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THE THERAPEUTIC EFFECT OF ETHANOL EXTRACT FROM *ARGYREIA ACUTA* LOUR. LEAVES IN THE TREATMENT OF INDOMETHACIN-INDUCED GASTRITIS IN MICE

Tran Thi Phuong Nhung, Le Pham Tan Quoc, Tran Thi Ai Vi*

Address(es): Le Pham Tan Quoc,
Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam,

*Corresponding author: lephamtanquoc@iuh.edu.vn

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly associated with gastric mucosal injury. Based on its known antioxidant and anti-inflammatory properties, this study hypothesized that the ethanol extract of Argyreia acuta leaves (EEAA) could attenuate indomethacin-induced gastric damage. Mice were allocated into six groups: normal control, indomethacin-induced ulcer model, omeprazole-treated, and three EEAA-treated groups (100, 200, 300 mg/kg). Gastric protection was assessed through gross ulceration, inflammatory cytokines, antioxidant biomarkers, and histopathological changes. Indomethacin administration led to marked mucosal lesions, inflammation, and oxidative stress. EEAA treatment, particularly at higher doses, significantly ameliorated these effects, improving gastric morphology, modulating cytokine expression, and restoring antioxidant balance. Histological findings further supported the protective effect. These results indicate that EEAA confers dose-dependent gastroprotection, mediated by its anti-inflammatory and antioxidant mechanisms, and offer therapeutic value against NSAID-induced gastric injury.

Keywords: Ethanol extract, Indomethacin-induced gastritis, Gastritis treatment, Anti-inflammatory properties

INTRODUCTION

Gastritis is an inflammation of the gastric mucosa, commonly triggered by nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin **(Sohail *et al.*, 2023)**. While NSAIDs are effective for pain and inflammation, they compromise the protective gastric barrier, increasing the risk of mucosal injury and ulceration **(Takeuchi, 2022)**. Gastritis is associated with symptoms like abdominal pain, nausea, and indigestion, and in severe cases, lead to bleeding or chronic ulcers. The widespread use of NSAIDs has contributed to a growing prevalence of gastritis, particularly in developed countries. This condition also imposes a substantial economic burden through direct healthcare costs and reduced productivity **(Guo and Leung, 2019)**. Epidemiological data indicate that up to 50% of chronic NSAID users develop some degree of gastric mucosal injury, and approximately 10-20% of these cases progress to clinically significant ulcers or complications. The risk increases with higher doses and longer durations of NSAID use **(McEvoy *et al.*, 2021; Guo and Leung, 2019)**.

Conventional treatment involves antacids, proton pump inhibitors (PPIs), and mucosal protectants. However, long-term use of these agents may result in side effects such as nutrient malabsorption, infections, and bone loss **(Maideen, 2023; Scarpignato *et al.*, 2016)**. These limitations have prompted interest in alternative therapies, particularly plant-based agents with mucosal protective properties **(Bi *et al.*, 2014)**. At the cellular level, NSAIDs such as indomethacin inhibit cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis in the gastric mucosa. Prostaglandins are essential for maintaining mucosal blood flow, mucus secretion, and epithelial cell regeneration. Their depletion results in increased susceptibility to acid-induced injury, inflammation, and oxidative stress.

Argyreia acuta Lour., a member of the Convolvulaceae family, is native to Southeast Asia and known for its antioxidant, antibacterial, and anti-inflammatory properties. Its ethanol leaf extract contains flavonoids, alkaloids, and tannins, which have demonstrated activity in modulating pro-inflammatory mediators such as cytokines and cyclooxygenase (COX) **(Li *et al.*, 2021)**. These compounds are also reported to enhance endogenous antioxidant defense systems by upregulating superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC), thereby neutralizing reactive oxygen species (ROS) that contribute to mucosal damage. Other species in this family, including *Ipomoea batatas*, *Ipomoea aquatica*, *Convolvulus arvensis*, and *Argyreia speciosa*, have shown protective effects against gastric inflammation and NSAID-induced injury **(Pasha *et al.*, 2022; Dua *et al.*, 2015; Azman *et al.*, 2015; Jaiswal *et al.*, 2011)**.

Based on these pharmacological properties and structural similarities to other gastroprotective species, we hypothesize that the ethanol extract of *A. acuta* leaves exerts a protective effect against NSAID-induced gastritis. Specifically, we propose that the extract reduces gastric inflammation by inhibiting pro-inflammatory cytokines (e.g., TNF-α, IL-1β), restores antioxidant balance, and limits mucosal erosion through histological preservation.

The objective of this study is to evaluate the gastroprotective efficacy of *A. acuta* extract in a murine model of indomethacin-induced gastritis. This investigation aims to provide scientific evidence supporting the use of *A. acuta* as a potential natural therapeutic agent for gastric mucosal protection.

MATERIAL AND METHODS

Hypothesis and study design

This study was conducted to test the hypothesis that ethanol extract of *Argyreia acuta* leaves (EEAA) exerts gastroprotective effects against NSAID-induced gastric injury by modulating oxidative stress and inflammatory responses. A combination of phytochemical analysis, biochemical assays, histopathology, and ulcer scoring was employed to validate this hypothesis using an indomethacin-induced murine model.

Plant material collection and extract preparation

Argyreia acuta Lour. leaves were collected in June 2024 from the Son Tra region of Quang Ngai province, Vietnam. A reference specimen (code AC090624VST) was deposited at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City. After manual inspection and washing, the leaves were shade-dried for four days to preserve their bioactive compounds, then ground using a FitzMill Laboratory Grinder (The Fitzpatrick Company, USA) and stored in airtight containers under dry, ventilated conditions.

Approximately 60 g of powdered leaves were extracted with 600 mL of ethanol using ultrasonic-assisted extraction (UAE) at a frequency of 50 kHz and power output of 200 W for 45 minutes with intermittent agitation. The extract was filtered through Whatman No. 1 filter paper and concentrated at 55°C using a rotary evaporator (IKA-Werke GmbH & Co. KG, Germany). The final extract (EEAA) was stored in sealed amber glass containers at 4 °C. The extraction yield was 28%.

Phytochemical screening and quantification

Qualitative phytochemical screening of the ethanol extract from *Argyreia acuta* leaves (EEAA) was conducted to identify the presence of key bioactive compounds, including tannins, flavonoids, terpenoids, polyphenols, saponins, alkaloids, steroids, and cardiac glycosides, using standard colorimetric and precipitation-based assays **(Tran and Tran, 2021)**. For quantitative assessment, spectrophotometric methods were employed. Flavonoid content was determined by complexation with aluminum chloride ($AlCl_3$), with absorbance measured at 415 nm **(Nhung and Quoc, 2024)**, while total polyphenols were quantified using the Folin-Ciocalteu reagent, read at 765 nm **(Nhung and Quoc, 2025)**. Terpenoid levels were evaluated via the vanillin-acetic acid method with absorbance at 538 nm **(Tran *et al.*, 2023)**, and saponin content was measured using the vanillin-sulfuric acid method at 560 nm **(Tran and Tran, 2024)**. Calibration curves were prepared using quercetin for flavonoids, gallic acid for polyphenols, and diosgenin for saponins. Results were expressed as milligram equivalents of each standard per gram of extract.

Animals and housing conditions

Healthy Swiss albino mice (29-31 g) were procured from the Pasteur Institute, Ho Chi Minh City. The animals were acclimatized for 14 days under controlled conditions: 23-25°C, 50-60% humidity, and 12 h light/dark cycles. Mice were housed in sawdust-lined cages (sawdust treated with odor-neutralizing bio-agents) and provided ad libitum access to filtered water and a standard pellet diet. All procedures complied with the guidelines of **NACLAR (2022)**.

Sample size (n = 5 per group) was determined based on prior ulcer model studies showing sufficient statistical power (≥0.8) to detect intergroup differences **(Alamr, 2024; Weng *et al.*, 2024)**.

Experimental design

Thirty Swiss albino mice were randomly divided into six groups (n = 5 per group): a normal control group (vehicle only, no ulcer induction), an ulcer control group receiving indomethacin (IND, 50 mg/kg), a positive control group treated with omeprazole (20 mg/kg), and three treatment groups administered EEAA at doses of 100, 200, and 300 mg/kg, respectively. Gastric ulcers were induced in all groups except the normal control using a single oral dose of indomethacin **(Alamr, 2024)**. EEAA and omeprazole were administered orally once daily for seven consecutive days following ulcer induction. Therapeutic efficacy was evaluated through ulcer index (UI), ulcer healing percentage (UHP), hematological and biochemical parameters, antioxidant and cytokine profiles, and histopathological examination.

Macroscopic ulcer scoring and gastric ulcer index (GUI)

Following euthanasia, the stomachs were dissected along the greater curvature, rinsed with cold saline, and pinned flat for macroscopic evaluation. Ulcer severity was assessed using a standardized gastric ulcer index (GUI) scoring system based on visible mucosal damage **(Eltahir, 2024)**, where scores ranged from 0 to 5: 0 indicated normal mucosa; 1, mild hyperemia or small hemorrhagic spots; 1.5-2, hemorrhagic streaks or up to five small ulcers; 3, more than five small ulcers; 4, a combination of small and large ulcers; and 5, extensive ulceration or perforation.

Ulcer index determination and percentage of ulcer healing

Ulcer severity was further quantified using the scoring method described by **Roy and Roy (2023)**, in which lesions were rated from 0 to 3: 0 indicated normal mucosa, 0.5 mild redness, 1 spot ulcers, 1.5 hemorrhagic streaks, 2 deep ulcers, and 3 perforation. The ulcer index (UI) was calculated using the formula: $UI = UN + US + (UP \times 10^{-1})$, where UN is the mean number of ulcers per animal, US the mean severity score, and UP the percentage of animals with ulcers. The ulcer healing percentage (UHP) was determined as: $UHP\,(\%) = \frac{(\text{UI of control} - \text{UI of treated})}{\text{UI of control}} \times 100$

Hematological and biochemical analyses

Blood was collected via cardiac puncture under anesthesia. Hematological parameters (RBC, WBC, platelets) were measured using a Mindray BC-2800 auto-analyzer (Mindray Bio-Medical Electronics, China). Serum for biochemical analysis was separated using SST tubes, centrifuged at 3,000 rpm for 10 min (Hermle Z206A), and stored at −20°C. Parameters measured included glucose, ALT, AST, BUN, creatinine, and albumin using a Hitachi 902 auto-analyzer (Hitachi High-Technologies, Japan).

Antioxidant assays

Gastric tissues were homogenized in phosphate buffer (pH 7.4) and centrifuged to obtain supernatants for antioxidant analysis. Superoxide dismutase (SOD) activity was determined based on the inhibition of pyrogallol autoxidation, with absorbance measured at 420 nm. Catalase (CAT) activity was assessed by monitoring the decomposition rate of hydrogen peroxide at 240 nm. Total antioxidant capacity (TAC) was evaluated using the ABTS radical scavenging assay, with absorbance recorded at 734 nm **(Nhung and Quoc, 2024; Tran and Tran, 2021)**. Quantification was performed using standard calibration curves, and results were expressed as mmol/min/mL for enzymatic activity or µM Trolox equivalents for TAC.

Cytokine analysis

Serum levels of TNF-α, IL-1β, and IL-6 were quantified using sandwich ELISA kits. Plates were coated with monoclonal antibodies, followed by biotinylated detection antibodies, streptavidin-HRP, and TMB substrate. Absorbance was read at 450 nm. Cytokine concentrations were calculated from standard curves and expressed in pg/mL **(Nhung and Quoc, 2024)**.

Histological evaluation

Gastric tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-5 µm (Leica RM2235), and stained with H&E, PAS, and Masson's trichrome. Histopathological changes were observed under an Olympus CX23 microscope, and images were captured using a Nikon DS-Fi3 camera. Scoring criteria included epithelial integrity, necrosis, inflammation, and mucosal regeneration **(Tran and Tran, 2024)**.

Statistical analysis

Data were expressed as mean ± SD. One-way ANOVA was performed using Statgraphics Centurion XX. Tukey's post hoc test was applied for multiple comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis of the extract

Table 1 Phytochemical screening and quantification of ethanol extract from *Argyreia acuta* leaves

Phytoconstituents	Test	Observation	Present in EEAA	Quantification of phytochemicals
Tannins	2 mL EEAA + 2 mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate	+	NT
Flavonoids	1 mL EEAA + 1 mL $Pb(OAc)_4$ (10%)	Yellow coloration	+	38.56 ± 1.25 (mg QE/g)
Terpenoids	2 mL EEAA + 2 mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration	+	66.43 ± 1.81 (mg TAE/g)
Polyphenol	2 mL EEAA + 2 mL $FeCl_3$	Bluish-green appearance	+	67.77 ± 1.45 (mg GAE/g)
Saponins	5 mL EEAA + 5 mL H_2O + heat	Froth appears	+	11.98 ± 0.63 (mg SE/g)
Steroids	2 mL EEAA + 2 mL $CHCl_3$ + 2mL H_2SO_4 (conc.)	The reddish-brown ring at the junction	+	NT
Cardiac glycosides	2 mL EEAA + 2 mL $CHCl_3$ + 2mL CH_3COOH	Violet to Blue to Green coloration	-	-
Alkaloids	2 mL EEAA + a few drops of Hager's reagent	Yellow precipitate	+	NT

Phytochemicals in EEAA are (+) present, (-) absent, and (NT) not tested.

The ethanol extract of *Argyreia acuta* (EEAA) was found to contain key phytochemical constituents, including tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, and alkaloids, while cardiac glycosides were absent **(Tran and Tran, 2021)**. Quantitative analysis revealed high levels of flavonoids (38.56 ± 1.25 mg QE/g), polyphenols (67.77 ± 1.45 mg GAE/g), and terpenoids (66.43 ± 1.81 mg TAE/g), with moderate saponin content (11.98 ± 0.63 mg SE/g) (Table 1). These compounds are known for their antioxidant and anti-inflammatory properties, which contribute to mucosal protection **(Park and Hahm, 2024)**. Flavonoids and polyphenols neutralize reactive oxygen species (ROS), inhibit lipid peroxidation, and modulate pro-inflammatory signaling pathways such as NF-κB and COX-2 **(Cherrada *et al.*, 2024)**. Terpenoids support mucosal healing by enhancing prostaglandin synthesis, inhibiting cytokine production, and promoting mucus secretion **(Tran and Tran, 2024)**. The absence of cardiac glycosides eliminates the risk of cardiotoxicity, supporting EEAA's safety profile.

Macroscopic findings and gastric ulcer index assessment

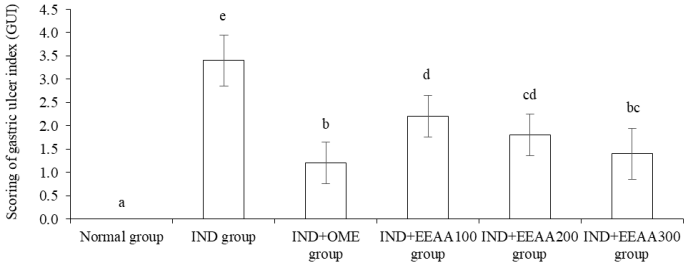

Figure 1 Effect of EEAA on gastric ulcer index in IND-induced ulcer model. Data are presented as Mean ± SD. Different letters (a, b, c, d, and e) denote statistically significant differences between groups ($p < 0.05$).

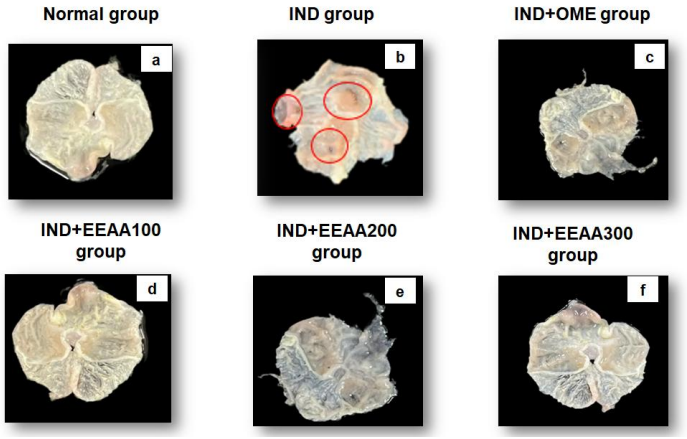

Figure 2 Macroscopic evaluation of gastric tissue in IND-induced ulcer model and treatment groups. (a) Normal group: Intact gastric mucosa; (b) IND group: Severe mucosal damage and ulceration; (c) IND+OME group: Reduced ulceration with preserved mucosa; (d) IND+EEAA100 group: Moderate ulcer protection; (e) IND+EEAA200 group: Improved gastric protection; (f) IND+EEAA300 group: Significant mucosal preservation, similar to normal.

Figure 1 shows significant differences ($p < 0.05$) in gastric ulcer index (GUI) among the groups. Indomethacin (IND) induced severe mucosal damage (3.40 ± 0.55), while EEAA reduced this damage in a dose-dependent manner: GUI values were 2.20 ± 0.45 (EEAA100), 1.80 ± 0.45 (EEAA200), and 1.40 ± 0.55 (EEAA300). Omeprazole (IND+OME) also showed substantial protection (1.20 ± 0.45). Macroscopic images (Figure 2) support these findings, with near-complete restoration of the gastric mucosa observed in the EEAA300 and IND+OME groups. The protective effects of EEAA are attributed to its high levels of flavonoids and polyphenols, which reduce oxidative stress and inflammatory responses **(Sharifi-Rad *et al.*, 2018)**.

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Ulcer index and healing percentage

Table 2 Ulcer index and percentage of ulcer healing in IND-induced ulcer model with EEAA

Parameters	Normal group	IND group	IND+OME group	IND+EEAA100 group	IND+EEAA200 group	IND+EEAA300 group
Ulcer index (UI)	0.00 ± 0.00^{a}	$4.16 \pm .09^{f}$	1.66 ± 0.01^{b}	2.77 ± 0.02^{e}	2.45 ± 0.01^{d}	1.89 ± 0.06^{c}
Percentage of ulcer healing (PUH) (%)	100.00 ± 0.00^{a}	$0.00 \pm .00^{f}$	60.03 ± 0.54^{b}	33.51 ± 1.01^{e}	41.19 ± 1.09^{d}	54.51 ± 0.71^{c}

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between groups ($p < 0.05$)

As shown in Table 2, the ulcer index (UI) was significantly reduced in the treatment groups, with the EEAA300 group showing the lowest UI (1.89 ± 0.06) among extract-treated groups and a corresponding ulcer healing percentage (PUH) of 54.51 ± 0.71%. The improvement correlates with the extract's ability to modulate oxidative stress and inflammatory mediators. Indomethacin-induced ulcers result from COX-1 inhibition, leading to decreased prostaglandins, reduced mucus production, and increased ROS **(Soydan *et al.*, 2024)**. Omeprazole improved healing by inhibiting acid secretion **(Pegg *et al.*, 2023)**, while EEAA exerted a protective effect through its bioactive constituents, which enhance mucosal defense and repair **(Sun *et al.*, 2025)**. To enhance the statistical robustness of the findings, future studies should consider increasing the sample size and employing more advanced analytical approaches such as repeated measures ANOVA or mixed-effects modeling, which can better account for intra-group variability and time-dependent effects.

Hematological and biochemical analysis

Table 3 Hematological and biochemical analysis in IND-induced ulcer model with EEAA

Parameters	Normal group	IND group	IND+OME group	IND+EEAA100 group	IND+EEAA200 group	IND+EEAA300 group
RBC ($\times 10^6$ cells/mm^3)	6.37 ± 0.23^{f}	3.03 ± 0.11^{a}	5.79 ± 0.21^{e}	3.94 ± 0.14^{b}	4.55 ± 0.15^{c}	5.31 ± 0.08^{d}
WBC ($\times 10^3$ cells/mm^3)	5.22 ± 0.18^{a}	10.96 ± 0.49^{f}	5.74 ± 0.21^{b}	8.35 ± 0.29^{e}	7.31 ± 0.26^{d}	6.26 ± 0.09^{c}
PLT ($\times 10^3$ cells/mm^3)	743.39 ± 19.92^{a}	1561.12 ± 95.15^{f}	817.73 ± 18.49^{b}	1189.42 ± 54.46^{e}	1040.75 ± 49.94^{d}	892.07 ± 24.11^{c}
CRP (mg/dL)	0.46 ± 0.02^{a}	0.97 ± 0.04^{d}	0.51 ± 0.02^{a}	0.74 ± 0.08^{c}	$0.64 \pm 0.18b^{c}$	0.55 ± 0.02^{ab}
ALT (U/L)	42.49 ± 1.87^{a}	89.23 ± 3.89^{f}	46.74 ± 2.03^{b}	67.98 ± 1.34^{e}	59.49 ± 2.61^{d}	50.99 ± 2.35^{c}
AST (U/L)	194.51 ± 11.82^{a}	408.47 ± 16.93^{f}	213.96 ± 5.81^{b}	311.22 ± 18.89^{e}	272.31 ± 16.48^{d}	233.41 ± 14.17^{c}
BUN (mg/dL)	17.42 ± 0.38^{a}	36.58 ± 0.65^{f}	19.16 ± 0.23^{b}	27.87 ± 0.55^{e}	24.39 ± 0.51^{d}	21.08 ± 0.41^{c}
Creatinine (mg/dL)	0.34 ± 0.01^{a}	0.71 ± 0.03^{f}	0.37 ± 0.01^{b}	0.54 ± 0.02^{e}	0.48 ± 0.02^{d}	0.41 ± 0.01^{c}
Albumin (g/dL)	3.25 ± 0.16^{f}	1.55 ± 0.02^{a}	2.95 ± 0.05^{e}	2.03 ± 0.07^{b}	2.32 ± 0.08^{c}	2.71 ± 0.11^{d}

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between groups ($p < 0.05$)

Indomethacin significantly reduced RBC count and elevated WBC, PLT, CRP, ALT, AST, BUN, and creatinine levels ($p < 0.05$) (Table 3), indicating systemic inflammation, hepatic stress, and renal dysfunction **(Zamani *et al.*, 2024)**. EEAA, particularly at 300 mg/kg, reversed these effects, restoring hematological balance and reducing hepatic and renal stress markers **(Nhung and Quoc, 2024)**. The hepatoprotective and nephroprotective effects of EEAA are mediated through its antioxidant mechanisms that reduce lipid peroxidation and support cellular homeostasis **(Al-Naemi *et al.*, 2024; Li *et al.*, 2022)**.

Antioxidant analysis in gastric ulcer treatment

As summarized in Table 4, EEAA reduced SOD, CAT, and TAC levels in gastric tissue in a dose-dependent manner ($p < 0.05$). Elevated oxidative markers in the IND group confirmed oxidative damage, while reductions in treated groups indicate restoration of redox balance. EEAA's polyphenolic and flavonoid content plays a key role in neutralizing ROS and inhibiting lipid peroxidation **(Nhung and Quoc, 2024)**. Although omeprazole had stronger antioxidant effects, EEAA300 showed substantial efficacy, suggesting its therapeutic potential. Future studies incorporating MDA and GSH measurements are recommended to further validate these mechanisms.

Table 4 Antioxidant enzyme activities and total antioxidant capacity in IND-induced ulcer model with EEAA

Parameters	Normal group	IND group	IND+OME group	IND+EEAA100 group	IND+EEAA200 group	IND+EEAA300 group
SOD (mmol H_2O_2/min/mL)	42.55 ± 1.86^a	80.85 ± 3.56^f	46.81 ± 1.15^b	63.83 ± 1.62^e	59.57 ± 1.52^d	51.06 ± 1.24^c
CAT (mmol H_2O_2/min/mL)	0.66 ± 0.02^a	1.25 ± 0.02^f	0.73 ± 0.03^b	0.99 ± 0.02^e	0.92 ± 0.03^d	0.79 ± 0.03^c
TAC (µM Trolox)	3.43 ± 0.06^a	6.52 ± 0.12^f	3.77 ± 0.07^b	5.15 ± 0.17^e	4.81 ± 0.18^d	4.12 ± 0.15^c

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between groups ($p < 0.05$)

Cytokine analysis in gastric ulcer treatment

Figure 3 demonstrates that EEAA significantly reduced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) in a dose-dependent manner ($p < 0.05$), with EEAA300 producing effects comparable to omeprazole. These reductions are attributed to the suppression of cytokine production and inhibition of inflammatory pathways such as NF-κB **(Júnior *et al.*, 2024; Shahzad *et al.*, 2024)**. Future studies using RT-PCR or Western blot could elucidate the molecular mechanisms underlying EEAA's cytokine-modulatory effects.

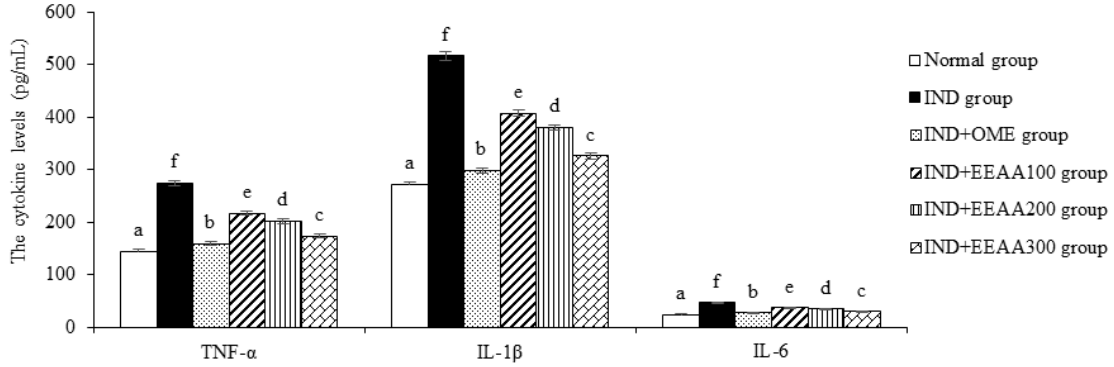

Figure 3 Cytokine levels (TNF-α, IL-1β, and IL-6) in IND-induced ulcer model with EEAA. Data are presented as Mean ± SD. Different letters (a, b, c, d, e, and f) denote statistically significant differences between groups ($p < 0.05$).

Histological analysis of gastric tissue

Histological evaluation (Figure 4) revealed severe epithelial disruption, inflammation, and hemorrhage in the IND group. Omeprazole and EEAA treatments resulted in progressive mucosal restoration, with EEAA300 showing nearly complete regeneration. These findings confirm the dose-dependent protective effect of EEAA, mediated by its antioxidant, anti-inflammatory, and epithelial-regenerative activities **(Soydan *et al.*, 2024; García-Pérez *et al.*, 2024)**.

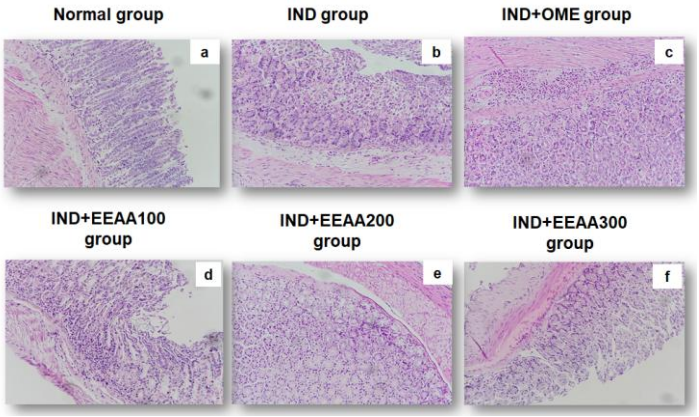

Figure 4 Histopathological analysis of gastric tissue in IND-induced ulcer model with EEAA (H&E staining, ×200). (a) Normal group: Intact mucosa with normal epithelial and glandular structure.; (b) IND group: Severe mucosal damage, inflammation, and ulcer formation.; (c) IND+OME group: Partial healing with reduced inflammation and improved integrity.; (d) IND+EEAA100 group: Moderate protection with signs of regeneration.; (e) IND+EEAA200 group: Enhanced epithelial restoration, less inflammation.; (f) IND+EEAA300 group: Significant mucosal preservation.

Translational and clinical relevance

While this study provides strong evidence for the gastroprotective effects of EEAA in a murine model, translation to human application requires caution. Pharmacokinetics, bioavailability, and safety profiling in humans remain unexplored. Moreover, molecular confirmation of mechanistic pathways is needed. Future clinical trials and molecular studies are essential to validate EEAA as a potential therapeutic agent for NSAID-induced gastric injuries. In addition, it is important to consider the pharmacodynamic differences between rodents and humans, which may influence the extract's efficacy and safety profile. Identifying optimal dosing strategies, long-term toxicity thresholds, and potential herb-drug interactions will be critical steps toward clinical application. Addressing these challenges through well-designed translational studies will help clarify EEAA's therapeutic relevance and support its development as a candidate for human use.

CONCLUSION

This study tested the hypothesis that Argyreia acuta ethanol leaf extract (EEAA) confers gastroprotection against NSAID-induced injury via anti-inflammatory and antioxidant mechanisms. Indomethacin caused significant gastric damage, systemic inflammation, oxidative stress, and organ dysfunction. Omeprazole mitigated these effects, and EEAA, particularly at 300 mg/kg, produced comparable outcomes, restoring mucosal integrity, reducing cytokine levels, and normalizing biochemical markers. Despite these promising findings, limitations include small sample size and absence of molecular validation. Further studies with expanded cohorts and mechanistic assays (e.g., RT-PCR, Western blot) are needed. While EEAA shows potential as a plant-based alternative, its clinical translation warrants investigation into bioavailability, safety, and therapeutic applicability in humans.

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