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Original Research Article

Therapeutic Potential and Dose-Response Correlation of *Argyreia acuta* Lour. Extract in Oxidative Stress and Gastric Mucosal Protection

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin can cause gastric mucosal injury through oxidative stress and immune imbalance. This study evaluated the dose-dependent gastroprotective, antioxidant, and immunomodulatory effects of ethanol extract from *Argyreia acuta* Lour. leaves (EE-AC) in a murine model of indomethacin-induced gastric damage. Mice were treated with extract (100, 200, or 300 mg/kg) for seven days following ulcer induction. The *A. acuta* leaf extract (EE-AC) significantly reduced ulcer scores and gastric acidity, while increasing pH and healing rates. It also enhanced antioxidant enzymes (SOD, CAT, GPx, GR), total antioxidant capacity, and reduced MDA and H_2O_2 levels. Immune parameters improved, with decreased levels of TNF-α, IL-1β, and IL-6, and enhanced phagocytic responses. Strong dose-response correlations ($|r| > 0.95$, $p < 0.05$) were observed across all markers. These findings support the therapeutic potential of EE-AC as a multi-target natural agent for gastric protection, warranting further preclinical and clinical validation.

Keywords: Gastroprotection, Antioxidant enzymes, Immunomodulation, Dose-dependent effects, *Argyreia acuta*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively prescribed for the treatment of pain and inflammation; however, their prolonged use is a major etiological factor in the development of gastric mucosal injury and peptic ulceration.[1] Among NSAIDs, indomethacin is widely employed in experimental studies due to its potent ulcerogenic effects, which are primarily mediated through the inhibition of cyclooxygenase (COX) enzymes, induction of oxidative stress, mitochondrial dysfunction, and the activation of pro-inflammatory cytokines.[2] Oxidative stress plays a pivotal role in the pathogenesis of NSAID-induced gastric damage. Excessive generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), leads to lipid peroxidation, protein oxidation, and DNA damage, collectively compromising mucosal integrity.[3] This oxidative insult is typically evidenced by elevated levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, and concurrent depletion of endogenous antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH).[4] Simultaneously, immunological disturbances, marked by increased expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), further aggravate mucosal inflammation. Hyperactivation of phagocytes also contributes to this pathology by releasing additional ROS and proteolytic enzymes.[5]

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In light of these mechanisms, medicinal plants have gained attention as alternative therapeutic agents owing to their multifaceted bioactivities, including antioxidant, anti-inflammatory, cytoprotective, and immunomodulatory effects.[6] Bioactive phytoconstituents such as flavonoids, polyphenols, alkaloids, terpenoids, and saponins exhibit protective actions through attenuation of oxidative stress, modulation of inflammatory pathways, and preservation of mucosal architecture.[7]

Argyreia acuta, a species within the family Convolvulaceae and indigenous to tropical Asia, has been traditionally utilized in folk medicine for the management of inflammation, wound healing, and gastrointestinal ailments.[8,9] Phytochemical investigations have demonstrated that its leaves contain abundant flavonoids, polyphenols, alkaloids, terpenoids, and saponins,[10] which are known to confer antioxidant, anti-inflammatory, and immunoregulatory activities. Despite these promising attributes, the gastroprotective efficacy of *A. acuta*, particularly concerning dose-response dynamics and correlations with oxidative and immunological biomarkers, has not been systematically evaluated.

Accordingly, the present study investigated the dose-dependent gastroprotective, antioxidant, and immunomodulatory effects of the ethanol extract derived from *A. acuta* leaves (EE-AC) in a murine model of indomethacin-induced gastric injury. Furthermore, the study elucidated the correlation between the dosage of ethanol extract from *Argyreia acuta* Lour. leaves (EE-AC) and key biochemical and immunological indices associated with ulcer healing.

Materials and Methods

Plant material and extract preparation

Mature leaves of *Argyreia acuta* Lour. were collected in October 2024 from the Son Tra region, Quang Ngai Province, Vietnam. A voucher specimen (Reference Code: AC131024VST) was authenticated and deposited at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, for future reference. The freshly harvested leaves were thoroughly washed with distilled water to eliminate surface impurities, then air-dried under shade at ambient temperature in a well-ventilated environment to preserve the phytochemical constituents. The dried material was pulverized into coarse powder using a mechanical grinder (IKA® MF 10 basic, IKA-Werke GmbH & Co. KG, Germany).

The powdered material was subjected to cold maceration in 70% ethanol (1:10 w/v) for 72 hours at room temperature. During the

extraction process, the mixture was intermittently agitated using a magnetic stirrer (Velp Scientifica AREX Digital, Italy) at 200 rpm for 30 minutes every 12 hours to maximize the yield. After maceration, the extract was filtered through Whatman No. 1 filter paper, and the filtrate was subsequently concentrated under reduced pressure at 40°C using a rotary evaporator (Büchi Rotavapor® R-300, Switzerland). The resulting crude ethanol extract (EE-AC) was dried and stored in amber-colored airtight containers at 4°C until further use.
Immediately before experimental administration, EE-AC was reconstituted in 0.5% carboxymethyl cellulose (CMC) to ensure homogeneous dispersion and precise dosage delivery.

Phytochemical screening and quantification
Preliminary phytochemical analysis of EE-AC was conducted to detect major bioactive constituents, including tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, alkaloids, and cardiac glycosides.[11]
Total flavonoid content was quantified by the aluminum chloride colorimetric method (absorbance at 415 nm).
Polyphenols were determined using the Folin-Ciocalteu assay (absorbance at 765 nm).
Terpenoids were assessed via a modified Salkowski test (absorbance at 538 nm).
Alkaloid content was quantified gravimetrically following Dragendorff's reagent precipitation.
These analyses supported the pharmacological evaluation by confirming the presence of key antioxidant-related compounds. [12]

Experimental animals
Thirty Swiss mice (29-31 g) were obtained from the Pasteur Institute (Ho Chi Minh City, Vietnam). Animals were housed in ventilated glass cages containing bedding of biologically treated rice husks. Standardized environmental conditions were maintained (24-26°C, 50-60% humidity, 12-hour light/dark cycle). Animals had free access to commercial rodent feed and filtered drinking water. After a 7-day acclimatization period, healthy mice were selected for the experiment.
Animal care and all procedures adhered to internationally recognized ethical standards, including the Basel Declaration and the International Guiding Principles for Biomedical Research Involving Animals.[13]
Humane endpoints were established: animals exhibiting severe distress, lethargy, loss of >20% body weight, severe dehydration, or inability to access food or water were humanely euthanized before the study endpoint.

Experimental design and treatment protocol
The mice were randomly assigned into six groups (n = 5 per group) to evaluate the gastroprotective, antioxidant, and immunomodulatory effects of the ethanol extract from *Argyreia acuta* leaves (EE-AC) against indomethacin-induced gastric injury.
Group 1 (Normal control) received 0.5% carboxymethyl cellulose (CMC) orally.
Group 2 (Ulcer control) was administered a single oral dose of indomethacin (30 mg/kg) to induce gastric ulcers.
Group 3 (Positive control) received indomethacin (30 mg/kg) followed by omeprazole (20 mg/kg) orally.
Groups 4 to 6 were treated with indomethacin (30 mg/kg) followed by EE-AC at doses of 100, 200, or 300 mg/kg, respectively.
Gastric ulcers were induced by a single oral administration of indomethacin (30 mg/kg) after a 24-hour fasting period, during which water was provided ad libitum. Treatments with omeprazole or EE-AC were given orally once daily for seven consecutive days using a blunt-ended gavage needle. The selected EE-AC doses were based on preliminary dose-finding experiments and previous studies on related phytochemical-rich plant extracts, demonstrating safety and therapeutic relevance.[4,6]
At the end of the treatment period, animals were euthanized by CO_2 inhalation. The stomachs were removed, opened along the greater curvature, and rinsed with saline. Gastric parameters (ulcer index, pH, and healing rate) were measured macroscopically. Ulcer scoring was conducted according to the method described by Prayola *et al*. (2025),[14] using a macroscopic scale ranging from 0 to 5 (0 = no lesion, 1 = superficial erosion, 2 = deep erosion, 3 = one or two ulcers, 4 = multiple ulcers, 5 = perforation). Gastric pH was measured using a calibrated digital pH meter. Oxidative stress biomarkers**,** including MDA, H_2O_2, SOD, CAT, GPx, GR, GSH, and TAC, were analyzed using spectrophotometric methods as described by Zahouani *et al*.[15]
Immune function parameters, such as total WBC count, thymus index, NBT reduction, phagocytic index, and cytokine levels (TNF-α, IL-1β, IL-6), were assessed using standard protocols from Bio-Rad ELISA kits and procedures adapted from Marimuthu *et al.* and Xu *et al*.[16,17]

Dose-response correlation and statistical analysis
To elucidate the dose-response relationships of the ethanol extract from *Argyreia acuta* leaves (EE-AC), pharmacological outcomes were systematically evaluated across four domains: (1) gastric parameters, including ulcer index, ulcer healing percentage, gastric pH, and total acidity; (2) pro-inflammatory cytokines, specifically tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6); (3) oxidative stress biomarkers, encompassing malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH), and myeloperoxidase (MPO); and (4) immunological parameters, including white blood cell (WBC) count, nitroblue tetrazolium (NBT) reduction, total immunoglobulin (TI), phagocytic rate (PR), and phagocytic index (PI).
For variables exhibiting normal distribution, Pearson's correlation coefficient was employed to assess the linear relationship between EE-AC dosage and the measured outcomes. In cases where data did not meet the assumptions of normality, Spearman's rank correlation was utilized. A p-value less than 0.05 was considered indicative of statistical significance. All statistical analyses were conducted using GraphPad Prism version 10.4.2.[18,19]
Data are presented as mean ± standard deviation (SD). Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify significant differences between treatment groups. Dose-response relationships were visualized through scatter plots for parameters demonstrating statistically significant correlations ($|r| > 0.7$, $p < 0.05$), with linear regression lines, correlation coefficients (r), and corresponding p-values annotated on the plots. For parameters lacking clear linear trends, group-wise comparisons were illustrated using column charts. Additionally, a summary correlation table was compiled to provide an overview of the relationships across all measured variables.

Results and Discussion

Phytochemical profile and pharmacological implications of EE-AC
Phytochemical analysis of the ethanol extract from *Argyreia acuta* leaves (EE-AC) revealed the presence of diverse secondary metabolites, including flavonoids, terpenoids, polyphenols, alkaloids, tannins, saponins, and steroids, while cardiac glycosides were not detected. Quantitative assessments indicated notably high concentrations of flavonoids, terpenoids, polyphenols, and alkaloids (Table 1).
The rich phytochemical profile of EE-AC provides a biochemical basis for its potential pharmacological activities. High levels of flavonoids and polyphenols are particularly significant, as these compounds are known to scavenge reactive oxygen species (ROS) and activate endogenous antioxidant defense mechanisms, including the Nrf2/HO-1 signaling pathway, as reported in similar plant-based extracts.[20] Terpenoids contribute to mucosal protection and exhibit anti-inflammatory properties by inhibiting pro-inflammatory mediators such as TNF-α, IL-1β, and COX-2.[21] Alkaloids have been reported to modulate immune responses by downregulating pro-inflammatory cytokines and enhancing the production of anti-inflammatory cytokines like IL-10.[22] Additionally, tannins, saponins, and steroids support mucosal defense by reinforcing epithelial integrity and exerting mild anti-inflammatory effects. These phytochemical characteristics align with previous findings from other medicinal plants, such as *Ocimum sanctum* and *Camellia sinensis*, which have demonstrated gastroprotective, antioxidant, and immunoregulatory activities in models of NSAID-induced gastric injury.[23,24] Thus, the chemical composition of EE-AC substantiates its potential as a multi-target therapeutic agent against gastric mucosal damage.

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

Phytochemical	Presence in EE-AC	Content
Flavonoids	+	37.67 ± 1.34 mg QE/g
Terpenoids	+	68.72 ± 2.12 mg TAE/g
Polyphenols	+	71.46 ± 1.68 mg GAE/g
Alkaloids	+	177.81 ± 8.43 µg/mL
Saponins	+	NT
Steroids	+	NT
Cardiac Glycosides	-	-

Note: Phytochemicals in EE-AC are (+) present, (-) absent, and (NT) not tested.

Correlation between EE-AC dose and gastroprotective effects

As shown in Table 2, a strong negative correlation was observed between EE-AC dose and both ulcer score and ulcer index (UI), whereas a strong positive correlation was established with ulcer healing percentage (PUH). These findings are visually supported by Figure 1, which illustrates the progressive reduction in ulcer score with increasing EE-AC doses, and Figure 2, which shows a linear dose-dependent enhancement in PUH values. Collectively, these data confirm the dose-responsive nature of EE-AC in mitigating indomethacin-induced gastric lesions.

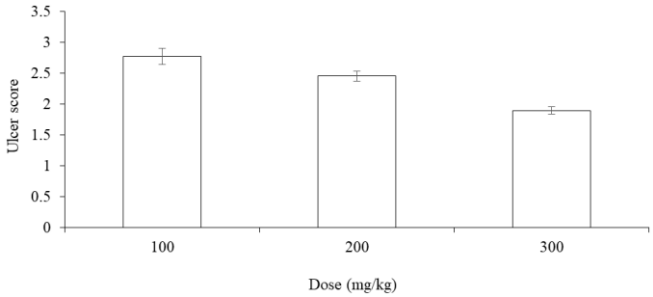

Figure 1: Effect of EE-AC at 100, 200, and 300 mg/kg on ulcer score in the indomethacin-induced gastric injury model in mice. EE-AC reduced ulcer severity in a dose-dependent manner.

The observed dose-response correlations between EE-AC and ulcer score, ulcer index (UI), and ulcer healing percentage (PUH) (%) highlight its therapeutic potential in gastric mucosal protection. Increasing EE-AC doses were associated with progressive reductions in ulcer severity and enhancement in mucosal healing, suggesting a biologically consistent and quantifiable protective effect.[25] This pattern is attributed to the extract's rich phytochemical composition, particularly flavonoids, polyphenols, and terpenoids, which are known to modulate oxidative stress via Nrf2/HO-1 signaling, suppress pro-inflammatory mediators (TNF-α, IL-1β, COX-2), and enhance epithelial regeneration and immune homeostasis.[26] These mechanisms align with prior reports on *Nigella sativa* and *Centella asiatica*, which have demonstrated dose-dependent gastroprotection and oxidative stress mitigation in NSAID-induced ulcer models.[27,28] Collectively, these findings substantiate the dose–dependent efficacy of EE-AC and support its application as a plant-based intervention for gastric injury with anti-inflammatory and immunomodulatory benefits.

Table 2: Dose-response correlation between ethanol extract from *Argyreia acuta* and gastric parameters

Parameters	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
Ulcer score	100	2.77 ± 0.13	0.9945	-0.996	0.0136	↓↓
	200	2.45 ± 0.08	0.9776	-0.989	0.217	↓↓
	300	1.89 ± 0.06	0.9932	-0.985	0.0164	↓↓
Ulcer index (UI)	100	2.85 ± 0.11	0.9801	-0.993	0.0332	↓↓
	200	2.39 ± 0.06	0.9964	-0.991	0.0255	↓↓
	300	1.92 ± 0.05	0.9885	-0.988	0.0158	↓↓
Ulcer healing (PUH) (%)	100	33.51 ± 1.01	0.9801	0.997	0.0025	↑↑
	200	41.19 ± 1.09	0.9939	0.988	0.0049	↑↑
	300	54.51 ± 0.71	0.9792	0.992	0.0066	↑↑

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

Correlation between EE-AC dose and anti-inflammatory cytokine modulation

The dose-response analysis revealed a consistent and significant inverse relationship between EE-AC administration and pro-inflammatory cytokine levels. As shown in Table 3, increasing doses of EE-AC corresponded to a marked reduction in TNF-α, IL-1β, and IL-6 levels, with strong negative correlations ($r < -0.97$, $p < 0.05$). These trends are further illustrated in Figure 3, depicting cytokine profiles across treatment groups, and Figure 4, confirming the linear dose-dependent decline in TNF-α concentrations. Collectively, these findings indicate that EE-AC exerts a dose-proportional immunomodulatory effect in the context of NSAID-induced gastric injury.

The dose-response relationship between EE-AC and cytokine suppression indicates a concentration-dependent immunomodulatory effect, in which escalating doses progressively downregulate TNF-α, IL-1β, and IL-6.[25] This inverse correlation is driven by the high content of bioactive constituents, particularly flavonoids, terpenoids, and polyphenols, that interfere with NF-κB and MAPK signaling pathways, mitigating inflammatory responses and oxidative damage.[29]

Table 3: Dose-response correlation between ethanol extract from *Argyreia acuta* and cytokine levels

Cytokines	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
TNF-α (pg/mL)	100	222.85 ± 7.34	0.9874	-0.983	0.0082	↓↓
	200	189.44 ± 6.12	0.9946	-0.9936	0.0045	↓↓
	300	165.27 ± 5.18	0.9794	-0.9874	0.0069	↓↓
IL-1β (pg/mL)	100	397.38 ± 9.56	0.9942	-0.991	0.0043	↓↓
	200	362.77 ± 7.81	0.9885	-0.9957	0.0076	↓↓
	300	317.46 ± 6.77	0.9922	-0.9918	0.0057	↓↓
IL-6 (pg/mL)	100	35.62 ± 2.41	0.9793	-0.9729	0.0117	↓↓
	200	28.84 ± 1.98	0.9935	-0.9865	0.0288	↓↓
	300	22.15 ± 1.52	0.9864	-0.9923	0.0149	↓↓

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

Such findings are consistent with previous reports on *Curcuma longa* and *Zingiber officinale*, which have demonstrated dose-dependent cytokine modulation and gastroprotective efficacy in NSAID-induced gastric models.[30,31] The results support the mechanistic plausibility of EE-AC as a multi-target therapeutic agent capable of attenuating immune-mediated gastric injury through antioxidant and anti-inflammatory pathways.

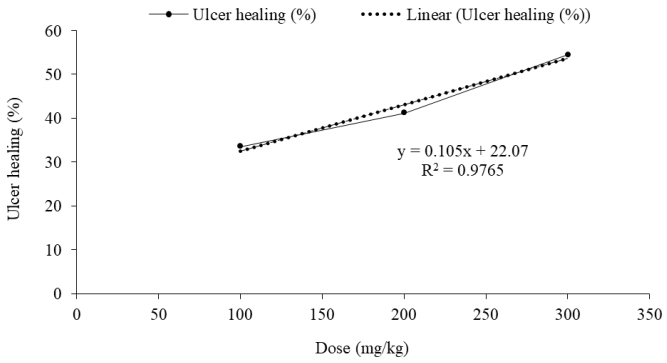

Figure 2: Dose-response relationship between EE-AC (100-300 mg/kg) and the percentage of ulcer healing (PUH) in indomethacin-induced gastric injury. A strong linear correlation (R^2 = 0.9765) was observed, indicating a dose-dependent enhancement of mucosal recovery.

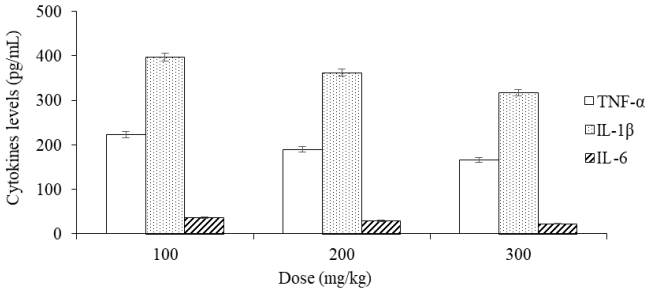

Figure 3: Effect of EE-AC at increasing doses (100, 200, 300 mg/kg) on pro-inflammatory cytokine levels (TNF-α, IL-1β, and IL-6) in mice with indomethacin-induced gastric injury. EE-AC treatment reduced cytokine production in a dose-dependent manner.

Correlation between EE-AC dose and oxidative stress reduction

A clear dose-response correlation was observed between EE-AC administration and oxidative stress regulation in gastric tissue. As shown in Table 4A, biomarkers of oxidative damage, including MDA, H_2O_2, and MPO, exhibited a consistent dose-dependent decrease, with strong negative correlations and statistically significant trends. These findings are visually supported by the linear regression in Figure 6, confirming the inverse relationship between EE-AC dose and MDA levels. Conversely, antioxidant defense markers, including GSH, GPx, and GR, demonstrated progressive increases in activity with escalating EE-AC doses (Table 4B), accompanied by strong positive correlations. The bar chart in Figure 5 further illustrates this upward trend in GPx and GR activity across treatment groups. These findings collectively support the dose-response relationship between EE-AC and redox homeostasis, reinforcing its therapeutic potential in oxidative stress regulation and gastric mucosal protection.

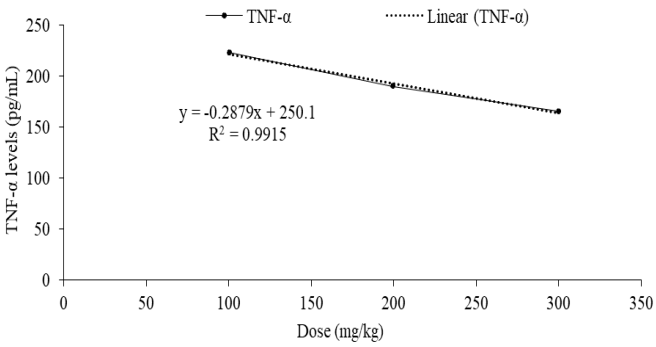

Figure 4: Dose-response relationship between EE-AC and TNF-α levels. Linear regression analysis reveals a strong negative correlation (R^2 = 0.9915). indicating progressive suppression of TNF-α expression with increasing EE-AC dose.

The observed dose-response relationship between EE-AC and oxidative stress biomarkers highlights its potential as a redox-modulating agent in gastric injury. The inverse correlation between EE-AC dose and oxidative mediators such as MDA, H_2O_2, and MPO, alongside a parallel increase in endogenous antioxidants (GSH, GPx, GR), suggests that EE-AC exerts protective effects via enhancement of the cellular antioxidant defense system.[32] This dual modulation reflects an integrated antioxidative and anti-inflammatory response, which is fundamental in mitigating gastric mucosal damage. The phytochemical richness of *Argyreia acuta*, particularly in flavonoids and phenolic acids, likely underpins this response through radical scavenging, enzyme regulation, and inhibition of ROS-generating pathways.[33] Similar dose-dependent protective effects have been reported in related studies involving polyphenol-rich extracts, supporting the hypothesis that EE-AC mediates gastroprotection through a concentration-dependent restoration of redox balance and immune homeostasis. These findings are consistent with prior research on *Gardenia stenophylla* and *Glycyrrhiza glabra*, which demonstrated that therapeutic efficacy against oxidative gastric injury is strongly influenced by both the dose and bioactive profile of the extract.[34,35] The present study therefore reinforces the translational value of EE-AC in oxidative stress-related

gastric disorders and supports its inclusion in further pharmacological development.

Table 4A: Dose-response correlation between ethanol extract from *Argyreia acuta* and oxidative stress biomarkers in stomach tissue

Parameters	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
MDA (nmol/mL)	100	1.63 ± 0.07	0.9874	-0.9945	0.0146	↓↓
	200	1.49 ± 0.03	0.9946	-0.9868	0.0237	↓↓
	300	1.31 ± 0.04	0.9794	-0.9794	0.0195	↓↓
H_2O_2 (nmol/g tissue)	100	1.58 ± 0.06	0.9862	-0.989	0.0251	↓↓
	200	1.46 ± 0.08	0.9931	-0.994	0.0193	↓↓
	300	1.29 ± 0.05	0.9919	-0.988	0.0162	↓↓
MPO (mU/mL)	100	0.13 ± 0.005	0.9955	-0.9934	0.0186	↓↓
	200	0.09 ± 0.004	0.9867	-0.9908	0.0213	↓↓
	300	0.05 ± 0.002	0.9918	-0.988	0.0158	↓↓

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

Table 4B: Dose-dependent effects of ethanol extract from *Argyreia acuta* on oxidative stress biomarkers (GSH, GPx, GR) and their correlation parameters in treated groups

Parameters	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
GSH (µmol/mL)	100	4.05 ± 0.07	0.9923	-0.9918	0.0015	↑↑
	200	3.82 ± 0.06	0.9914	-0.9872	0.0062	↑↑
	300	3.29 ± 0.04	0.9876	-0.9954	0.0038	↑↑
GPx (U/mg protein)	100	16.78 ± 0.39	0.9879	-0.9925	0.0056	↑↑
	200	19.92 ± 0.54	0.9952	-0.9899	0.0064	↑↑
	300	21.48 ± 0.61	0.9943	-0.9947	0.0073	↑↑
GR (U/mg protein)	100	18.79 ± 0.34	0.9985	-0.9924	0.0079	↑↑
	200	20.53 ± 0.49	0.9896	-0.9839	0.0084	↑↑
	300	22.85 ± 0.72	0.9927	-0.9942	0.0062	↑↑

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

Correlation between EE-AC dose and immunomodulatory responses

A dose-dependent immunomodulatory effect of EE-AC was observed across hematological, oxidative, and phagocytic parameters. As shown in Table 5A, all assessed immune-inflammatory indicators demonstrated strong negative correlations with increasing EE-AC dose, indicating suppression of inflammatory burden. This trend was visually confirmed in Figure 8, where a clear inverse relationship was established between EE-AC concentration and the percentage of NBT-positive neutrophils. In contrast, Table 5B demonstrates consistent dose-responsive enhancement of phagocytic activity, with phagocytic response (PR) and phagocytic index (PI) increasing in a strong positive correlation with EE-AC administration. This trend was further illustrated in Figure 7, where the phagocytic index in blood, liver, and spleen showed progressive elevation with increasing extract doses. These findings collectively support a robust and quantifiable dose-response relationship between EE-AC and immune regulation in the gastric inflammation model.

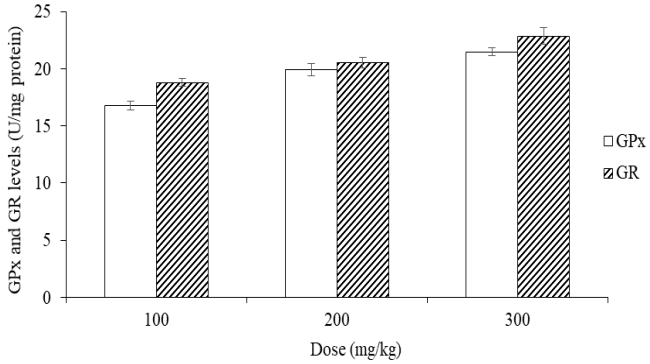

Figure 5: Dose-dependent effects of EE-AC on glutathione peroxidase (GPx) and glutathione reductase (GR) activities in treated mice. Data are expressed as mean ± SD (n = 3).

Table 5A: Dose-response correlation between ethanol extract from *Argyreia acuta* and hematological or immune-inflammatory indicators (WBC, NBT, and TI) in mice with gastric injury

Parameters	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
WBC ($\times 10^3$ cell/mm^3)	100	8.77 ± 0.11	0.9945	-0.9945	0.0056	↓↓
	200	7.65 ± 0.07	0.9898	-0.9936	0.0074	↓↓
	300	6.23 ± 0.04	0.9837	-0.9844	0.0092	↓↓
NBT (%)	100	22.59 ± 0.12	0.9964	-0.9952	0.0149	↓↓
	200	20.64 ± 0.11	0.9949	-0.9919	0.0201	↓↓
	300	17.98 ± 0.08	0.9897	-0.9927	0.0137	↓↓
TI (mg/mL)	100	21.56 ± 0.18	0.9884	-0.9895	0.0082	↓↓
	200	19.75 ± 0.13	0.9925	-0.9889	0.0064	↓↓
	300	16.87 ± 0.11	0.9943	-0.9933	0.0051	↓↓

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

Table 5B: Dose-dependent effects of ethanol extract from *Argyreia acuta* on phagocytic response (PR) and phagocytic index (PI) in blood, liver, and spleen tissues, and their correlation parameters

Parameters	Dose (mg/kg)	Mean ± SD	R^2	r	p-value	Correlation
PR (%) - Blood	100	38.25 ± 0.17	0.9941	0.9961	0.0053	↑↑
	200	41.57 ± 0.24	0.9926	0.9894	0.0049	↑↑
	300	48.78 ± 0.36	0.9865	0.9978	0.0078	↑↑
PI (particles/cell) - Blood	100	374.00 ± 5.16	0.9894	0.9926	0.0162	↑↑
	200	399.00 ± 6.32	0.9937	0.9899	0.0214	↑↑
	300	466.00 ± 8.41	0.9848	0.9867	0.0188	↑↑
PI (particles/cell) - Liver	100	193.00 ± 7.27	0.9962	0.9955	0.0074	↑↑
	200	204.00 ± 9.64	0.9917	0.9943	0.0065	↑↑
	300	242.00 ± 11.18	0.9924	0.9886	0.0047	↑↑
PI (particles/cell) - Spleen	100	268.00 ± 13.29	0.9885	0.9959	0.0163	↑↑
	200	267.00 ± 15.45	0.9897	0.9898	0.0149	↑↑
	300	305.00 ± 16.58	0.9933	0.9924	0.0156	↑↑

Note: ↑↑: Strong positive correlation; ↓↓: Strong negative correlation.

The observed dose-response relationship between EE-AC and immuno-redox parameters suggests that EE-AC exerts its therapeutic effects through a concentration-dependent modulation of oxidative and immune pathways. The progressive suppression of pro-inflammatory and oxidative stress markers, coupled with enhanced phagocytic competence, indicates a dual mechanism involving both attenuation of reactive oxygen species and reinforcement of innate immune function. This coordinated response likely reflects the synergistic action of phenolic and flavonoid constituents in EE-AC, which are known to regulate redox-sensitive transcription factors such as NF-κB and Nrf2. Previous studies have reported similar dose-responsive effects of Argyreia species, particularly in models of gastric injury, where escalating extract concentrations resulted in amplified antioxidant enzyme activity and reduced mucosal inflammation. The present findings are consistent with this pharmacological profile, reinforcing the hypothesis that EE-AC mediates gastroprotection via a dose-dependent restoration of oxidative balance and immune homeostasis.

The progressive suppression of pro-inflammatory and oxidative stress markers, coupled with enhanced phagocytic competence, indicates a dual mechanism involving both attenuation of reactive oxygen species and reinforcement of innate immune function.[36] This coordinated response likely reflects the synergistic action of phenolic and flavonoid constituents in EE-AC, which are known to regulate redox-sensitive transcription factors such as NF-κB and Nrf2.[37] Previous studies have reported similar dose-responsive effects of *Caryota urens* and *Plukenetia volubilis*, particularly in models of gastric injury, where escalating extract concentrations resulted in amplified antioxidant enzyme activity and reduced mucosal inflammation.[5,11]

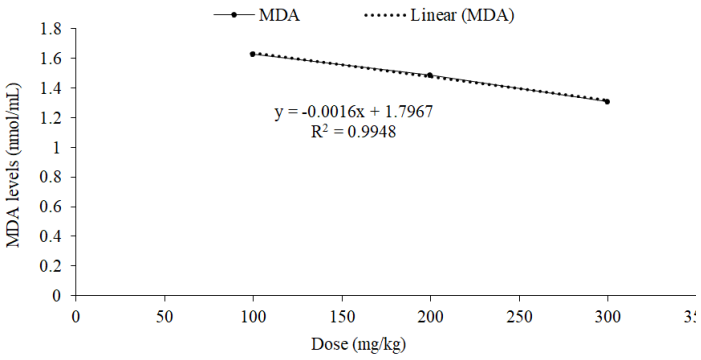

Figure 6: Linear regression analysis showing a strong inverse correlation between EE-AC dose and malondialdehyde (MDA) levels in serum, as evidenced by the regression equation and a high R^2 value.

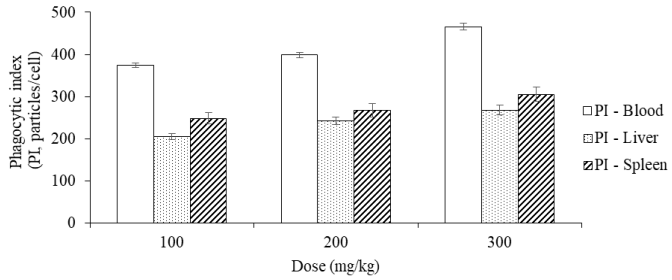

Figure 7: Dose-dependent effects of EE-AC on phagocytic index (PI) in blood, liver, and spleen tissues of treated mice. Data are expressed as mean ± SD.

The present findings are consistent with this pharmacological profile, reinforcing the hypothesis that EE-AC mediates gastroprotection via a dose-dependent restoration of oxidative balance and immune homeostasis.

Summary of dose-response correlation analysis

Table 6 presents an integrated overview of dose–response correlations between Argyreia acuta ethanol extract (EE-AC) and key pharmacological parameters related to gastric protection, inflammation, oxidative stress, and immune function. The analysis reveals robust and statistically significant correlations across all parameter groups, with R^2 values consistently ranging from 0.98 to 0.99 and correlation coefficients (r/ρ) indicating strong associations ($|r| \geq 0.986$). Notably, ulcer-related indices, including ulcer score and ulcer index, exhibited strong inverse correlations with increasing EE-AC doses, while the percentage of ulcer healing showed a direct positive trend, supporting the gastroprotective potential of the extract. Similarly, cytokine levels (TNF-*α*, IL-1*β*, IL-6) demonstrated dose-dependent suppression, suggesting anti-inflammatory modulation.

Oxidative stress biomarkers displayed a bifunctional correlation profile, where lipid peroxidation and pro-oxidant markers (MDA, H_2O_2, MPO) were inversely correlated, while endogenous antioxidants (GSH, GPx) exhibited positive correlations. This dual trend reinforces the hypothesis that EE-AC contributes to redox homeostasis by mitigating oxidative damage and restoring antioxidant defenses. Furthermore, immunological indicators, including WBC counts, NBT reduction, TI levels, and phagocytic parameters (PR and PI), also showed dose-responsive modulation, indicating enhanced innate immune function and inflammation resolution. The consistency of the statistical parameters across diverse biological pathways underscores the systemic efficacy of EE-AC and supports its translational relevance in inflammation-associated gastric injury.

Table 6: Summary of dose-response correlations between ethanol extract from *Argyreia acuta* and pharmacological parameters

Parameter	Correlation type	R^2	r / ρ	p-value
Ulcer score	↓↓	0.98-0.99	(-0.99) to (-0.988)	< 0.05
Ulcer index (UI)	↓↓	0.98-0.99	(-0.99 to (-0.986)	< 0.05
Ulcer healing (PUH)	↑↑	0.98-0.99	(0.98) to (1.00)	< 0.01
Cytokines levels (TNF-*α*, IL-1*β*, and IL-6)	↓↓	0.98-0.99	(-0.99) to (-0.989)	< 0.01
Oxidative stress biomarkers (MDA, H_2O_2, MPO, GSH, and GPx)	↓↓ or ↑↑	0.98-0.99	(-0.99) to (0.996)	< 0.03
Immunological and inflammatory response indicators [WBC, NBT, TI, PR (Blood), PI (Blood), PI (Liver), and PI (Spleen)]	↓↓ or ↑↑	0.98-0.99	(-0.99) to (0.987)	< 0.01

Conclusion

Argyreia acuta ethanol extract (EE-AC) exhibits clear dose-dependent protective effects against indomethacin-induced gastric injury, primarily through antioxidant, anti-inflammatory, and mucosal healing mechanisms. With its phytochemical richness and multi-target actions, EE-AC shows strong potential as a natural adjuvant for gastroprotective therapies. These findings support its further development toward clinical applications in gastric mucosal protection. Further studies should explore its mechanism of action in chronic ulcer models and evaluate its safety profile in long-term applications

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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