design and treatment

Rats were allocated to four groups (n = 6/group): untreated ulcer control, omeprazole-treated positive control (100 mg/kg), BOE 50 mg/kg, and BOE 150 mg/kg. Allocation was performed by simple random assignment after acclimatization. Treatments were administered by oral gavage for 7 consecutive days. BOE was dissolved in distilled water. The ulcer-control group received the same administration route without active plant extract, and omeprazole served as the reference anti-ulcer drug.

Ulcer induction

On day 6, rats were fasted for 24 h according to the approved protocol. On day 7, 1 h after the final treatment, gastric ulcers were induced using 1.0 mL HCl/ethanol solution (60 mL ethanol, 1.7 mL HCl, and 38.3 mL distilled water). One hour after ulcer induction, animals were sacrificed by pentobarbitone anesthetic overdose in accordance with the UPM IACUC-approved procedure. The stomachs were removed and opened along the greater curvature (Zabidi et al., 2012).

Macroscopic ulcer-index scoring and histology

Gastric damage was assessed using a macroscopic ulcerative lesion index adapted from published experimental ulcer-scoring approaches (Mizui & Doteuchi, 1983; Zabidi et al., 2012). The scoring considered loss of normal morphology, mucosal discoloration, edema, hemorrhage, petechial points, and visible ulcerative lesions. Scores were recorded as semi-quantitative lesion severity values, where higher values indicated more severe macroscopic injury. The present study did not quantify ulcerated area (%), ulcer inhibition (%), hemorrhagic area, or gastric mucus weight; these endpoints are now stated as limitations and are recommended for future confirmatory work.

Stomach tissues were processed for hematoxylin and eosin (H&E) staining. Congestion, edema, neutrophil/lymphocyte infiltration, and necrosis were evaluated microscopically using a semi-quantitative histopathological scoring system adapted from published histopathological descriptions of gastritis and gastric injury (Zhang et al., 2023). Histological scoring was performed by a consultant histopathologist who was blinded to group allocation. Macroscopic ulcer scoring was also treated as blinded during revision; where full blinding was not documented in the original records, this is acknowledged as a limitation. Representative photomicrographs were recorded at 10x objective and 10x ocular magnification (100x total magnification).

Statistical analysis

Data are presented as mean ± standard deviation (SD). Because the sample size was small and the outcomes were ordinal or semi-quantitative, between-group comparisons were analyzed using the Kruskal-Wallis test. Dunn post hoc multiple-comparison testing was not interpreted because the omnibus Kruskal-Wallis tests were non-significant. A value of $p < 0.05$ was considered statistically significant. All findings were interpreted as exploratory and trend-based, not as evidence of statistically confirmed efficacy.

RESULTS

The HCl/ethanol challenge produced clear macroscopic and microscopic gastric mucosal injury in the untreated ulcer control group, confirming successful induction of acute gastric ulceration. However, the overall Kruskal-Wallis result for ulcer index did not reach statistical significance ($H = 6.57$, $p = 0.087$). Therefore, the ulcer-index findings demonstrate a biological trend rather than statistically confirmed gastroprotective efficacy.

Mean ± SD values for the ulcer index and histological scores are presented in Table 1. The untreated ulcer control and BOE 50 mg/kg groups showed the highest mean ulcer-index values, whereas the omeprazole and BOE 150 mg/kg groups showed lower numerical values. BOE 150 mg/kg produced an ulcer-index pattern closer to the omeprazole-treated group than to the untreated ulcer control, while BOE 50 mg/kg remained close to the ulcer control. These numerical differences must be interpreted cautiously because the overall test was non-significant. The group-wise ulcer-index values are illustrated in Figure 1.

Semi-quantitative histological scoring also showed no statistically significant differences among groups for congestion, edema, inflammatory infiltration, or necrosis. The BOE 150 mg/kg group showed numerically lower edema, inflammatory infiltration, and necrosis scores than the ulcer control, but these observations remain trend-based only. The corresponding group-wise histological injury scores are presented in Figure 2. The histological results therefore support a possible dose-related biological trend but do not establish a significant protective effect. A conservative summary of the numerical trends observed across the macroscopic and histological outcomes is presented in Table 2.

Representative H&E-stained sections showed severe mucosal injury in the untreated ulcer control, relatively preserved mucosal architecture in the omeprazole group, persistent mucosal injury in the BOE 50 mg/kg group, and

less severe tissue disruption in the BOE 150 mg/kg group. Representative H&E-stained gastric sections from all four experimental groups are shown in Figure 3.

Table 1

Numerical summary of macroscopic and histological outcomes

Outcome	Untreated ulcer control	Omeprazole 100 mg/kg	BOE 50 mg/kg	BOE 150 mg/kg	Overall p value
Ulcer index	5.16 ± 1.51	3.92 ± 1.11	5.21 ± 1.66	3.85 ± 1.34	0.087
Congestion score	1.18 ± 0.60	0.22 ± 0.22	0.67 ± 0.52	0.35 ± 0.35	0.458
Edema score	1.20 ± 0.61	0.50 ± 0.29	1.34 ± 0.46	0.32 ± 0.33	0.158
Inflammatory infiltration score	1.22 ± 0.57	0.20 ± 0.20	1.21 ± 0.52	0.20 ± 0.20	0.126
Necrosis score	1.33 ± 0.49	0.18 ± 0.18	1.16 ± 0.28	0.68 ± 0.32	0.089

Note. Values are mean ± SD (n = 6/group). BOE = Bixa orellana extract. p values are from Kruskal-Wallis tests. No outcome reached statistical significance at p < 0.05.

Table 2

Conservative interpretation of outcome trends

Outcome	Main numerical pattern	Interpretation
Ulcer index	Lower mean values in omeprazole and BOE 150 mg/kg than untreated ulcer control; BOE 50 mg/kg remained close to ulcer control.	Possible macroscopic protective trend only; not statistically confirmed.
Congestion	Lower score in BOE 150 mg/kg than untreated ulcer control; omeprazole lowest.	Non-significant histological trend.
Edema	BOE 150 mg/kg numerically lower than untreated ulcer control and BOE 50 mg/kg.	Non-significant trend that requires confirmatory testing.
Inflammatory infiltration	BOE 150 mg/kg and omeprazole showed lower scores than untreated ulcer control and BOE 50 mg/kg.	Non-significant trend; no inflammatory mediator assay was performed.
Necrosis	BOE 150 mg/kg showed a lower mean score than untreated ulcer control and BOE 50 mg/kg.	Non-significant trend; biochemical or morphometric confirmation is required.

Note. This table intentionally uses conservative language because all measured outcomes were non-significant.

DISCUSSION

The present study demonstrates biological trends rather than statistically confirmed gastroprotective efficacy. The main finding is that BOE 150 mg/kg produced the most favorable numerical pattern across the macroscopic ulcer index and several histological injury markers, whereas BOE 50 mg/kg did not show a consistent protective pattern. Nevertheless, the ulcer index and all histological outcomes were non-significant. The study therefore cannot claim that BOE significantly reduced gastric ulceration in this model.

The cautious interpretation is important because the small sample size (n = 6 per group), ordinal or semi-quantitative scoring, and absence of biochemical endpoints limit statistical power and mechanistic inference. In this context, p values should remain the main basis for statistical interpretation. Directional consistency across endpoints is useful only for generating hypotheses for larger studies, not for confirming efficacy.

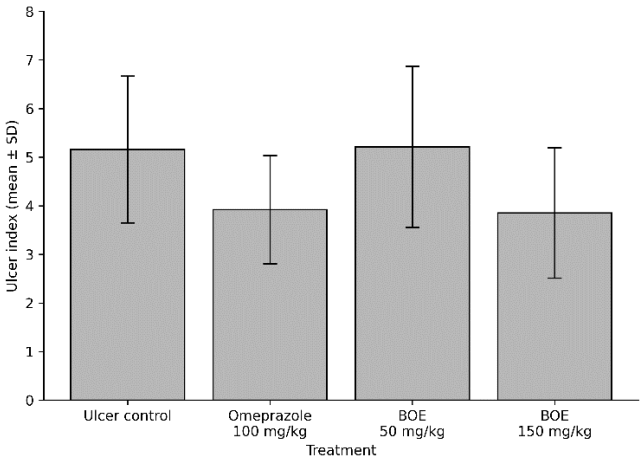
The HCl/ethanol model causes acute epithelial injury, congestion, edema, inflammatory cell infiltration, and necrosis (Badr et al., 2019; Mizui & Doteuchi, 1983). The numerically lower edema, inflammatory infiltration, and necrosis scores in the BOE 150 mg/kg group are compatible with a possible cytoprotective pattern, but they do not prove that BOE prevents vascular leakage, suppresses inflammatory recruitment, or attenuates oxidative injury. These mechanisms were not measured experimentally in the present study.

Previous studies provide a rationale for future mechanistic testing. *B. orellana* leaf extract has been reported to inhibit bradykinin-induced inflammation, serotonin- and histamine-induced vascular permeability, leukocyte infiltration, and endothelial hyperpermeability in non-gastric experimental systems (Yong et al., 2011, 2013a, 2013b,

2015, 2018).

Figure 1

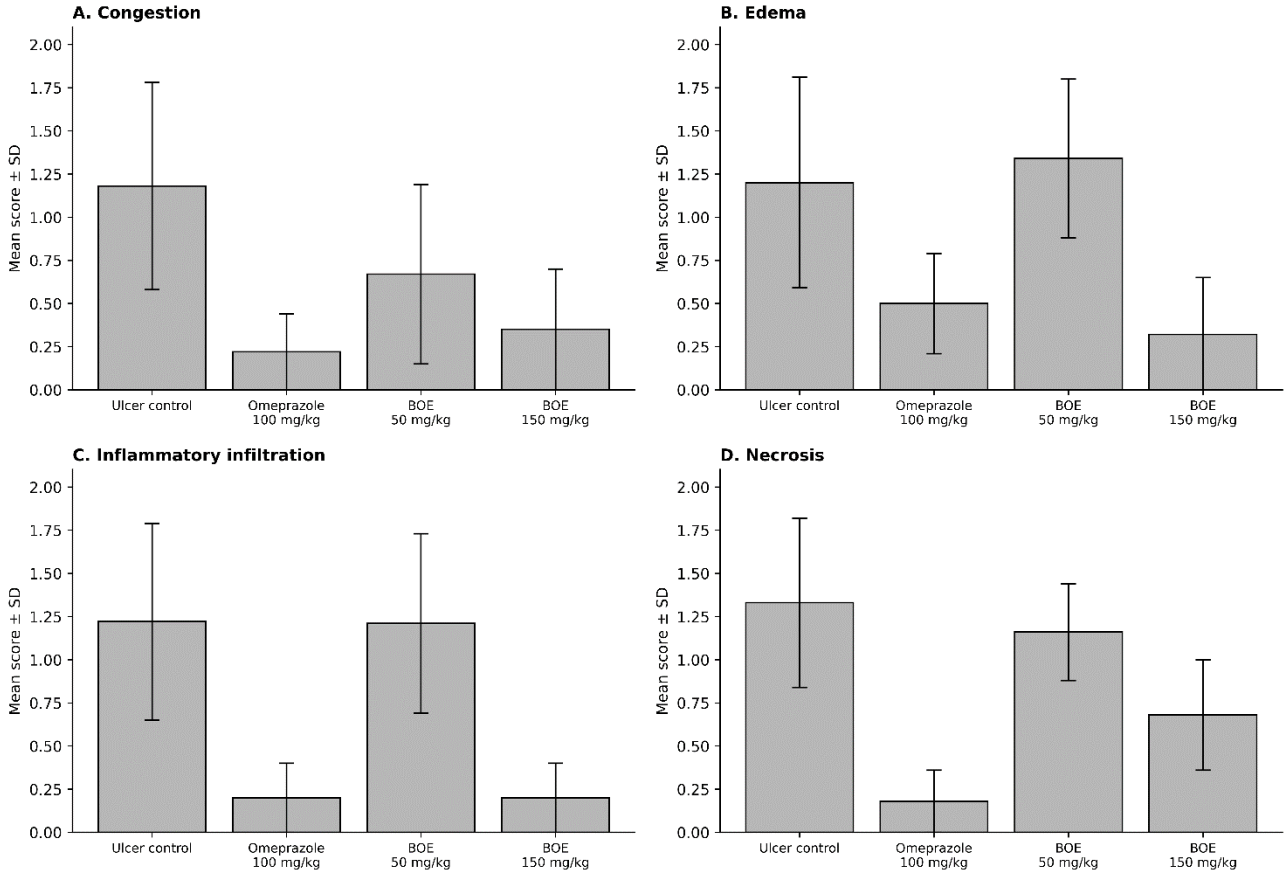
Ulcer index in experimental groups



Note: Data are presented as mean ± SD (n = 6/group). Ulcer index was determined using a macroscopic gastric lesion scoring system. Statistical analysis was performed using the Kruskal-Wallis test. No significant overall difference was observed among groups (H = 6.57, p = 0.087).

Figure 2

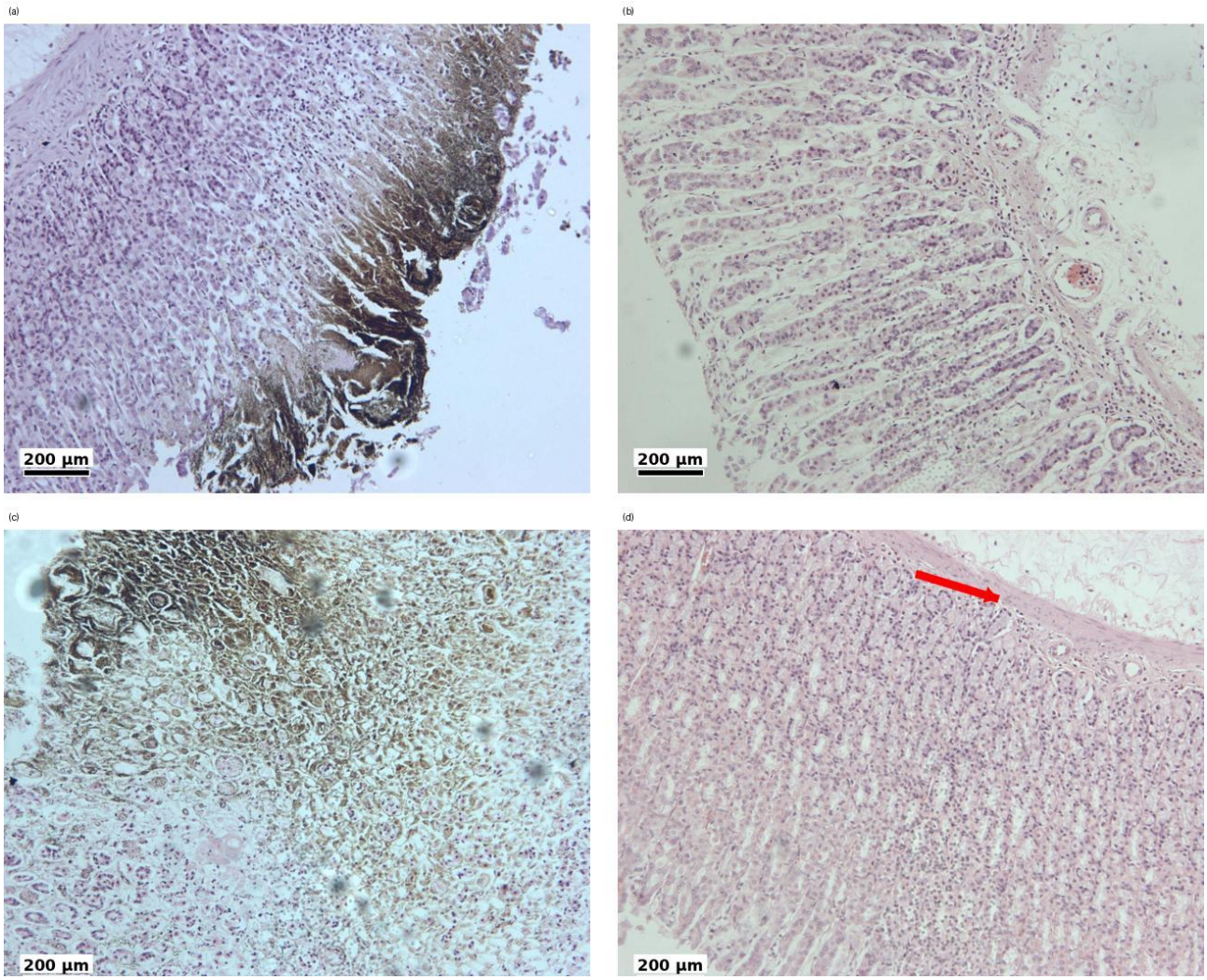
Histological injury scores in gastric tissue following treatment with Bixa orellana extract (BOE)



Note: Histological scores for congestion, edema, inflammatory infiltration, and necrosis are presented as mean ± SD (n = 6/group). No significant differences were observed among groups for congestion (p = 0.458), edema (p = 0.158), inflammatory infiltration (p = 0.126), or necrosis (p = 0.089). BOE = Bixa orellana extract; SD = standard deviation.

Figure 3

Representative histopathological sections of gastric tissues stained with hematoxylin and eosin



Note: (a) Untreated ulcer control showing severe mucosal injury with congestion, inflammatory cell infiltration, and necrosis. (b) Omeprazole-treated group showing preservation of gastric architecture with minimal inflammatory changes. (c) BOE 50 mg/kg-treated group demonstrating persistent mucosal injury and inflammatory infiltration with mild necrosis. (d) BOE 150 mg/kg-treated group showing comparatively less severe tissue disruption in the representative section; the arrow indicates the relatively preserved mucosal region. All sections were photographed at 100× total magnification. Scale bars = 200 µm.

Evidence from non-gastric experimental systems provides additional context for the inflammatory and vascular processes that may warrant examination in future BOE studies. Other natural compounds have been shown to modulate inflammatory mediators including nitric oxide, prostaglandin E2, tumor necrosis factor-α, and reactive oxygen species (Ahmad et al., 2006), while cryptotanshinone has been investigated in tumor necrosis factor-α-induced endothelial activation (Ahmad et al., 2016). Endothelial permeability studies have also implicated nitric oxide–cyclic guanosine monophosphate signaling in interferon-γ-associated barrier dysfunction (Ng et al., 2016). These findings provide mechanistic context only and should not be interpreted as evidence that BOE acts through the same pathways. Other natural products in ethanol-induced gastric injury models have been linked to antioxidant and anti-inflammatory pathways such as Nrf2/HO-1 signaling, HMGB1/TLR4/NF-kappa B signaling, and inflammatory mediator suppression (Raish et al., 2021; Badr et al., 2019; Zhou et al., 2020). However, these pathways should be treated as hypotheses for BOE rather than as mechanisms demonstrated by this experiment.

The lower response observed at 50 mg/kg suggests that this dose was insufficient under the severe acidified ethanol challenge. However, only two BOE doses were tested, and the absence of an intermediate 100 mg/kg group prevents dose-response modeling. A future design should include a vehicle control, at least three BOE doses such as 50, 100, and 150 mg/kg or a broader range, and a predefined primary endpoint such as ulcerated area or ulcer inhibition percentage.

The macroscopic assessment should also be strengthened. Ulcer index alone is less robust than a combined

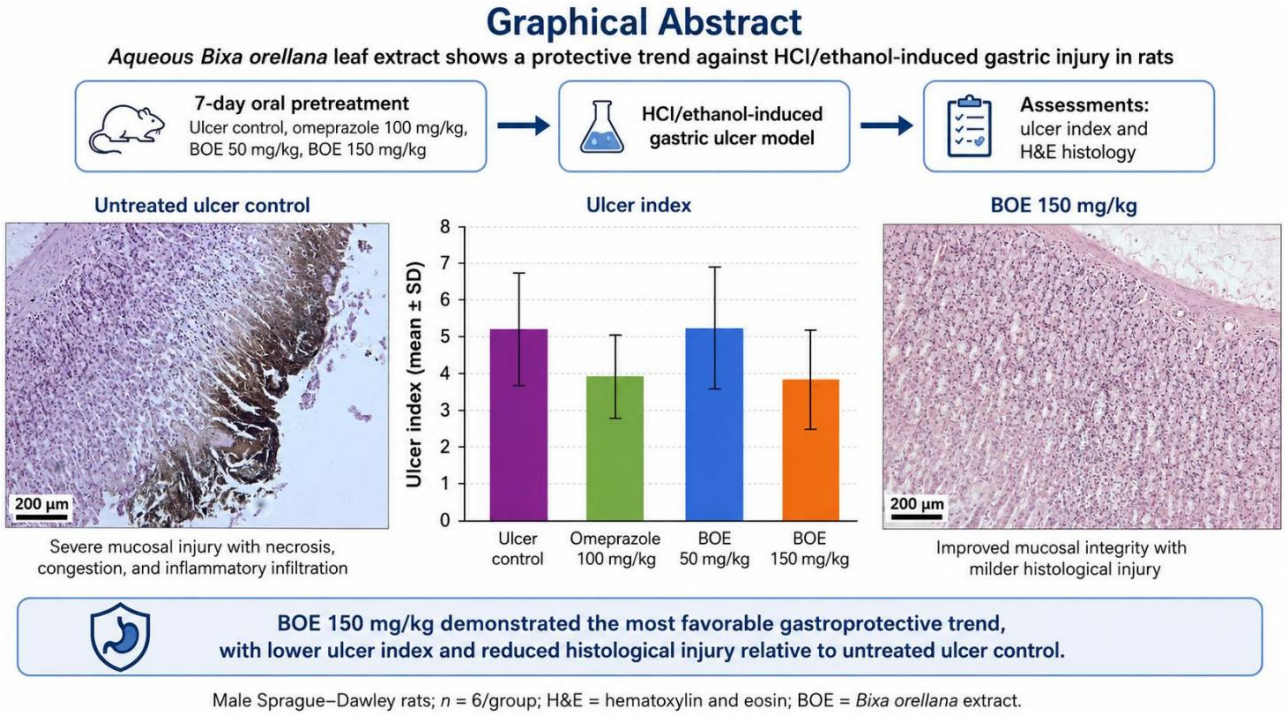
assessment that includes ulcerated area (%), ulcer inhibition (%), hemorrhagic area, gastric mucus weight, and digital morphometry. These endpoints would reduce subjectivity and allow more complete comparison with other gastroprotection studies. Similarly, histology should be supported by fully blinded scoring, calibrated image analysis, and clear reporting of scale bars and magnification.

Several limitations must be emphasized. First, the small group size limited power and increased the risk of Type II error. Second, only two BOE doses were examined, which is insufficient to establish dose-response relationships. Third, the extract was not chemically standardized, and the concentrations of key constituent classes were not quantified. Fourth, no biochemical assays were performed for oxidative stress, inflammatory cytokines, prostaglandins, gastric mucus, gastric acidity, or Nrf2/HO-1-related pathways. Fifth, the study did not include ulcerated area, ulcer inhibition, hemorrhagic area, or gastric mucus weight. Sixth, the acute HCl/ethanol model does not represent all clinically relevant ulcer mechanisms, such as NSAID-induced or chronic ulcer-healing processes.

Overall, this short communication provides a cautious preliminary basis for follow-up work. A confirmatory study should be powered a priori, include intermediate and higher doses, use a clearly defined vehicle group, quantify ulcerated area and mucus-related endpoints, chemically standardize BOE, and test mechanistic biomarkers such as malondialdehyde, glutathione, superoxide dismutase, catalase, prostaglandin E2, TNF-alpha, IL-1beta, IL-6, NF-kappaB, and Nrf2/HO-1. Until such data are available, BOE should be described as showing a possible gastroprotective trend rather than proven efficacy.

Figure 4

Exploratory study design and summary of the observed numerical gastroprotective trends



Note: Male Sprague–Dawley rats (n = 6/group) received oral treatment for 7 days with the ulcer-control vehicle, omeprazole (100 mg/kg), or aqueous Bixa orellana leaf extract (BOE; 50 or 150 mg/kg), followed by HCl/ethanol-induced gastric injury. Macroscopic ulcer index and hematoxylin and eosin (H&E) histopathology were assessed. BOE 150 mg/kg showed a lower mean ulcer index (3.85 ± 1.34) than the untreated ulcer control (5.16 ± 1.51); however, the overall Kruskal–Wallis test was not statistically significant (H = 6.57, p = 0.087). Histological outcomes were likewise non-significant. Representative H&E sections illustrate severe mucosal injury in the untreated ulcer control and comparatively less severe tissue disruption in the BOE 150 mg/kg group. Accordingly, the figure illustrates a possible gastroprotective trend and does not represent statistically confirmed efficacy. BOE = Bixa orellana extract; H&E = hematoxylin and eosin; SD = standard deviation.

CONCLUSION

Aqueous *B. orellana* leaf extract did not demonstrate a statistically significant protective effect in the HCl/ethanol-induced gastric ulcer rat model. The 150 mg/kg dose showed the most favorable numerical trend, with lower ulcer-index and histological injury scores than the untreated ulcer control, whereas 50 mg/kg did not show consistent

protection. These results should be interpreted as exploratory biological trends rather than conclusive gastroprotective efficacy. Future studies require larger sample sizes, intermediate dose groups, vehicle control clarity, chemical standardization, ulcer-area and mucus endpoints, fully blinded morphometry, and biochemical assays before any mechanistic or translational claims can be made. The experimental design and principal exploratory findings of the present study are summarized graphically in Figure 4.

AUTHOR CONTRIBUTIONS

Zuraini Ahmad and Muhammad Nazrul Hakim Abdullah conceptualized the study. Fong Lai Yen and Ng Chin Theng developed the methodology. Asma Syuhaily Mohd Yusof and Yong Yoke Keong conducted the investigation. Shiran Mohd Sidik, Saidi Abdul Moin, and Mohd Sofian Omar Fauzee performed the formal analysis. Asma Syuhaily Mohd Yusof and Zuraini Ahmad prepared the original draft. Muhammad Nazrul Hakim Abdullah, Mohd Sofian Omar Fauzee, Yong Yoke Keong, Fong Lai Yen, Ng Chin Theng, Shiran Mohd Sidik, Saidi Abdul Moin, and Zuraini Ahmad reviewed and edited the manuscript. Shiran Mohd Sidik, Saidi Abdul Moin, and Zuraini Ahmad supervised the study. Zuraini Ahmad and Muhammad Nazrul Hakim Abdullah were responsible for project administration.

ETHICS APPROVAL

Ethical approval for this animal study was obtained from the Animal Care and Use Committee, Universiti Putra Malaysia (approval reference no. UPM/FPSK/PADS/BR-UUH/00495). All animal procedures were conducted in accordance with the approved protocol and applicable institutional guidelines for the care and use of laboratory animals. Informed consent was not applicable because the study involved laboratory animals and no human participants.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest in this work.

ACKNOWLEDGEMENTS

Not applicable.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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