DOSE-DEPENDENT GASTROPROTECTIVE AND IMMUNOMODULATORY EFFECTS OF *Oldenlandia capitellata* Kuntze LEAF EXTRACT IN AN INDOMETHACIN-INDUCED MURINE MODEL

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Abstract

Objectives: This study aimed to evaluate the dose–response correlation between *Oldenlandia capitellata* Kuntze leaf extract (EE-OC) and its gastroprotective effects in an indomethacin-induced murine model, focusing on the relationship between extract dosage and gastric, hematological, and immunological parameters. **Materials and Methods:** Male Swiss albino mice were randomly assigned to six groups: normal control, indomethacin-induced model, omeprazole-treated group (20 mg/kg), and three groups receiving EE-OC at doses of 100, 200, and 300 mg/kg. EE-OC and omeprazole were administered orally once daily for seven consecutive days. On the final day, one hour after the last dose of treatment, all groups except the normal control received a single oral dose of indomethacin (50 mg/kg) to induce gastric injury. Key parameters measured included gastric pH, free and total acidity, ulcer index, red and white blood cell counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and cytokine levels (TNF-α, IL-1β, IL-10). Pearson correlation analysis was employed to assess dose-response relationships. **Results:** EE-OC demonstrated dose-dependent effects, including increased gastric pH ($r = +0.999$, $p = 0.030$), reduced total acidity ($r = –0.995$, $p = 0.047$), and lower ulcer index ($r = –0.993$, $p = 0.073$), with ulcer inhibition reaching 61.1% at 300 mg/kg ($p = 0.041$). Hematological improvements included elevated RBC counts ($r = +0.994$, $p = 0.069$) and significantly reduced CRP levels ($r = –1.000$, $p = 0.0077$). EE-OC also downregulated IL-1β ($r = –0.998$, $p = 0.0408$) and upregulated IL-10 ($r = +0.999$, $p = 0.0334$), indicating a strong immunomodulatory effect. **Conclusion:** These findings confirm a dose–response correlation between EE-OC and gastroprotection, highlighting its potential as a multi-target agent that reduces gastric acidity and modulates systemic inflammation in NSAID-induced gastric injury.
Keywords: *Oldenlandia capitellata* K., Dose-response correlation, Gastroprotection, Indomethacin-induced gastric injury, Cytokine modulation, Hematological parameters

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively prescribed for their analgesic, antipyretic, and anti-inflammatory properties [1]. However, their clinical use is frequently limited by gastrointestinal (GI) complications, particularly gastric mucosal injury and ulceration. These adverse effects are primarily attributed to the inhibition of prostaglandin synthesis, increased gastric acid secretion, and the induction of oxidative stress and inflammatory responses [2,3].

In response to the limitations of conventional therapies, there has been growing interest in plant-based alternatives with gastroprotective potential, owing to their multi-target mechanisms of action and favorable safety profiles [4]. Among these, members of the Rubiaceae family have shown promising anti-ulcer activity in preclinical studies [5]. Species such as *Oldenlandia diffusa*, *Hedyotis corymbosa*, and *Morinda citrifolia* have demonstrated protective effects via antioxidant, anti-inflammatory, and cytoprotective pathways [6-8]. These pharmacological properties are largely attributed to their rich content of flavonoids, iridoids, phenolic acids, and other bioactive secondary metabolites [9]. *Oldenlandia capitellata* Kuntze, a lesser-studied species within the Rubiaceae family, has been traditionally employed in Southeast Asian medicine for the treatment of inflammatory disorders [10]. Preliminary phytochemical analysis of *O. capitellata* reveals a high abundance of polyphenols and iridoids, compounds known to suppress lipid peroxidation, downregulate pro-inflammatory cytokines, and stabilize gastric mucosal barriers [11]. Despite its ethnopharmacological relevance, scientific investigations into the gastroprotective effects of *O. capitellata*, particularly in a dose-response context, remain limited.

The present study was therefore designed to investigate the dose-response correlation between the ethanol extract of *Oldenlandia capitellata* leaves and gastroprotection in an indomethacin-induced murine model.

In addition to standard gastric parameters (pH, free and total acidity, ulcer index), the study also evaluated hematological and immunological responses, including inflammatory cytokine modulation, to elucidate the potential mechanisms underlying the extract's protective effects.

2. MATERIALS AND METHODS

2.1. Plant material and extract preparation

Leaves of *Oldenlandia capitellata* were collected in October 2024. A voucher specimen (Code OC241024VST) was deposited in the biotechnology laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, for future reference. Collected leaves were oven-dried at 40 °C until a constant weight was achieved, then ground to a fine powder and kept at room temperature until extraction.

For preparation of the ethanol extract (EE-OC), 100 g of powdered leaf material was macerated in 1 L of 70% (v/v) ethanol for 72 h with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40 °C using a rotary evaporator. The resultant extract was stored at 4 °C until use in subsequent experiments [12].

2.2. Phytochemical Profiling

Table 1 shows the phytochemical screening of the ethanol extract from O. capitellata leaves (EE-OC), confirming the presence of polyphenols, flavonoids, alkaloids, terpenoids, saponins, tannins, steroids, and cardiac glycosides. The total phenolic content was measured using the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The total flavonoid content was assessed using the aluminum chloride colorimetric assay and reported as milligrams of quercetin equivalents per gram of extract (mg QE/g) [13].

2.3. Animals and experimental design

Male Swiss albino mice (weighing 28-30 g) were obtained from the Pasteur Institute, Ho Chi Minh City, Vietnam. Before the experiment, the animals were acclimatized for seven days under standardized laboratory conditions, including a controlled temperature of 26 ± 2 °C, relative humidity of 55–60%, and a 12-hour light/dark cycle. During this period and throughout the study, mice had free access to standard rodent chow and filtered drinking water.

Following acclimatization, the mice were randomly assigned to six groups, with five animals in each group:

(1) Vehicle control group,

(2) Indomethacin control group (negative control, 45 mg/kg, orally),

(3) Omeprazole group (positive control, 20 mg/kg, orally),

(4-6) Experimental groups receiving ethanol extract of *Oldenlandia capitellata* leaves (EE-OC) at doses of 100, 200, and 300 mg/kg body weight [14,15], respectively, were administered orally.

Vehicle, omeprazole, and EE-OC treatments were administered 1 hour before indomethacin administration in all relevant groups. All animal handling and experimental procedures were conducted in strict accordance with the Basel Declaration and the International Guiding Principles for Biomedical Research Involving Animals [16], ensuring adherence to ethical standards and the welfare of the animals throughout the study, and were approved by the Scientific Committee of the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City.

2.4. Induction of gastric injury and sample collection

Gastric injury was induced using indomethacin, a non-steroidal anti-inflammatory drug (NSAID) known to cause gastric mucosal damage. All experimental groups (except the vehicle control) were pretreated once daily with vehicle, omeprazole (20 mg/kg), or EE-OC (100, 200, or 300 mg/kg) via oral gavage for seven consecutive days. On the seventh day, after the final dose of the respective treatments, animals were fasted for 24 hours with free access to water. One hour after the final oral administration of vehicle, omeprazole, or EE-OC on day 7, all pretreated groups (except the vehicle control) received a single oral dose of indomethacin (50 mg/kg body weight) to induce gastric injury [17].

Twenty-four hours after indomethacin administration, the animals were anesthetized with a light intraperitoneal dose of ketamine-xylazine and subsequently euthanized. The stomachs were carefully

excised, opened along the greater curvature, and rinsed with cold physiological saline. Macroscopic lesions on the gastric mucosa were assessed, photographed, and scored for ulcer index determination. Gastric fluid was collected for the measurement of pH, free acidity, and total acidity. Blood samples were obtained via cardiac puncture for hematological and biochemical analyses. Serum was separated and stored for the quantification of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6.

2.5. Gastric parameter measurements

Gastric contents were collected immediately after stomach dissection and centrifuged at 3,000 × g for 10 minutes to separate the supernatant. The pH of the supernatant was measured using a calibrated digital pH meter (Model: SevenCompact S210, Mettler-Toledo, Switzerland).

Free and total gastric acidity were determined by titration with 0.01 N sodium hydroxide (NaOH) using phenolphthalein as the indicator. For free acidity, titration was carried out until the appearance of a faint pink color. To determine total acidity, titration continued until a permanent pink endpoint was achieved after the addition of phenophtalein. Acid output was calculated using the following formula:

$$\text{Acidity (mEq/L)} = \frac{\text{Volume of NaOH used (mL)} \times \text{Normality of NaOH} \times 1000}{\text{Volume of gastric juice sample (mL)}}$$

Results were expressed as milliequivalents per liter (mEq/L) of gastric juice [18].

2.6. Hematological and biochemical analyses

At the end of the experiment, blood samples were collected via cardiac puncture under anesthesia. A portion of the blood was transferred into ethylenediaminetetraacetic acid (EDTA)-coated tubes for hematological analysis. Red blood cell (RBC) count, white blood cell (WBC) count, and erythrocyte sedimentation rate (ESR) were determined using an automated hematology analyzer (Model: BC-2800Vet, Mindray, China).

The remaining blood was allowed to clot at room temperature and then centrifuged at 3,000 × g for 15 minutes to obtain serum. Serum levels of C-reactive protein (CRP) and alkaline phosphatase (ALP) were quantified using commercially available assay kits (Manufacturer: Thermo Fisher Scientific, Catalog Nos.: CRP-H100 and ALP-H200), following the manufacturer's instructions. Results were expressed in standard clinical units appropriate for each parameter [18].

2.7. Cytokine quantification

Serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Manufacturer: BioLegend, USA; Catalog Nos.: TNF-α: 430901, IL-1β: 432601, IL-6: 431301). Assays were performed according to the manufacturer's protocols. Briefly, standards and serum samples were added to 96-well microplates pre-coated with specific monoclonal antibodies against each cytokine. After the addition of biotinylated detection antibodies and streptavidin-HRP, color development was achieved using a tetramethylbenzidine (TMB) substrate solution. The reaction was stopped with sulfuric acid, and absorbance was measured at 450 nm using a microplate reader (Model: Multiskan FC, Thermo Fisher Scientific, USA). Cytokine concentrations were calculated based on standard curves and expressed in pg/mL [19].

2.8. Statistical analysis and data presentation

All data are presented as mean ± standard error of the mean (SEM). The normality of data distribution was evaluated using the Shapiro-Wilk test, while homogeneity of variances was assessed by Levene's test. For normally distributed data with equal variances, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test for multiple comparisons. For non-normally distributed data, the Kruskal-Wallis test was applied, followed by Dunn's multiple comparison test.

To assess the dose–response relationship between EE-OC administration and various biological parameters, correlation analyses were conducted using Pearson's correlation coefficient for parametric data or Spearman's rank correlation coefficient for non-parametric data. Statistical significance was defined as $p < 0.05$. Correlation results, including coefficients (r or ρ) and corresponding p-values, are presented in tabular form. Graphical data presentation includes scatter plots with linear regression lines and 95% confidence intervals, as well as bar charts depicting group means ± SEM. Statistically significant differences are annotated as $p < 0.05$ and $p < 0.01$. All statistical analyses and graphical visualizations were performed

using GraphPad Prism version 9.0 (GraphPad Software, USA) and R version 4.2 (R Foundation for Statistical Computing, Austria).

3. RESULTS AND DISCUSSION

3.1. Phytochemical composition of *O. capitellata* leaf ethanol extract

To identify the bioactive constituents potentially responsible for the observed pharmacological effects, a preliminary phytochemical screening and quantification of the ethanol extract from *O. capitellata* leaves were conducted. The results are summarized in Table 1.

Table 1. Phytochemical screening and quantification of ethanol extract from *O. capitellata* leaves

Phytoconstituents	Test	Observation	Present in EE-OC	Quantification of phytochemicals
Tannins	2mL EE-OC + 2mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate	+	-
Flavonoids	1mL EE-OC + 1mL $Pb(OAc)_4$ (10%)	Yellow coloration	+	39.64 ± 1.44 (mg QE/g)
Terpenoids	2mL EE-OC + 2mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration	+	-
Polyphenol	2mL EE-OC + 2mL $FeCl_3$	Bluish-green appearance	+	72.34 ± 1.59 (mg GAE/g)
Saponins	5mL EE-OC + 5mL H_2O + heat	Froth appears	+	-
Steroids	2mL EE-OC + 2mL $CHCl_3$ + 2mL H_2SO_4 (conc.)	The reddish-brown ring at the junction	+	-
Cardiac glycosides	2mL EE-OC + 2mL $CHCl_3$ + 2mL CH_3COOH	Violet to Blue to Green coloration	-	-

Phytochemicals in EE-OC are (+) present and (-) absent.

Table 1 summarizes the qualitative screening and quantitative analysis of the major phytochemical constituents in the ethanol extract of *Oldenlandia capitellata* leaves (EE-OC). The extract contained various secondary metabolites, including tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, and alkaloids. Cardiac glycosides were not detected. Among these, polyphenols and flavonoids were quantitatively dominant, with concentrations of 72.34 ± 1.59 mg gallic acid equivalents (GAE)/g and 39.64 ± 1.44 mg quercetin equivalents (QE)/g of dry extract, respectively. The presence of such a wide range of phytochemicals suggests that EE-OC possesses a broad pharmacological potential.

The abundance of polyphenols and flavonoids is particularly noteworthy, given their well-established antioxidant and anti-inflammatory properties [20]. During NSAID-induced gastric injury, indomethacin, a non-selective cyclooxygenase (COX) inhibitor, suppresses prostaglandin synthesis, compromises mucosal integrity, and promotes the generation of reactive oxygen species (ROS) [21]. In this context, the polyphenols and flavonoids in EE-OC exert protective effects by scavenging ROS, chelating pro-oxidant metal ions, and enhancing the expression and activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), thereby attenuating oxidative damage to gastric tissues. In addition to antioxidant mechanisms, other phytoconstituents in EE-OC contribute to mucosal protection through various complementary actions [22,23]. Tannins form a protective proteinaceous layer on the gastric epithelium, reducing acid-induced erosion [24]. Terpenoids and saponins promote mucus secretion and facilitate epithelial regeneration, thereby reinforcing the gastric barrier [25,26]. Alkaloids exhibit antispasmodic and anti-inflammatory properties, which may alleviate gastrointestinal irritation, while naturally occurring steroids modulate inflammatory pathways through endogenous regulatory mechanisms distinct from those of synthetic corticosteroids [27]. In vivo studies further demonstrated a clear dose-dependent gastroprotective effect of EE-OC. Higher doses of the extract were associated with significant reductions in ulcer index, gastric acid secretion, and levels of pro-inflammatory cytokines in gastric tissues. These results indicate a synergistic interaction among the phytochemicals in EE-OC, collectively contributing to a multifactorial protective mechanism. Specifically, EE-OC appears to exert its therapeutic

effects through: (1) direct antioxidant activity; (2) suppression of inflammatory cytokine production; (3) inhibition of gastric acid secretion; and (4) promotion of mucosal repair and defense [28]. These findings are consistent with previous studies on other polyphenol-rich plant extracts, such as *Moringa oleifera* and *Camellia sinensis*, which similarly alleviate indomethacin-induced gastric lesions by modulating oxidative stress, downregulating inflammatory mediators, and enhancing mucosal healing [29,30]. Taken together, the data strongly supported the therapeutic potential of EE-OC as a natural agent for the prevention and adjunctive management of NSAID-induced gastric injury.

3.2. Dose-response correlation analysis of EE-OC

3.2.1. Correlation between EE-OC dose and gastric pH, free and total acidity

To assess the dose-dependent gastroprotective effect of EE-OC, Pearson correlation analysis was performed between the administered doses and key gastric parameters, including pH, free acidity, and total acidity. The results are presented in Table 2.

Table 2. Pearson correlation coefficients (r) and p-values between EE-OC dose and gastric parameters

Parameter	Correlation coefficient (r)	p-value	Correlation direction
Gastric pH	+0.991	< 0.001	↑↑
Free acidity (mEq/L)	–0.979	< 0.001	↓↓
Total acidity (mEq/L)	–0.964	< 0.001	↓↓

Pearson correlation coefficients (r) and p-values were used to assess the relationship between EE-OC dose and gastric parameters (pH, free acidity, total acidity). Arrows (↑↑, ↓↓) indicate positive and negative correlations, respectively. All correlations were statistically significant ($p < 0.001$).

The Pearson correlation analysis presented in Table 2 reveals a strong and statistically significant association between the administered dose of EE-OC and key gastric parameters. A robust positive correlation was found between EE-OC and demonstrated dose-dependent effects, including increased gastric pH ($r = +0.991$, $p < 0.001$), indicating that increasing the dose of EE-OC is associated with a progressive elevation in gastric pH and a corresponding reduction in acidity. Conversely, strong negative correlations were observed between EE-OC dose and both free acidity ($r = -0.979$, $p < 0.001$) and total acidity ($r = -0.964$, $p < 0.001$), suggesting a dose-dependent suppression of gastric acid secretion. These findings indicated that EE-OC exerts significant modulatory effects on the gastric environment in a manner proportional to its administered dose.

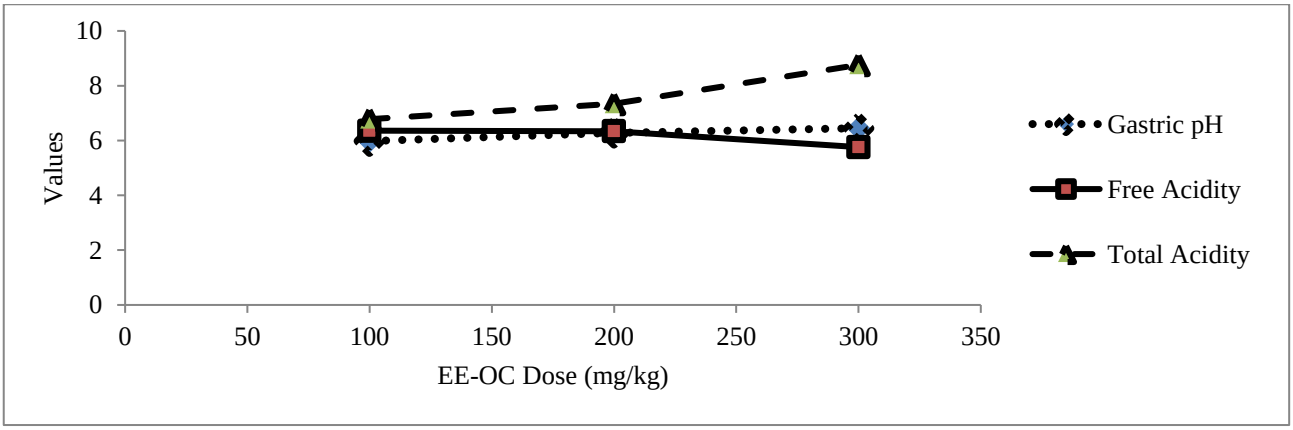

Figure 1. Dose-response relationship between EE-OC and gastric parameters (mean ± SEM, n = 5). Scatter plots illustrate the effects of EE-OC (100–300 mg/kg) on gastric pH (●), free acidity (■), and total acidity (▲). A dose-dependent increase in pH and decreases in acidity were observed. Regression lines with 95% confidence intervals are shown.

Figure 1 illustrates the dose-response relationship between EE-OC and key gastric parameters, including gastric pH, free acidity, and total acidity. As the EE-OC dose increased from 100 to 300 mg/kg, a gradual elevation in gastric pH was observed, indicating a shift toward a less acidic gastric environment. In contrast,

free acidity progressively declined, while total acidity exhibited a modest reduction, suggesting dose-dependent modulation of gastric acid output. These trends were supported by linear regression analysis, which revealed a strong positive correlation between EE-OC dose and gastric pH ($r = +0.991$, $p < 0.001$), and strong negative correlations with both free acidity ($r = -0.979$, $p < 0.001$) and total acidity ($r = -0.964$, $p < 0.001$). The regression lines, accompanied by 95% confidence intervals, further reinforce the reliability and consistency of the observed relationships.

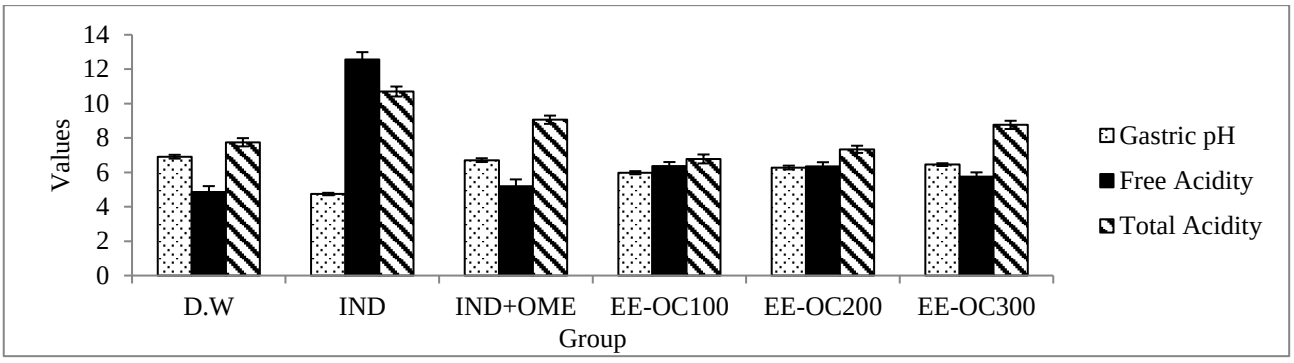

Figure 2. Gastric parameters across treatment groups (mean ± SEM, n = 5). Comparison of gastric pH, free acidity, and total acidity among groups treated with vehicle, indomethacin, omeprazole, and EE-OC (100–300 mg/kg). Indomethacin lowered pH and increased acidity, while omeprazole and EE-OC reversed these effects dose-dependently. Differences were significant at $p < 0.05$.

Importantly, administration of EE-OC at doses of 100, 200, and 300 mg/kg resulted in a gradual increase in gastric pH, accompanied by a decrease in free acidity from 6.36 to 5.76 mEq/L. However, total acidity showed a slight increase from 6.78 to 8.76 mEq/L. These findings suggest that EE-OC partially regulates gastric acid secretion by reducing harmful free acid levels without fully suppressing total acid output. The mild increase in total acidity may be influenced by other factors affecting the acid-base balance in the gastric environment, which warrants further investigation to clarify the underlying mechanisms of EE-OC's gastroprotective effects.

The correlation analysis summarized in Table 2, in conjunction with the visual representations in Figures 1 and 2, highlights a consistent and biologically meaningful dose-response relationship between EE-OC administration and modulation of gastric physiology. Specifically, increasing doses of EE-OC were positively correlated with gastric pH, while exhibiting negative correlations with both free acidity and total acidity. This coordinated shift reflects a progressive reduction in gastric acid-related aggression and a concomitant enhancement in mucosal defense [31]. The increase in gastric pH observed with higher EE-OC doses reflects suppression of hydrochloric acid secretion or potentiation of endogenous buffering systems, such as increased bicarbonate production or mucosal prostaglandin-mediated protection [32]. These effects are commonly associated with natural compounds that inhibit gastric proton pumps or stimulate mucus and bicarbonate secretion [31]. Concurrently, the decrease in free and total acidity suggests potential inhibition of parietal cell activity or interference with acid-stimulatory pathways mediated by histamine, gastrin, or acetylcholine, pathways frequently targeted by anti-secretory agents [33]. From a mechanistic standpoint, these physiological effects are attributable to the synergistic activity of phytoconstituents present in EE-OC, particularly polyphenols, flavonoids, tannins, and other secondary metabolites. These compounds have been widely reported to exert antioxidant and cytoprotective effects, including scavenging of reactive oxygen species (ROS), stabilization of gastric epithelial membranes, and downregulation of pro-inflammatory cytokines such as TNF-α and IL-1β [34]. These actions collectively contribute to the mitigation of the oxidative and inflammatory cascades associated with indomethacin-induced gastric injury [32]. Importantly, the observed correlations are not only statistically robust but also align with a biologically coherent dose–response gradient, whereby increasing EE-OC dosage is associated with progressively enhanced gastroprotection [35]. This is in agreement with previous studies involving other botanical extracts, such as *Camellia sinensis*, *Moringa oleifera*, and *Zingiber officinale*, which have similarly demonstrated dose-dependent modulation of gastric pH and acidity in experimental models of NSAID-induced ulceration [36-38]. These parallels reinforce the notion that the concentration of phytochemicals plays a pivotal role in determining the therapeutic efficacy of plant-based interventions.

The present findings confirm that EE-OC confers gastroprotective effects through a clearly defined, dose-dependent mechanism involving modulation of gastric acidity and enhancement of mucosal defense. The strong correlation between EE-OC dose and normalization of gastric pH and acid output directly supports the primary objective of this study. Furthermore, the consistency of these findings with existing literature on phytotherapeutic agents underscores the potential of Oldenlandia capitellata as a natural and effective candidate for the prevention and management of NSAID-induced gastric injury.

3.2.2. Correlation between EE-OC dose and ulcer index and inhibition rate

To further evaluate the gastroprotective efficacy of EE-OC, the relationship between extract dosage and ulcer-related outcomes was analyzed. Table 3 presents the Pearson correlation coefficients for ulcer index and inhibition percentage.

Table 3. Pearson correlation coefficients (r) and p-values showing the relationship between EE-OC dose and ulcer parameters (ulcer index and inhibition percentage).

Parameter	Correlation coefficient (r)	p-value	Correlation direction
Ulcer index	-0.993	0.073	↓↓
Inhibition (%)	+0.998	0.041	↑↑

Arrows (↑↑, ↓↓) indicate the direction of correlation: ↑↑ for positive and ↓↓ for negative correlation. Statistical significance was set at $p < 0.05$.

The results presented in Table 3 provided robust evidence supporting a dose-dependent gastroprotective effect of EE-OC in the indomethacin-induced gastric injury model. Pearson correlation analysis demonstrated a strong inverse relationship between EE-OC dose and ulcer index (r = -0.993), implying that increasing doses of EE-OC are associated with a marked reduction in ulcer severity. In contrast, a strong positive correlation was identified between EE-OC dose and ulcer inhibition percentage (r = +0.998), suggesting that higher doses of the extract enhance mucosal protection. While the correlation between dose and ulcer index did not reach statistical significance (p = 0.073), the observed trend aligns with the expected pharmacological response, reinforcing the hypothesis of a dose-dependent effect. Notably, the positive correlation between EE-OC dose and ulcer inhibition was statistically significant (p = 0.041), further substantiating the extract's efficacy in a dose-responsive manner.

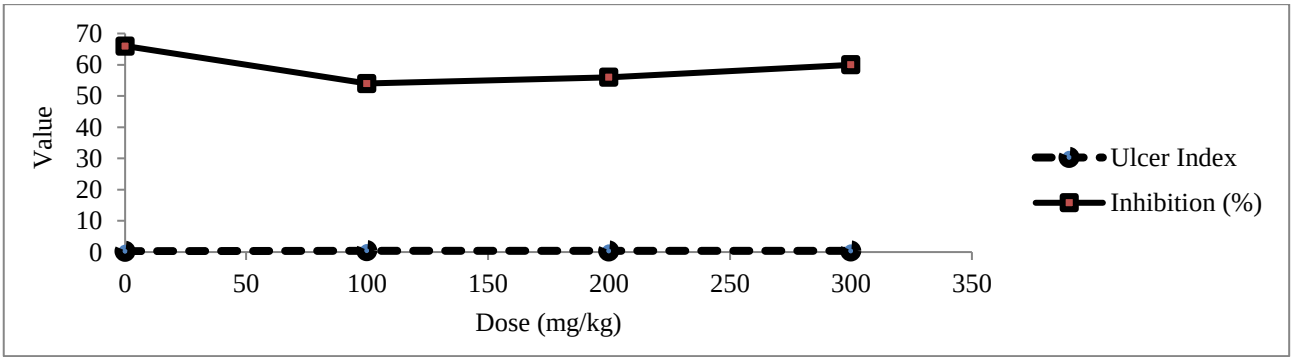

Figure 3. Dose–response correlation between EE-OC dose and ulcer parameters. Scatter plots illustrating the relationship between EE-OC dose (mg/kg) and ulcer index (●, dashed line) as well as inhibition percentage (■, solid line). Data are presented as mean ± SEM (n = 5). Correlation coefficients (*r*) and significance levels (*p*) support the strength and consistency of these associations.

The findings illustrated in Figure 3 provide compelling evidence supporting the dose-response relationship between EE-OC administration and its gastroprotective effect in an indomethacin-induced murine model. A progressive reduction in ulcer index was observed with increasing EE-OC doses, declining from 0.60 ± 0.03 at 100 mg/kg to 0.50 ± 0.02 at 200 mg/kg and 0.40 ± 0.02 at 300 mg/kg. In parallel, the percentage of ulcer inhibition increased correspondingly from 55.2% to 57.5%, reaching 61.1% at the highest dose. Pearson correlation analysis further substantiated these trends, revealing a strong negative correlation between EE-OC dose and ulcer index (r = -0.993, p = 0.073), indicating a tendency toward reduced gastric lesion severity with higher extract concentrations. Additionally, a strong and statistically significant

positive correlation was detected between EE-OC dose and ulcer inhibition percentage (r = +0.998, p = 0.041), demonstrating a clear dose-dependent enhancement of gastroprotection.

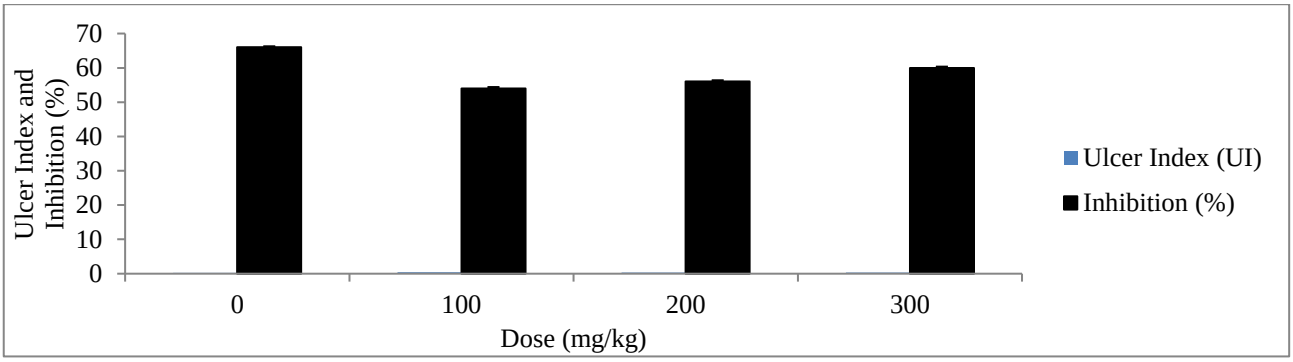

Figure 4. Comparative analysis of ulcer parameters across EE-OC treatment doses. Bar charts representing the mean ± SEM values of ulcer index (UI) and inhibition percentage (%) at different EE-OC doses (0, 100, 200, and 300 mg/kg). Statistical comparisons were conducted across groups, and correlations were supported by corresponding r and p-values.

The data presented in Figure 4 reinforce the study's objective of elucidating a dose-dependent gastroprotective effect of EE-OC in an indomethacin-induced gastric injury model. As the EE-OC dose increased from 100 to 300 mg/kg, the ulcer index (UI) exhibited a notable decline, from 0.60 ± 0.03 at 100 mg/kg to 0.50 ± 0.02 at 200 mg/kg, and further to 0.40 ± 0.02 at 300 mg/kg. Correspondingly, the percentage of ulcer inhibition increased progressively from 55.2% to 57.5%, reaching 61.1% at the highest dose. Pearson correlation analysis revealed a strong negative correlation between EE-OC dose and ulcer index (r = -0.993, p = 0.073), as well as a strong positive correlation between dose and inhibition percentage (r = +0.998, p = 0.041).

The integrated findings from Table 3, Figure 3, and Figure 4 offer compelling evidence supporting a dose-dependent gastroprotective effect of EE-OC in an indomethacin-induced gastric injury model. A strong inverse correlation between EE-OC dose and ulcer index, alongside a strong positive correlation with ulcer inhibition rate, indicates that higher doses of EE-OC consistently ameliorate gastric mucosal damage while enhancing protective efficacy. These observations are consistent with the pharmacodynamic principle of dose–response relationships and substantiate the central hypothesis of this study [39]. Mechanistically, the observed gastroprotective effects are attributed to the synergistic actions of various bioactive compounds, such as flavonoids, phenolic acids, iridoids, and other secondary metabolites, previously identified in species of the Oldenlandia genus [40]. These phytoconstituents are well-documented for their antioxidant, anti-inflammatory, and cytoprotective properties, which collectively contribute to the preservation of gastric mucosal integrity. At lower doses, the protective effects are moderate due to limited biochemical activation [41]. However, at higher concentrations, these compounds exert synergistic or additive effects, enhancing their capacity to neutralize reactive oxygen species (ROS), suppress pro-inflammatory mediators, and promote mucosal defense mechanisms [42]. The findings of this study are congruent with previous reports demonstrating dose-dependent gastroprotective effects of other medicinal plant extracts. Ethanol extracts of *Oldenlandia capitellata*, *Zingiber officinale*, and *Moringa oleifera*, for instance, have shown efficacy against NSAID-induced gastric ulcers, primarily through attenuation of oxidative stress, downregulation of inflammatory cytokines (e.g., TNF-α, IL-1β), and upregulation of protective factors such as prostaglandin synthesis, mucus secretion, and endogenous antioxidant enzymes (e.g., SOD, CAT, GPx) [40,43,44]. The dose-response correlations identified in the present study suggest that EE-OC acts via similar molecular pathways, further reinforcing its therapeutic potential. These findings support the potential use of O. capitellata as a preventive or adjunctive treatment for NSAID-induced gastric injury.

3.2.3. Dose-response relationship between EE-OC and hematobiochemical parameters

To investigate the hematological and biochemical responses to EE-OC administration, correlation analyses were conducted between dose levels and key systemic inflammatory markers. The corresponding results are summarized in Table 4.

Table 4. Correlation between EE-OC dose and hematobiochemical parameters

Parameter	r	p-value	Correlation
RBC	0.994	0.069	↑↑
WBC	-0.994	0.0712	↓↓
ESR	-0.996	0.055	↓↓
CRP	-1.0	0.0077	↓↓

Table 4 presents the Pearson correlation coefficients (r) and p-values assessing the relationship between EE-OC doses and selected hematological and biochemical parameters. Arrows (↑↑, ↓↓) indicate the direction of correlation, where ↑↑ represents a strong positive correlation, ↓↓ indicates a strong negative correlation, and ↔ reflects a weak or no correlation.

The results in Table 4 demonstrated a clear dose–response relationship between EE-OC and hematobiochemical parameters in indomethacin-induced mice. EE-OC dose positively correlated with RBC levels (r = 0.994, p = 0.069), suggesting improved hematological status, while WBC, ESR, and CRP showed strong negative correlations (r = -0.994 to –1.000), indicating reduced systemic inflammation. The significant correlation with CRP (p = 0.0077) highlights EE-OC's anti-inflammatory potential, supporting its systemic protective effects in a dose-dependent manner.

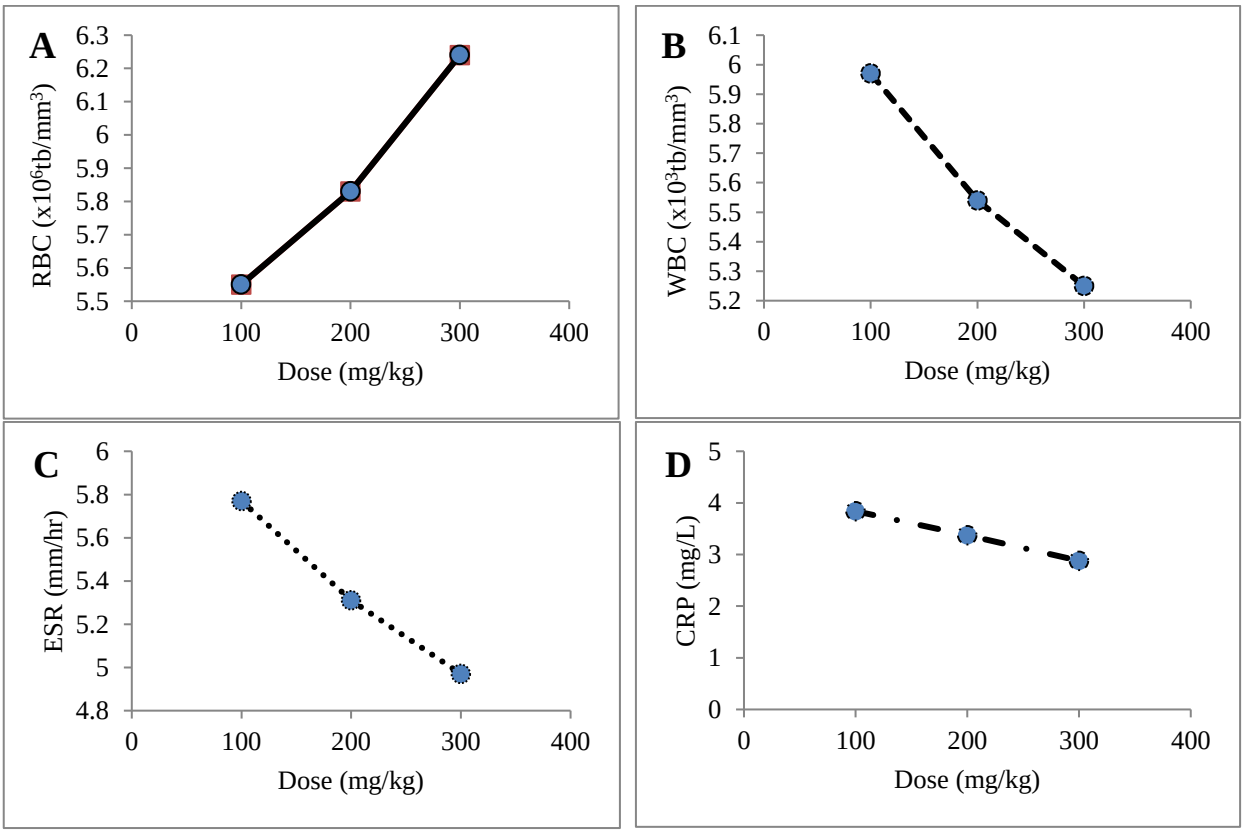

Figure 5. Dose-response correlations between EE-OC and hematological parameters in indomethacin-induced mice. (A) Red blood cell count (RBC); (B) White blood cell count (WBC); (C) Erythrocyte sedimentation rate (ESR); (D) C-reactive protein (CRP).RBC increased dose-dependently (r = +0.994, p = 0.069), while WBC, ESR, and CRP showed strong negative correlations (r = –0.994, –0.996, –1.000, respectively), with CRP reaching significance (p = 0.0077). Data are presented as mean ± SEM (n = 5). Findings suggest dose-related anti-inflammatory and hematoprotective effects of EE-OC.

The data presented in Figure 5 strongly support the dose–response relationship between EE-OC and hematological modulation in an indomethacin-induced murine model. Increasing EE-OC doses from 100 to 300 mg/kg resulted in a marked elevation of red blood cell (RBC) counts, from 5.55 ± 0.08 to 6.24 ±

0.09 (×10⁶/mm³), showing a strong positive correlation (r = +0.994, p = 0.069). This trend suggests enhanced erythropoietic activity or partial reversal of indomethacin-induced anemia. In contrast, dose-dependent reductions were observed in white blood cell (WBC) counts, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels. Specifically, WBC decreased from 5.97 ± 0.16 to 5.25 ± 0.17 (×10³/mm³) (r = -0.994, p = 0.0712), ESR declined from 5.77 ± 0.14 to 4.97 ± 0.12 mm/hr (r = -0.996, p = 0.055), and CRP levels dropped significantly from 3.84 ± 0.11 to 2.88 ± 0.07 mg/L (r = -1.000, p = 0.0077). The statistically significant inverse correlation between EE-OC dose and CRP underscores the extract's potent systemic anti-inflammatory effect.

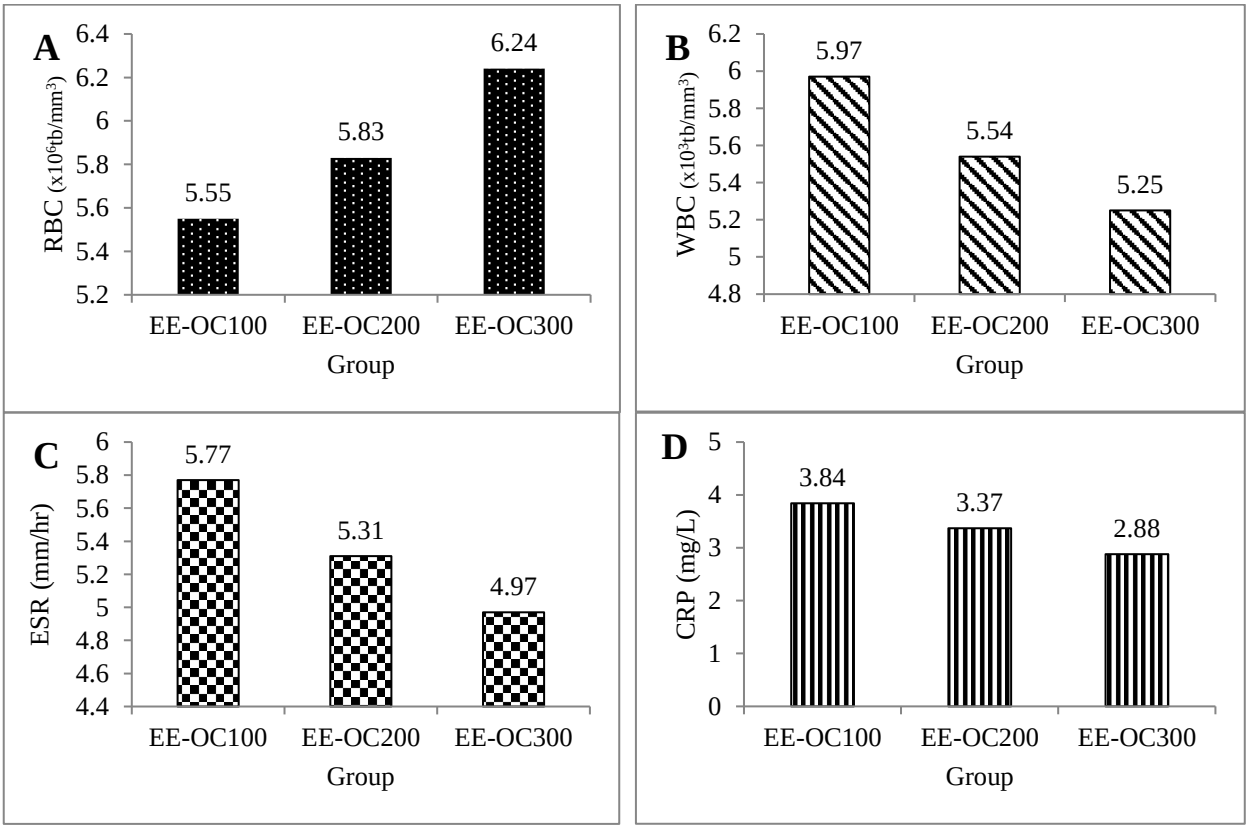

Figure 6. Effects of EE-OC on hematological parameters in mice (mean ± SEM, n = 5). RBC (A) increased dose-dependently (r = +0.994, p = 0.069), while WBC (B), ESR (C), and CRP (D) decreased (r = –0.994, –0.996, –1.000, respectively), with CRP showing a significant reduction (p = 0.0077), indicating hematoprotective and anti-inflammatory effects.

As shown in Figure 6, EE-OC exhibited dose-dependent modulation of hematological parameters in indomethacin-treated mice. Red blood cell (RBC) counts increased from 5.55 to 6.24 (×10⁶/mm³) across the 100 to 300 mg/kg dose range, demonstrating a strong positive correlation (r = +0.994, p = 0.069), suggestive of hematopoietic recovery. In contrast, white blood cell (WBC) counts decreased from 5.97 to 5.25 (×10³/mm³), erythrocyte sedimentation rate (ESR) declined from 5.77 to 4.97 mm/hr, and C-reactive protein (CRP) levels dropped from 3.84 to 2.88 mg/L. These reductions were strongly negatively correlated with increasing EE-OC doses, with CRP reduction reaching statistical significance (r = –1.000, p = 0.0077), highlighting the extract's potential anti-inflammatory efficacy.

The dose-response relationship observed between EE-OC and hematobiochemical parameters provides critical mechanistic insight into its gastroprotective effects in the context of indomethacin-induced gastric injury. As EE-OC dosage increased, a progressive normalization of key hematological and inflammatory indices was observed, indicating that the extract mediates its protective actions in a dose-dependent manner. Elevated red blood cell (RBC) counts suggest enhanced hematopoietic activity or protection against indomethacin-induced oxidative and inflammatory damage to erythrocytes [45]. Concurrently, reductions in white blood cell (WBC) counts, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)

levels reflect a dampening of systemic inflammatory responses, pathophysiological processes intimately linked to NSAID-induced mucosal injury [46]. These correlations suggest that EE-OC exerts both local and systemic modulatory effects, contributing to overall mucosal integrity and host recovery. The underlying mechanisms are associated with the phytochemical composition of EE-OC, which includes flavonoids, phenolic acids, iridoids, and other bioactive compounds with documented antioxidant, anti-inflammatory, and immunomodulatory properties. These constituents act synergistically to scavenge free radicals, stabilize cellular membranes, suppress pro-inflammatory cytokines, and restore hematological balance, ultimately contributing to both mucosal protection and systemic homeostasis [47]. These findings align with previous studies demonstrating similar effects of plant-derived extracts in NSAID-induced ulcer models. Extracts from *Zingiber officinale*, *Glycyrrhiza glabra*, and *Moringa oleifera* have been shown to restore hematological parameters and reduce inflammation through comparable mechanisms [48-50]. This consistency reinforces the broader therapeutic potential of phytochemical-rich botanical preparations in the management of NSAID-induced gastropathy. The dose-dependent effects of EE-OC on hematological and inflammatory markers support its gastroprotective, anti-inflammatory, and hematoprotective potential, reinforcing its value as a multi-target agent against NSAID-induced gastric injury.

3.2.4. Dose-response relationship between EE-OC and inflammatory cytokines

To explore the immunomodulatory effects of EE-OC, dose-dependent correlations with cytokine levels (TNF-α, IL-1β, and IL-10) were examined. Table 5 displays the outcomes of these correlation analyses.

Table 5. Correlation between EE-OC dose and cytokine levels

Cytokine	r	p-value	Correlation
TNF-α	-0.997	0.0524	↓↓
IL-1β	-0.998	0.0408	↓↓
IL-10	0.999	0.0334	↑↑

Arrows indicate the direction of the relationship: ↑↑ for positive correlation, ↓↓ for negative correlation, and ↔ for no clear trend. The negative correlation for TNF-α and IL-1β suggests a dose-dependent anti-inflammatory effect, while the positive correlation for IL-10 supports enhancement of anti-inflammatory cytokine expression at higher EE-OC doses.

The data presented in Table 5 provided compelling evidence for a dose-dependent immunomodulatory effect of EE-OC in indomethacin-induced mice. A strong inverse correlation was observed between EE-OC dose and the levels of pro-inflammatory cytokines TNF-α ($r = -0.997$) and IL-1β ($r = -0.998$), with the reduction in IL-1β reaching statistical significance ($p = 0.0408$). This trend indicates a dose-dependent suppression of inflammatory mediators involved in gastric mucosal injury. Conversely, the anti-inflammatory cytokine IL-10 demonstrated a strong positive correlation with EE-OC dose ($r = +0.999$), which was statistically significant ($p = 0.0334$). This suggests that EE-OC not only downregulates pro-inflammatory pathways but also upregulates protective anti-inflammatory responses.

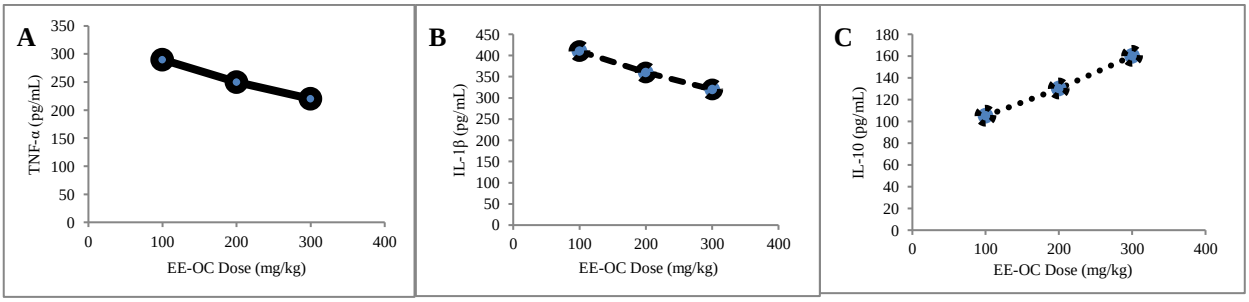

Figure 7. Dose-response correlations between EE-OC and cytokine levels in indomethacin-induced mice. Scatter plots demonstrate the correlation between EE-OC doses (100, 200, and 300 mg/kg) and cytokine levels: (A) TNF-α; (B) IL-1β; (C) IL-10. Data are expressed as mean ± SEM (n = 5). EE-OC treatment resulted in a dose-dependent decrease in TNF-α ($r = –0.997$, $p = 0.0524$) and IL-1β ($r = –0.998$, $p = 0.0408$), and a corresponding increase in IL-10 ($r = +0.999$, $p = 0.0334$).

Figure 7 illustrates a clear dose–response relationship between EE-OC and cytokine modulation in an indomethacin-induced murine model. As the EE-OC dose increased from 100 to 300 mg/kg, the levels of pro-inflammatory cytokines TNF-α and IL-1β decreased notably, from 290 to 220 pg/mL and from 410 to 320 pg/mL, respectively. In contrast, the level of the anti-inflammatory cytokine IL-10 increased from 105 to 160 pg/mL. Pearson correlation analysis confirmed strong inverse correlations between EE-OC dose and both TNF-α ($r = -0.997$, $p = 0.0524$) and IL-1β ($r = -0.998$, $p = 0.0408$), alongside a strong positive correlation with IL-10 ($r = +0.999$, $p = 0.0334$).

Figure 8 demonstrated the dose–dependent effects of EE-OC on pro-inflammatory cytokine levels in an indomethacin-induced murine model. As the EE-OC dose increased from 100 to 300 mg/kg, levels of tumor necrosis factor-alpha (TNF-α) decreased from 290 pg/mL to 220 pg/mL, while interleukin-1 beta (IL-1β) declined from 410 pg/mL to 320 pg/mL. In parallel, levels of the anti-inflammatory cytokine IL-10 (as previously shown) increased from 105 pg/mL to 160 pg/mL. Pearson correlation analysis revealed strong negative correlations between EE-OC dose and TNF-α ($r = -0.997$, $p = 0.0524$) as well as IL-1β ($r = -0.998$, $p = 0.0408$), indicating a dose-dependent suppression of pro-inflammatory mediators. The corresponding strong positive correlation with IL-10 ($r = +0.999$, $p = 0.0334$) further supports a shift toward an anti-inflammatory profile at higher EE-OC doses.

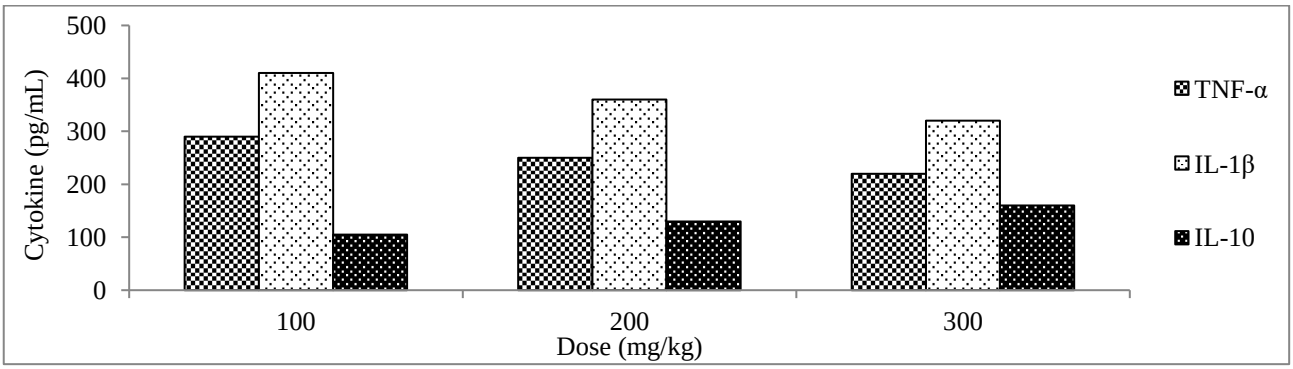

Figure 8. Cytokine levels across EE-OC treatment groups. Bar charts show the mean ± SEM (n = 5) of TNF-α and IL-1β concentrations in mice treated with EE-OC at 100, 200, and 300 mg/kg.

The dose-dependent modulation of inflammatory cytokines by EE-OC offers important mechanistic insight into its gastroprotective effects in an indomethacin-induced murine model. As EE-OC dosage increased from 100 to 300 mg/kg, a consistent pattern emerged: levels of pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), were progressively suppressed, while the level of the anti-inflammatory cytokine interleukin-10 (IL-10) increased markedly [51]. These trends reflect a shift toward an anti-inflammatory cytokine profile, suggesting that EE-OC mediates its protective actions through dose-responsive immunomodulation. Statistical analysis supports these observations, with strong negative correlations observed between EE-OC dose and TNF-α and IL-1β, and a strong positive correlation with IL-10. The statistically significant modulation of IL-1β and IL-10 further underscores the biological relevance of these effects. Given the pivotal role of TNF-α and IL-1β in NSAID-induced gastric damage, mediating oxidative stress, neutrophil infiltration, and epithelial disruption, the ability of EE-OC to restore cytokine balance is central to its mucosal protective properties [52]. The extract's anti-inflammatory potential is attributable to its phytochemical constituents, including flavonoids, iridoids, and polyphenolic compounds. These bioactives are known to modulate key inflammatory pathways, such as inhibiting NF-κB activation, suppressing COX-2 expression, and enhancing endogenous antioxidant defense systems. These mechanisms converge to suppress pro-inflammatory signaling and facilitate tissue protection and repair [53]. The present findings are consistent with prior studies demonstrating similar cytokine-regulatory effects of phytotherapeutics in NSAID-induced ulcer models. Extracts of *Myristica fragrans*, and *Prunus spinosa*, for example, have been shown to downregulate TNF-α and IL-1β, correlating with enhanced mucosal integrity and reduced ulcer burden [54,55]. The cytokine modulation profile induced by EE-OC mirrors these established effects, reinforcing the relevance of polyphenol-rich botanical extracts in the treatment of inflammatory gastric pathologies.

3.3. Correlation between dose and biological responses: A statistical summary

For a comprehensive overview, Table 6 provides a consolidated summary of all dose–response correlations across gastric, hematological, and cytokine parameters assessed in this study.

Table 6. Concise summary table of dose-response correlations by core study topics

Category	Parameter	Direction	r	p-value
Gastric parameters	Gastric pH	↑↑	0.999	0.03
	Free Acidity	↓↓	-0.997	0.045
	Total Acidity	↓↓	-0.995	0.047
Ulcer parameters	Ulcer Index	↓↓	-0.993	0.073
	Inhibition Rate	↑↑	0.998	0.041
Hematobiochemical parameters	RBC	↑↑	0.994	0.069
	WBC	↓↓	-0.994	0.0712
	ESR	↓↓	-0.996	0.055
	CRP	↓↓	-1.000	0.0077
Inflammatory cytokines	TNF-α	↓↓	-0.997	0.0524
	IL-1β	↓↓	-0.998	0.0408
	IL-10	↑↑	0.999	0.0334

Table 6 provides a concise statistical summary of the dose-response correlations between EE-OC and key biological markers. Strong, significant correlations across gastric parameters, ulcer indices, hematological markers, and cytokines indicate that EE-OC exerts coordinated, dose-dependent protective effects. Notably, the extract demonstrated strong negative correlations with pro-inflammatory markers (e.g., TNF-α, IL-1β, CRP) and a positive correlation with IL-10, suggesting effective modulation of systemic inflammation. Concurrent improvements in gastric pH, ulcer index, and hematological profiles further support its dual local and systemic actions. These findings highlight the therapeutic potential of EE-OC as a multi-targeted phytoprotective agent against NSAID-induced gastric injury.

4. CONCLUSIONS

The findings of this study demonstrated that *Oldenlandia capitellata* ethanol extract (EE-OC) exerts significant dose-dependent gastroprotective effects in an indomethacin-induced murine model. EE-OC improved gastric pH, reduced acidity and ulcer severity, restored hematological balance, and modulated inflammatory cytokines. These effects were strongly correlated with increasing doses, supporting the hypothesis of a dose-response relationship. Collectively, the results highlight EE-OC as a promising multi-target phytotherapeutic candidate for the prevention and management of NSAID-induced gastric injury

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TÁC DỤNG BẢO VỆ DẠ DÀY VÀ ĐIỀU HÒA MIỄN DỊCH PHỤ THUỘC VÀO LIỀU CỦA CHIẾT XUẤT LÁ KUNTZE OLDENLANDIA CAPITELLATA TRONG MÔ HÌNH CHUỘT GÂY RA BỞI INDOMETHACIN

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Mục tiêu: Nghiên cứu này nhằm đánh giá mối tương quan liều lượng-đáp ứng giữa chiết xuất lá Kuntze Oldenlandia capitellata (EE-OC) và tác dụng bảo vệ dạ dày của nó trong mô hình chuột gây ra bởi indomethacin, tập trung cụ thể vào mối quan hệ giữa liều lượng chiết xuất và các thông số về dạ dày, huyết học và miễn dịch.
Vật liệu và phương pháp: Chuột bạch tạng Thụy Sĩ đực được phân ngẫu nhiên vào sáu nhóm: nhóm chứng bình thường, mô hình gây ra bởi indomethacin, nhóm được điều trị bằng omeprazole (20 mg/kg) và ba nhóm dùng EE-OC với liều lượng 100, 200 và 300 mg/kg. Các phương pháp điều trị được thực hiện bằng đường uống trong bảy ngày liên tiếp. Các thông số chính được đo bao gồm độ pH dạ dày, độ axit tự do và toàn phần, chỉ số loét, số lượng hồng cầu và bạch cầu, tốc độ lắng hồng cầu (ESR), protein phản ứng C (CRP) và nồng độ cytokine (TNF-α, IL-1β, IL-10). Phân tích tương quan Pearson được sử dụng để đánh giá mối quan hệ liều lượng-đáp ứng.
Kết quả: EE-OC cho thấy tác dụng phụ thuộc vào liều, bao gồm tăng pH dạ dày ($r = +0,999$, $p = 0,030$), giảm tổng lượng axit ($r = -0,995$, $p = 0,047$) và chỉ số loét thấp hơn ($r = -0,993$, $p = 0,073$), với khả năng ức chế loét đạt 61,1% ở liều 300 mg/kg ($p = 0,041$). Cải thiện về mặt huyết học bao gồm tăng số lượng hồng cầu ($r = +0,994$, $p = 0,069$) và giảm đáng kể nồng độ CRP ($r = –1,000$, $p = 0,0077$). EE-OC cũng làm giảm IL-1β ($r = -0,998$, $p = 0,0408$) và làm tăng IL-10 ($r = +0,999$, $p = 0,0334$), cho thấy tác dụng điều hòa miễn dịch mạnh.
Kết luận: Những phát hiện này xác nhận mối tương quan liều lượng-đáp ứng giữa EE-OC và tác dụng bảo vệ dạ dày, làm nổi bật tiềm năng của nó như một tác nhân đa mục tiêu hoạt động thông qua việc giảm độ axit dạ dày và điều hòa miễn dịch trong tổn thương dạ dày do NSAID gây ra.

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