ANTIOXIDANT AND IMMUNOMODULATORY EFFECTS OF ETHANOL EXTRACT FROM *Oldenlandia capitellata* Kuntze LEAVES IN INDOMETHACIN-INDUCED GASTRIC INFLAMMATION IN MICE

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Abstract. Objective: This study evaluates the antioxidant and immunomodulatory effects of ethanol extract from *Oldenlandia capitellata* (EEOC) in a mouse model of indomethacin (IND)-induced gastric inflammation. **Methods:** Mice were treated with IND (45 mg/kg), omeprazole (20 mg/kg), or EEOC (100, 200, and 300 mg/kg). Key biomarkers of oxidative stress and immune response, including malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glutathione (GSH), total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD), white blood cell (WBC) counts, Nitroblue tetrazolium reduction (NBT), total immunoglobulin (TI), and phagocytic activity (PA), were assessed. **Results:** The IND group showed increased MDA (3.57 ± 0.03 nmol/mg protein) and H_2O_2 (2.15 ± 0.04 nmol/g tissue) levels ($p < 0.05$). EEOC treatment, particularly at 300 mg/kg, significantly reduced oxidative stress markers (MDA: 2.26 ± 0.01 nmol/mg, H_2O_2: 1.36 ± 0.02 nmol/g, $p < 0.05$) and restored antioxidant enzyme activities. EEOC also modulated immune responses by reducing WBC, NBT, TI, and PA counts while improving phagocytic activity. **Conclusion:** EEOC mitigates oxidative stress and modulates immune responses in gastric inflammation, suggesting its potential as a therapeutic agent for oxidative stress-related conditions.

Keywords: *Oldenlandia capitellata* K., Ethanol extract, Antioxidant activity, Immunomodulation, Gastric protection, Phytotherapy

1. INTRODUCTION

Gastritis is a prevalent gastrointestinal disorder characterized by inflammation of the gastric mucosa, often induced by Helicobacter pylori infection, excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), stress, and poor dietary habits [1]. This condition results in oxidative stress, disrupting the balance between free radicals and antioxidant defenses, leading to mucosal damage, impaired immune function, and increased susceptibility to infections [2]. Chronic gastritis is a global health concern, with an estimated 10% of the population affected at some point in their lives. Moreover, 40-50% of patients report a decline in quality of life, and untreated cases may progress to gastric ulcers or even gastric cancer [3,4]. The economic burden is also substantial, with annual healthcare costs reaching billions of dollars due to treatment expenses and productivity losses [5]. Current treatment strategies primarily rely on proton pump inhibitors (PPIs), antibiotics, and anti-inflammatory drugs. However, long-term use of these medications is associated with adverse effects, including gastric mucosal damage, altered gut microbiota, and antibiotic resistance [6]. These limitations have intensified interest in alternative therapies, particularly plant-based remedies with potent antioxidant and immunomodulatory properties.

Vietnam has a rich tradition of using medicinal plants for treating gastrointestinal disorders, with numerous species documented in traditional medicine for their gastroprotective effects [7]. *Oldenlandia capitellata* (*O. capitellata*), a species belonging to the Rubiaceae family, has been widely used in Vietnamese folk medicine for its anti-inflammatory, detoxifying, and digestive-supporting properties [8]. Its close relatives, such as *Oldenlandia diffusa* and *Oldenlandia corymbosa*, have been extensively studied for their antioxidant, antimicrobial, and anticancer activities [9]. Phytochemical analyses indicate that species of the *Oldenlandia* genus are rich in flavonoids, phenolics, alkaloids, and saponins, compounds known for their strong antioxidant and immunoregulatory activities [10]. Prior studies on *Oldenlandia diffusa* demonstrated its effectiveness in alleviating indomethacin-induced gastritis by reducing oxidative stress and enhancing immune function [11]. However, research specifically investigating the efficacy of *O. capitellata* in treating gastritis remains limited.

This study aims to bridge this gap by evaluating the antioxidant and immunomodulatory effects of ethanol extract from *O. capitellata* in a murine model of indomethacin-induced gastritis. By assessing key oxidative

stress markers and immune response parameters, this research provides novel insights into the therapeutic potential of *O. capitellata* as a natural remedy for gastritis and related conditions.

2. MATERIALS AND METHODS

2.1. Collection of materials and preparation of the extract

Oldenlandia capitellata leaves were systematically collected in November 2023 from the Cẩm Mỹ District in Đồng Nai Province, Vietnam. Each collected leaf was meticulously examined and selected to exclude damaged or diseased specimens. Following the selection process, the leaves were transferred to a clean, shaded environment for drying until a constant weight was achieved, indicating thorough moisture removal. Once adequately dried, the leaves were mechanically ground into a fine powder using a laboratory grinder. This powdered form was selected to enhance the surface area for subsequent extraction processes, thereby improving the efficiency of compound recovery. The processed material was then stored in airtight containers to prevent moisture absorption and contamination. The final powdered sample has been designated as OCT031123VST and is preserved at the Biotechnology Laboratory of the Institute of Biotechnology and Food Technology at Ho Chi Minh City University of Industry for future research and analysis.

The extraction of bioactive compounds from *Oldenlandia capitellata* leaves was carried out using ethanol as the solvent. 100 grams of the powdered leaves were then mixed with 1000 mL of the 70% ethanol solution in a clean, sterile container, ensuring an appropriate leaf-to-solvent ratio for efficient extraction. The mixture was soaked for 72 hours at room temperature, during which it was periodically shaken to enhance solvent penetration and facilitate thorough extraction. Following the soaking period, the mixture was filtered using fine mesh filter paper to separate the liquid extract from the solid plant material. The collected ethanol extract was concentrated using a rotary evaporator at a low temperature to remove the ethanol solvent, ensuring the preservation of the bioactive compounds. The resulting concentrated extract was transferred to a pre-weighed amber glass container to protect it from light exposure and was labeled as ethanol extract from *Oldenlandia capitellata* (EEOC). Finally, the EEOC was stored in a cool, dark place at 4°C to maintain its stability until further analysis and testing were conducted [13].

2.2. Qualitative and quantification phytochemical analysis of bioactive compounds in the extract

The qualitative phytochemical analysis of the ethanol extract of *O. capitellata* leaf was conducted following the method by Tran et al. (2023) [14]. Key phytochemicals identified included alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and phenolic compounds. Alkaloids were confirmed using Mayer's and Wagner's tests, while flavonoids produced a yellow hue with $Pb(OAc)_4$. Tannins and phenolic compounds showed dark blue or green color changes with $FeCl_3$. Saponins formed foam upon heating with water, and terpenoids yielded a dark red hue with the Liebermann-Burchard test. Steroids created a rust-colored ring with $CHCl_3$ and sulfuric acid, while cardiac glycosides exhibited a color change from purple to blue to green with acetic acid. The findings are summarized in Table 1, underscoring the extract's rich phytochemical profile.

Total polyphenol quantification: To determine the total polyphenol content in EEBS, we followed the initial protocol outlined by Tran and Le (2023b) [15], utilizing the Folin-Ciocalteu colorimetric method. Specifically, we combined 0.3 mL of the extract with 2.25 mL of Folin-Ciocalteu reagent. After a 5-minute incubation period, 2.25 mL of 6% sodium carbonate solution was added to the mixture, which was then allowed to stand at room temperature for 90 minutes. Subsequently, the absorbance of the solution was measured at 725 nm. A standard curve of gallic acid was also established within the concentration range of 0-200 μg/mL using a similar procedure. Results are expressed in milligrams of gallic acid equivalents (GAE) per gram of extract.

Quantification of total flavonoids: The total flavonoid content in EEBS was assessed using the aluminum colorimetric method, initially established by Tran and Le (2023b) [15]. Quercetin served as the standard reference material, and a calibration curve for quercetin was generated within the concentration range of 0-200 μg/mL. For this analysis, 0.5 mL of the extract solution and 0.5 mL of the standard solution were separately placed into individual test tubes. To each tube, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, 1.5 mL of 80% methanol, and distilled water (2.8 mL) were added and thoroughly

mixed. A blank sample was prepared similarly, replacing 0.5 mL of the sample or standard solution with distilled water and substituting aluminum chloride with distilled water. All tubes were then incubated at room temperature for 30 minutes, followed by absorbance measurement at 415 nm. Flavonoid concentrations are expressed in milligrams of quercetin equivalents (QE) per gram of extract.

2.3. Experimental animals

Swiss albino mice, weighing between 28 and 30 grams, were utilized as the experimental subjects in this study. These mice were procured from the Pasteur Institute in Ho Chi Minh City and housed in glass cages with bedding composed of wood shavings, which were treated with a biological agent for odor control three times per week. To minimize environmental variability, all cages were maintained in a designated experimental room with controlled temperature (28–30°C) and humidity (55–60%), ensuring consistency throughout the study. The facility followed strict hygiene protocols, including regular bedding replacement and scheduled cleaning procedures to reduce external stressors.

The mice were provided a standardized rodent diet and filtered, clean drinking water, ensuring adequate nutrition and hydration. Before the initiation of the experiments, a 7-day acclimatization period was implemented, during which the mice were closely monitored for signs of stress, illness, or abnormal behavior. This period allowed for physiological stabilization and adaptation to the experimental conditions, reducing potential confounding factors in subsequent analyses. The light/dark cycle was maintained at 12 hours each to synchronize circadian rhythms, preventing stress-induced fluctuations in metabolic and immune responses.

All experimental procedures adhered to the ARRIVE 2.0 guidelines and were approved by the relevant ethical review board to ensure compliance with ethical standards for animal research [16].

2.4. Experimental design

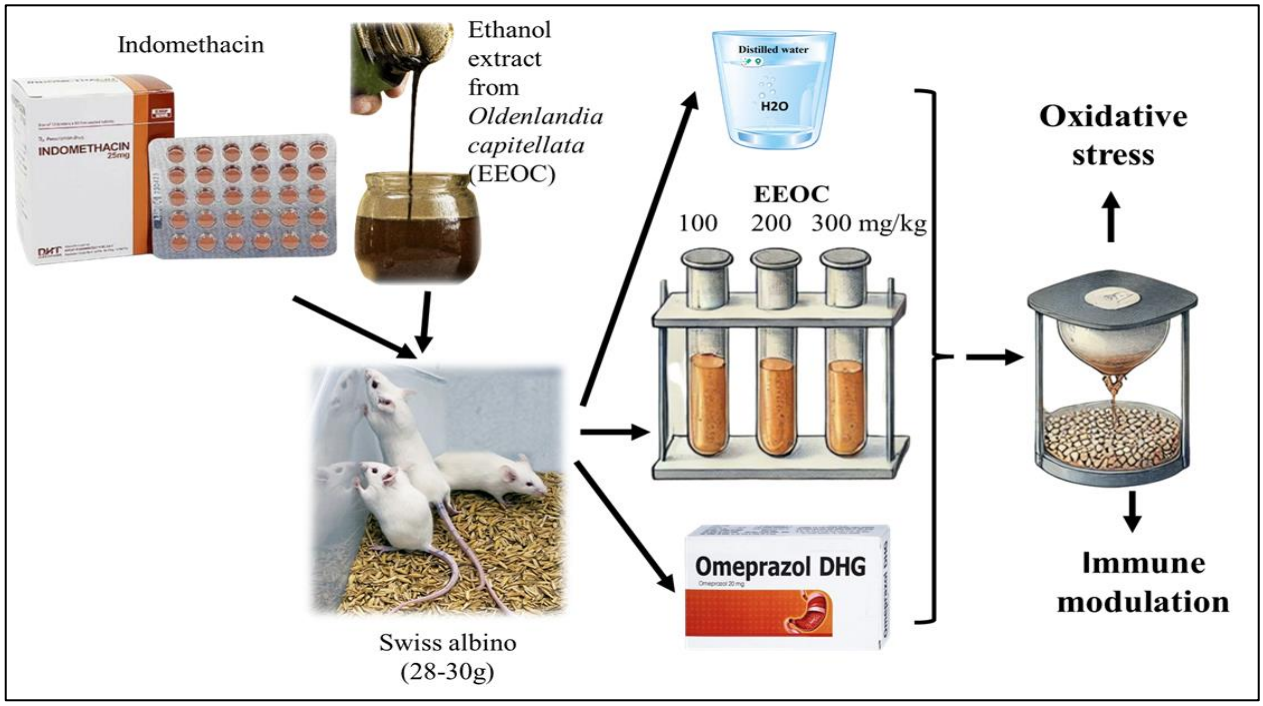

Figure 1. Antioxidant and immunomodulatory effects of ethanol extract from *Oldenlandia capitellata* Kuntze leaves in indomethacin-induced gastric inflammation in mice

The experimental animals were weighed and randomly divided into six groups, with five mice in each group.

Normal control group: Mice received distilled water at a dose of 10 mL/kg, serving as the untreated control group (Normal group).

Negative control group: Mice were administered a single dose of indomethacin (IND) at 45 mg/kg to induce damage, without any protective or therapeutic intervention (IND group).

Positive control group: Mice were given indomethacin (45 mg/kg, single dose) along with omeprazole (OME) at 20 mg/kg to assess the protective effects of OME against indomethacin-induced damage (IND+OME group).
Experimental groups: Mice in these groups were administered indomethacin (45 mg/kg, single dose) along with EEOC at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively, to evaluate the protective effects of EEOC at different dosages (IND+EEOC100, IND+EEOC200, IND+EEOC300 groups).
The EEOC and OME were administered orally via gavage (using a tube inserted into the esophagus to deliver the substances directly into the stomach), ensuring full drug delivery and preventing reflux. This intervention continued for 21 consecutive days, with treatments given daily. Before starting the treatment, the animals were fasted for 24 hours, though water was provided ad libitum. The fasting ensured the removal of dietary influences, allowing accurate monitoring of the treatment effects. After the 21-day treatment period, all groups underwent evaluations to assess the recovery or progression of indomethacin-induced damage. These assessments included biochemical analysis of oxidative stress and immunomodulatory potential of EEOC (Figure 1).

2.5. Evaluation of the extract's activity in reducing oxidative stress

Malondialdehyde (MDA) determination: MDA is measured by its reaction with thiobarbituric acid (TBA) to form a pink MDA-TBA complex that absorbs light at 532 nm. This reaction occurs under acidic conditions at 95°C for 30–60 minutes. The MDA concentration is calculated from the absorbance of processed samples like tissue homogenates or plasma, compared to a standard curve. Results are expressed as nmol MDA per mg of protein [17].
Hydrogen peroxide (H_2O_2) determination: The method measures H_2O_2 by reacting it with ferrous ions (Fe^{2+}), producing ferric ions (Fe^{3+}) that bind to xylenol orange, forming a colored complex. After incubating the sample with the reagent mixture for 30 minutes at room temperature, the absorbance is measured at 560 nm. H_2O_2 concentration is calculated from absorbance values using a standard curve, expressed as μmol H_2O_2 per mg of protein or per mL of sample [17].
Glutathione (GSH) determination: Glutathione (GSH) is measured using its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), or Ellman's reagent. This reaction produces a yellow compound, 5-thio-2-nitrobenzoic acid (TNB), which absorbs light at 412 nm. After preparing the sample (typically tissue homogenate or plasma) in a buffer and mixing it with DTNB, the absorbance is measured at room temperature. GSH concentration is calculated using a standard curve and is expressed as μmol GSH per mg of protein or per mL of sample [18].
Total antioxidant capacity (TAC) determination: Total antioxidant capacity (TAC) is evaluated via the Trolox equivalent antioxidant capacity (TEAC) assay, where antioxidants reduce the $ABTS^+$ radical, causing a decrease in blue-green color measured at 734 nm, and the ferric reducing antioxidant power (FRAP) assay, which detects the reduction of Fe^{3+} to Fe^{2+} by antioxidants, forming a blue complex measured at 593 nm. TAC is calculated using Trolox as a standard and expressed in Trolox equivalents or μmol antioxidant equivalents per mL or mg of protein [18].
Catalase (CAT) determination: Catalase (CAT) activity is commonly measured by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The reaction involves the conversion of H_2O_2 to water and oxygen, and the rate of decrease in absorbance at this wavelength is directly proportional to CAT activity [18].
Superoxide dismutase (SOD) determination: Superoxide dismutase (SOD) activity is typically determined using a colorimetric assay that measures the inhibition of the formazan dye formation from the reaction of superoxide radicals with a substrate such as nitroblue tetrazolium (NBT). The absorbance is measured at 560 nm, with the percentage inhibition correlating to SOD activity [18].
Glutathione peroxidase (GPx) determination: Glutathione peroxidase (GPx) activity is assessed by monitoring the reduction of hydrogen peroxide or organic peroxides in the presence of glutathione. This reaction is coupled with the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH), and the decrease in NADPH absorbance at 340 nm is measured to quantify GPx activity. Results for CAT, SOD, and GPx are typically expressed in units per milligram of protein [18].

2.6. Evaluation of the extract's ability to modulate the immune response

White blood cell (WBC) determination is done using a hematology analyzer MEK-7300K (Nihon Kohden, Japan), which counts and differentiates leukocytes in a diluted blood sample through electrical impedance and optical light scatter [18].
Nitroblue tetrazolium reduction test (NBT): The Nitroblue tetrazolium (NBT) reduction test evaluates neutrophil activity by measuring its ability to produce reactive oxygen species (ROS). In this assay, neutrophils are incubated with NBT, which is reduced to a blue formazan precipitate when exposed to superoxide radicals generated by activated neutrophils. After incubation at 37°C, the cells are fixed, and the formazan crystals are solubilized. The absorbance is measured at 620 nm [18].
T-independent (TI) determination: T-independent (TI) determination is performed using an enzyme-linked immunosorbent assay (ELISA). In the ELISA setup, serum samples are coated with antigens, followed by the addition of a secondary antibody conjugated to an enzyme. A substrate is then added, and the resulting color change is measured spectrophotometrically, indicating the antibody response that can be generated in response to TI antigens [18].
Phagocytic activity (PA) determination is conducted using the phagocytic index (PI) and phagocytic rate (PR). In the PI method, phagocytic cells are incubated with a suspension of fluorescently labeled pathogens or particles, such as latex beads. After incubation, the cells are washed to remove any unengulfed particles and are analyzed using flow cytometry or fluorescence microscopy. The PR is calculated as the percentage of phagocytic cells that have consumed the particles, while the PI measures the average number of particles ingested per phagocytic cell [18].

2.7. Statistical analysis

All statistical analyses were conducted using Statgraphics Centurion XIX software. One-way analysis of variance (ANOVA) was employed to assess significant differences among groups. The Student-Newman-Keuls test was utilized for post hoc comparisons between groups. Results are presented as mean ± standard deviation, and a p-value of less than 0.05 was considered indicative of statistically significant differences

3. RESULTS AND DISCUSSION

3.1. Qualitative and quantitative phytochemical analysis of bioactive compounds in the extracts

Table 1. Qualitative and quantitative assessment of phytochemicals in EEOC

Plant constituents	Test	Examination	Present in EEOC	Measurement of plant compounds
Tannins	2mL EEOC + 2mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green sediment	+	-
Flavonoids	1mL EEOC + 1mL $Pb(OAc)_4$ (10%)	Yellow hue	+	41.41 ± 2.22 (mg QE/g)
Terpenoids	2mL EEOC + 2mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Dark red hue	+	-
Polyphenol	2mL EEOC + 2mL $FeCl_3$	Blue-green look	+	71.44 ± 1.27 (mg GAE/g)
Saponins	5mL EEOC + 5mL H_2O + heat	Foam forms	+	-
Steroids	2mL EEOC + 2mL $CHCl_3$ + 2mL H_2SO_4 (conc.)	The rust-colored ring at the interface	+	-
Cardiac glycosides	2mL EEOC + 2mL $CHCl_3$ + 2mL CH_3COOH	Purple to blue to green hue	-	-
Alkaloids	2mL EEOC + a few drops of Hager's reagent	Yellow sediment	+	-

The existence of phytochemicals in EEOC is indicated by (+) for present and (-) for absent.

Table 1 demonstrates that the ethanol extract of *O. capitellata* leaves (EEOC) contains several bioactive compounds, most notably flavonoids (41.41 ± 2.22 mg QE/g) and polyphenols (71.44 ± 1.27 mg GAE/g), both recognized for their potent antioxidant properties. These compounds are key in neutralizing free

radicals and reducing oxidative stress, and they are critical for protecting tissues from damage. Additionally, terpenoids, saponins, steroids, tannins, and alkaloids suggest potential roles in immune regulation and anti-inflammatory activity. In a model of IND-induced gastric inflammation in mice, EEOC's antioxidant constituents help mitigate gastric mucosal damage by suppressing inflammation and oxidative stress, contributing to improved mucosal protection and immune response modulation. The broad spectrum of chemical constituents highlights EEOC's potential for oxidative stress reduction and immune regulation, despite the absence of cardiac glycosides in the extract.

Natural herbs are promising for reducing oxidative stress and modulating immune responses, especially in inflammation-induced conditions like IND-induced gastritis. Rich in bioactive compounds such as flavonoids and polyphenols, the herbs act as antioxidants, protecting tissues from oxidative damage and regulating immune responses to reduce inflammation and promote healing, making them valuable in managing gastric inflammation [19]. The ethanol extract of Oldenlandia capitellata (EEOC) contains a variety of bioactive compounds, including alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids, and saponins, all of which contribute to its therapeutic potential. Notably, flavonoids and polyphenols are present in high concentrations, playing a key role in reducing oxidative stress, a major factor in IND-induced gastric damage. These compounds, known for their antioxidant properties, effectively scavenge free radicals, preventing oxidative damage to the gastric mucosa [20]. In addition to their antioxidant activity, flavonoids, and polyphenols, along with alkaloids, terpenoids, and saponins, exhibit anti-inflammatory effects by modulating immune responses and inhibiting pro-inflammatory mediators [21]. Steroids and tannins further support this process by stabilizing cell membranes and reducing inflammation [22]. Together, these compounds act synergistically to limit tissue damage, reduce oxidative stress, and regulate immune responses [23], demonstrating EEOC's potential in protecting against gastric injury caused by indomethacin. Previous studies have extensively documented the therapeutic potential of plant-derived compounds in managing oxidative stress and inflammation, particularly in gastrointestinal disorders. For instance, research by Ahmadi et al. (2022) highlighted the protective effects of flavonoid-rich extracts from *Scutellaria baicalensis* against indomethacin-induced gastric lesions, demonstrating significant reductions in oxidative damage and inflammation [24]. Similarly, Chantree et al. (2023) found that polyphenol-rich extracts from *Garcinia dulcis* exhibited strong antioxidant activities and effectively mitigated gastric mucosal injury by inhibiting pro-inflammatory cytokines [25]. Furthermore, a study by Alves et al. (2023) emphasized the role of terpenoids in reducing oxidative stress in gastric tissues, supporting the idea that a diverse array of phytochemicals can work synergistically to enhance mucosal protection [26]. This growing body of research provides a solid foundation for investigating the effects of herbal extracts like O. capitellata in protecting against IND-induced gastric injury.

3.2. Evaluation of the extract's activity in reducing oxidative stress

Table 2. Effect of EEOC on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) level

Groups	Malondialdehyde (MDA) (nmol/mg protein)			Hydrogen peroxide (H_2O_2) (nmol/g tissue)		
	Livers	Kidneys	Spleens	Livers	Kidneys	Spleens
Normal group	1.88 ± 0.01^{a}	1.29 ± 0.01^{a}	1.46 ± 0.01^{a}	1.13 ± 0.04^{a}	0.47 ± 0.02^{a}	0.41 ± 0.01^{a}
IND group	3.57 ± 0.03^{f}	2.45 ± 0.02^{f}	2.77 ± 0.02^{f}	2.15 ± 0.04^{f}	2.34 ± 0.04^{f}	0.78 ± 0.02^{f}
IND+OMEgroup	2.07 ± 0.01^{b}	1.42 ± 0.01^{b}	1.61 ± 0.02^{b}	1.24 ± 0.02^{b}	0.52 ± 0.03^{b}	0.54 ± 0.03^{b}
EEOC100 group	2.82 ± 0.02^{e}	1.94 ± 0.02^{e}	2.19 ± 0.01^{e}	1.71 ± 0.03^{e}	0.71 ± 0.01^{e}	0.74 ± 0.02^{e}
EEOC200 group	2.63 ± 0.02^{d}	1.81 ± 0.02^{d}	2.04 ± 0.02^{d}	1.58 ± 0.02^{d}	0.66 ± 0.02^{d}	0.69 ± 0.02^{d}
EEOC300 group	2.26 ± 0.01^{c}	1.55 ± 0.02^{c}	1.75 ± 0.02^{c}	1.36 ± 0.02^{c}	0.56 ± 0.02^{c}	0.59 ± 0.03^{c}

The values are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

The data in Table 2 illustrate the antioxidant effects of the EEOC by evaluating malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels in a model of IND-induced gastric inflammation. In the IND group, both MDA and H_2O_2 levels were significantly elevated, reaching 3.57 ± 0.03 nmol/mg protein and 2.15 ± 0.04 nmol/g tissue, respectively ($p < 0.05$). This highlights the considerable oxidative stress and lipid peroxidation resulting from paracetamol toxicity. In contrast, the IND + OME group exhibited moderate levels of oxidative stress, with MDA at 2.07 ± 0.01 nmol/mg protein and H_2O_2 at 1.24 ± 0.02 nmol/g tissue

($p < 0.05$), indicating ongoing oxidative damage due to IND. Treatment with EEOC resulted in significant reductions in both MDA and H_2O_2 levels, particularly in a dose-dependent manner. The EEOC 300 group showed a notable decrease in MDA levels to 2.26 ± 0.01 nmol/mg protein and H_2O_2 levels to 1.36 ± 0.02 nmol/g tissue ($p < 0.05$). Other doses of EEOC also exhibited reductions, with the EEOC 100 group showing MDA at 2.82 ± 0.02 nmol/mg protein and H_2O_2 at 1.71 ± 0.03 nmol/g tissue ($p < 0.05$), while the EEOC 200 group recorded MDA at 2.63 ± 0.02 nmol/mg protein and H_2O_2 at 1.58 ± 0.02 nmol/g tissue ($p < 0.05$). These results indicate that EEOC effectively alleviates oxidative stress in gastric tissues induced by IND. EEOC effectively counteracts oxidative stress in IND-induced gastric inflammation in a dose-dependent manner. Higher doses resulted in greater reductions in oxidative stress markers (MDA, H_2O_2).

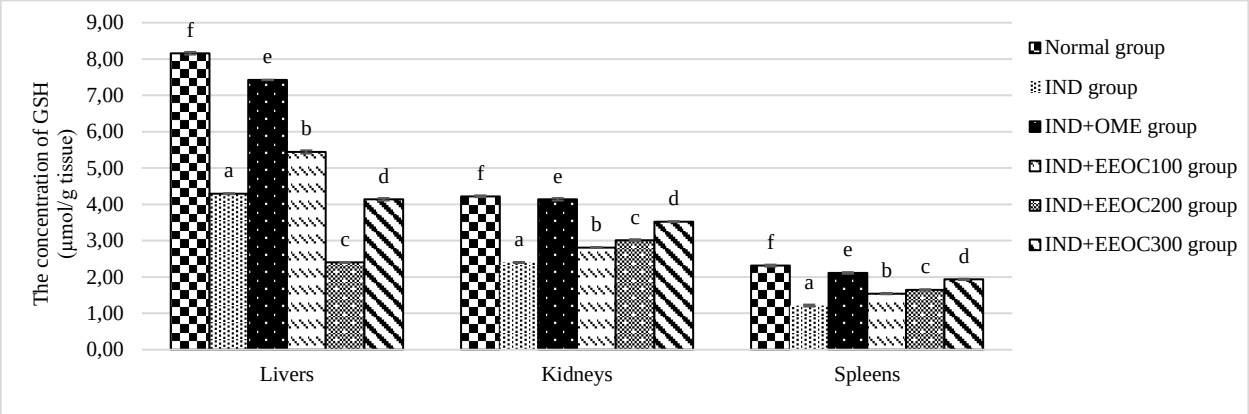

Figure 2. Effect of EEOC on glutathione (GSH) levels. Results are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

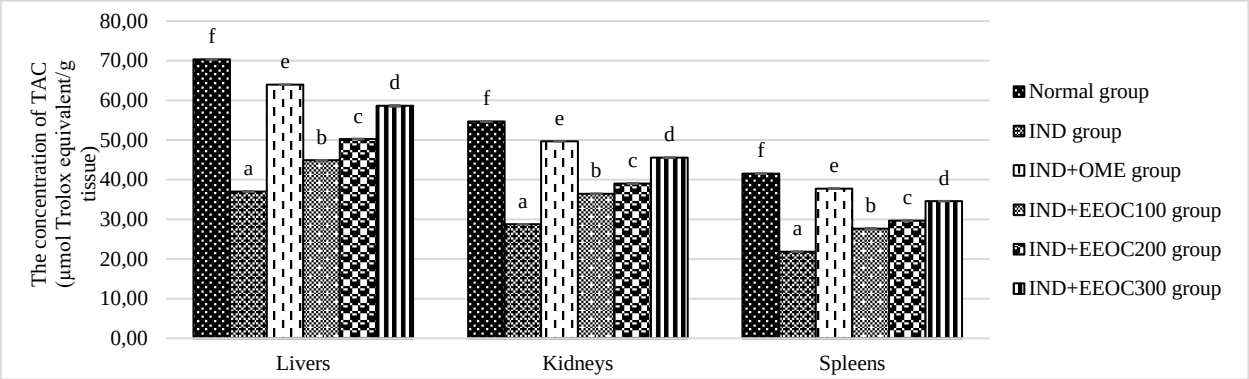

Figure 3. Effect of EEOC on total antioxidant capacity (TAC) levels. Results are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

The EEOC demonstrates significant potential in combating oxidative stress associated with IND-induced gastric inflammation, as evidenced by its effects on glutathione (GSH) levels and total antioxidant capacity (TAC). In Figure 2, GSH levels in the liver were notably decreased in the IND group (4.29 ± 0.01 nmol/mg protein) compared to the Normal group (8.16 ± 0.01 nmol/mg protein) ($p < 0.05$), indicating a pronounced oxidative stress response due to IND administration. However, treatment with EEOC resulted in a recovery of GSH levels, particularly in the EEOC 300 group (4.14 ± 0.01 nmol/mg protein) ($p < 0.05$), showcasing a dose-dependent effect. Similarly, in Figure 3, TAC levels also exhibited a marked decline in the IND group (0.01 ± 0.01 nmol/mg protein) compared to the Normal group (0.04 ± 0.01 nmol/mg protein) ($p < 0.05$), reflecting diminished antioxidant defenses. Remarkably, the treatment groups, especially EEOC 300, showed an increase in TAC levels to 0.19 ± 0.01 nmol/mg protein ($p < 0.05$). EEOC effectively counteracts oxidative stress in IND-induced gastric inflammation in a dose-dependent manner. Higher doses led to a greater restoration of antioxidant defenses (GSH, TAC), confirming its therapeutic potential in gastric protection.

Table 3. Effect of EEOC on superoxide dismutase (SOD), and catalase (CAT) levels

Groups	Catalase (CAT) (U/mg protein)			Superoxide dismutase (SOD) (U/mg protein)		
	Livers	Kidneys	Spleens	Livers	Kidneys	Spleens
Normal group	87.14 ± 0.05^{f}	40.52 ± 0.03^{f}	21.36 ± 0.05^{f}	34.14 ± 0.09^{f}	24.65 ± 0.07f	15.49 ± 0.07^{f}
IND group	45.86 ± 0.03^{a}	21.33 ± 0.04^{a}	11.24 ± 0.03^{a}	17.97 ± 0.05^{a}	12.97 ± 0.04^{a}	8.15 ± 0.07^{a}
IND+OME group	79.22 ± 0.06^{e}	36.84 ± 0.04^{e}	19.42 ± 0.03^{e}	31.04 ± 0.02e	22.41 ± 0.04^{e}	14.08 ± 0.03^{e}
EEOC100 group	58.09 ± 0.07^{b}	27.01 ± 0.04^{b}	14.24 ± 0.06^{b}	22.76 ± 0.16^{b}	16.43 ± 0.08^{b}	10.31 ± 0.07^{b}
EEOC200 group	62.24 ± 0.10^{c}	28.94 ± 0.04^{c}	15.26 ± 0.09^{c}	24.39 ± 0.07^{c}	17.61 ± 0.05^{c}	11.06 ± 0.07^{c}
EEOC300 group	72.62 ± 0.04^{d}	33.77 ± 0.04^{d}	17.81 ± 0.06^{d}	28.45 ± 0.02^{d}	20.54 ± 0.07^{d}	12.91 ± 0.08^{d}

The values are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

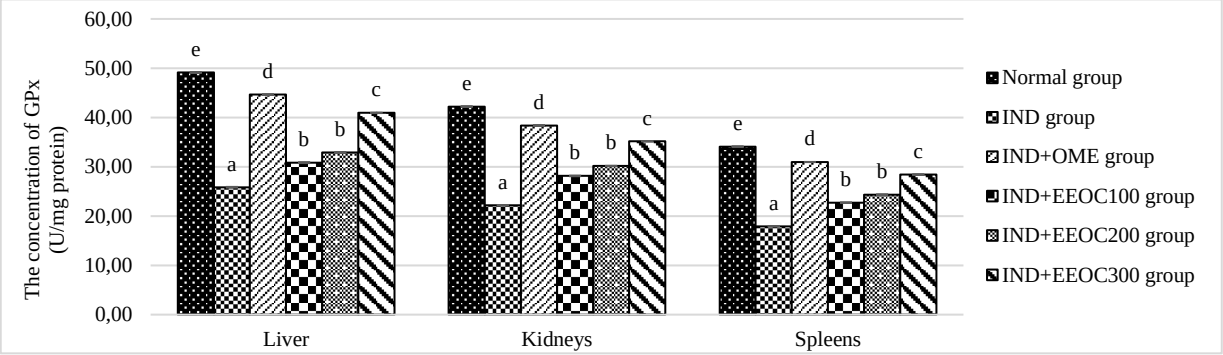

Figure 4. Effect of EEOC on glutathione peroxidase (GPx) levels. Results are presented as Mean ± SD, with the letters a - e denoting significant differences between groups ($p < 0.05$).

The EEOC exhibits significant antioxidant properties against oxidative stress induced by IND in gastric inflammation models, by its effects on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels. Table 3 shows, that in the Normal group, CAT activity was highest, measuring 87.14 ± 0.05 U/mg protein in the liver, 40.52 ± 0.03 U/mg protein in the kidneys, and 21.36 ± 0.05 U/mg protein in the spleen ($p < 0.05$). Conversely, the IND group exhibited reduced CAT levels, dropping to 45.86 ± 0.03 U/mg protein in the liver, 21.33 ± 0.04 U/mg protein in the kidneys, and 11.24 ± 0.03 U/mg protein in the spleen ($p < 0.05$), indicating compromised antioxidant defenses. Treatment with EEOC restored CAT activity, particularly in the EEOC300 group, which recorded levels of 72.62 ± 0.04 U/mg protein in the liver, 33.77 ± 0.04 U/mg protein in the kidneys, and 17.81 ± 0.06 U/mg protein in the spleen ($p < 0.05$). SOD levels were similar, with the Normal group at 34.14 ± 0.09 U/mg protein ($p < 0.05$) and the IND group significantly reduced to 17.97 ± 0.05 U/mg protein ($p < 0.05$). Following EEOC treatment, SOD levels in the EEOC300 group rose to 28.45 ± 0.02 U/mg protein ($p < 0.05$). GPx activity also showed notable variation (Figure 4), the Normal group had GPx levels of 49.12 in the liver, 42.21 in the kidneys, and 34.08 in the spleen ($p < 0.05$), while the IND group levels fell significantly ($p < 0.05$). After EEOC treatment, the EEOC300 group improved GPx levels to 40.93 in the liver, 35.18 in the kidneys, and 28.41 in the spleen ($p < 0.05$). EEOC significantly and dose-dependently restores SOD, CAT, and GPx activity, reducing oxidative stress and enhancing antioxidant defenses. Higher doses (EEOC300) showed the most pronounced recovery, reinforcing the extract's therapeutic potential in gastric protection.

The EEOC has shown significant antioxidant effects in alleviating oxidative stress induced by IND in a gastric inflammation model. This is evident from the regulation of various oxidative stress markers, including MDA, H_2O_2, GSH, TAC, SOD, CAT, and GPx in the liver, spleen, and kidneys of affected mice. MDA serves as a reliable marker for lipid peroxidation, directly indicating oxidative damage. In the IND-treated group, elevated MDA levels suggest an increase in oxidative stress and lipid peroxidation. In contrast, the significant reduction of MDA levels in the EEOC-treated groups demonstrates the extract's

efficacy in preventing lipid peroxidation and minimizing oxidative damage. Similarly, H_2O_2 is found at higher levels in the IND group, reinforcing the presence of oxidative damage. EEOC treatment, however, leads to a marked decrease in H_2O_2 levels, further confirming its protective role in counteracting oxidative stress. IND exerts its effects by inhibiting COX enzymes, reducing PGE synthesis, and leading to elevated oxidative stress. Increased levels of MDA and H_2O_2 indicate lipid peroxidation and cellular damage. The rise in MDA levels reflects heightened oxidative stress and increased susceptibility of hepatocytes, renal cells, and splenic cells to damage, thereby contributing to the development of inflammatory diseases [27]. Conversely, EEOC administration significantly reduces MDA and H_2O_2 levels, highlighting its ability to inhibit lipid peroxidation through the scavenging of reactive oxygen species (ROS) by its bioactive components, such as flavonoids, polyphenols, and tannins. These compounds convert harmful free radicals into less toxic forms, protecting cells from oxidative stress [28]. In the IND-treated group, GSH is significantly depleted, reflecting compromised antioxidant defenses, and the TAC is also diminished. In contrast, EEOC treatment effectively restores GSH levels and enhances TAC, indicating its ability to replenish antioxidant reserves and strengthen the body's defense mechanisms. Flavonoids and polyphenols in EEOC act by upregulating nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of cellular antioxidant response, leading to increased expression of antioxidant proteins such as heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1), which collectively enhance TAC and protect cells from oxidative injury. The restoration of GSH and improvement in TAC by EEOC are attributed to its promotion of antioxidant enzyme activity. Specifically, IND reduces the levels of key antioxidant enzymes, including SOD, CAT, and GPx, which are essential for neutralizing reactive oxygen species (ROS). However, EEOC counteracts this reduction by elevating SOD, CAT, and GPx levels, suggesting that the bioactive compounds in EEOC stimulate the expression and activity of these critical antioxidant enzymes. Flavonoids and polyphenols achieve this effect by modulating mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) pathways, which play a role in oxidative stress responses. By suppressing NF-κB activation, EEOC may reduce oxidative damage and inflammation while simultaneously enhancing the expression of SOD, CAT, and GPx through MAPK signaling modulation.

This combined action of restoring GSH, enhancing TAC, and upregulating antioxidant enzyme activity underscores EEOC's role in mitigating oxidative stress and restoring antioxidant defenses compromised by IND treatment. IND exerts its harmful effects primarily through the inhibition of COX enzymes, leading to decreased PGE synthesis and heightened oxidative stress in the liver, spleen, and kidneys of mice with gastric inflammation [29]. The reduction in PGE disrupts the cellular redox balance, leading to a depletion of critical antioxidants like GSH, which plays a central role in neutralizing reactive oxygen species (ROS) and maintaining oxidative equilibrium [30]. Additionally, the increase in ROS, such as H_2O_2, and lipid peroxidation products, overwhelms the body's antioxidant defenses, causing a significant reduction in total antioxidant capacity (TAC) [31]. This oxidative stress impairs the activity of essential antioxidant enzymes, including SOD, CAT, and GPx, further exacerbating cellular damage and inflammation in these organs. The present findings align with previous research on the protective effects of plant extracts in IND-induced gastric inflammation models. For instance, Park and Hahm (2024) demonstrated that flavonoid-rich extracts, such as those from walnuts, exhibit antioxidant properties by restoring GSH levels and enhancing total antioxidant capacity (TAC) in the liver, spleen, and kidneys of IND-treated mice [32]. Similarly, Danisman et al. (2023) showed that carnosic acid, a natural antioxidant found in *Rosmarinus officinalis*, significantly increased SOD, CAT, and GPx levels in these same tissues of mice with IND-induced gastric inflammation [33]. These findings suggest that polyphenols and flavonoids in EEOC function through similar mechanisms as other well-documented plant-based antioxidants, supporting their role in ROS scavenging, lipid peroxidation inhibition, and antioxidant enzyme upregulation.

This consistency in findings suggests that the mechanism of EEOC, involving the activation of antioxidant pathways and enzyme expression, mirrors well-established research on plant-based therapies for oxidative stress. For example, Nabil et al. (2021) found that an extract from *Albizia anthelmintica* leaves also decreased levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), markers of oxidative stress, similar to the observations in this study [34]. This comparative analysis further confirms that EEOC's phytochemical profile is capable of protecting against oxidative damage and inflammation, in line with other plant-based antioxidant treatments.

3.3. Evaluation of the extract's ability to modulate the immune response

Table 4. Effect of EEOC on white blood cell (WBC) count, nitroblue tetrazolium (NBT) positivity, and total immunoglobulin (TI) levels

Groups	Blood cell (WBC) (x 10^3cells/mm^3)	Percent NBT-positive cells (PNPC) (%)	Total immunoglobulin (TI) (mg/mL)
Normal group	4.49 ± 0.09^a	12.85 ± 0.13^a	16.66 ± 0.17^f
IND group	8.53 ± 0.11^f	24.42 ± 0.22^f	8.77 ± 0.11^a
IND+OMEgroup	4.94 ± 0.08^b	14.14 ± 0.16^b	15.21 ± 0.17^e
EEOC100 group	6.74 ± 0.11^e	19.28 ± 0.16^e	11.11 ± 0.13^b
EEOC200 group	6.29 ± 0.22^d	17.99 ± 0.14^d	11.91 ± 0.12^c
EEOC300 group	5.39 ± 0.19^c	15.42 ± 0.15^c	13.88 ± 0.26^d

The values are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

Table 4 summarizes the effects of EEOC on white blood cell (WBC) count, nitroblue tetrazolium (NBT) positivity, and total immunoglobulin (TI) levels in mice with IND-induced gastric inflammation. The normal group had a WBC count of 4.49 ± 0.09 x 10^3 cells/mm^3, which increased significantly to 8.53 ± 0.11 x 10^3 cells/mm^3 in the IND group ($p < 0.05$). Treatment with EEOC resulted in WBC counts of 6.74 ± 0.11, 6.29 ± 0.22, and 5.39 ± 0.19 x 10^3 cells/mm^3 for the EEOC100, EEOC200, and EEOC300 groups, respectively ($p < 0.05$). NBT positivity rose from 12.85 ± 0.13% in the normal group to 24.42 ± 0.22% in the IND group ($p < 0.05$), while EEOC treatment reduced positivity to 19.28 ± 0.16%, 17.99 ± 0.14%, and 15.42 ± 0.15% in the respective EEOC groups ($p < 0.05$). Total immunoglobulin levels decreased from 16.66 ± 0.17 mg/mL in the normal group to 8.77 ± 0.11 mg/mL in the IND group ($p < 0.05$), with partial restoration in the EEOC groups (11.11 ± 0.13, 11.91 ± 0.12, and 13.88 ± 0.26 mg/mL) ($p < 0.05$). EEOC treatment significantly and dose-dependently mitigated immune dysfunction caused by IND, reducing WBC count and NBT positivity while restoring total immunoglobulin levels. The EEOC300 group demonstrated the strongest immunomodulatory effects, suggesting its potential for therapeutic application in gastric protection.

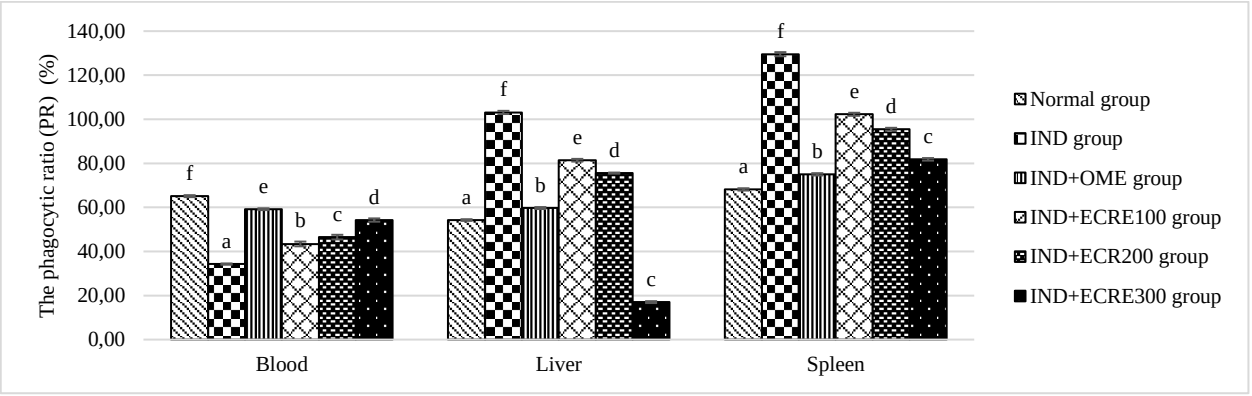

Figure 5. Effect of EEOC on the phagocytic ratio (PR). Results are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

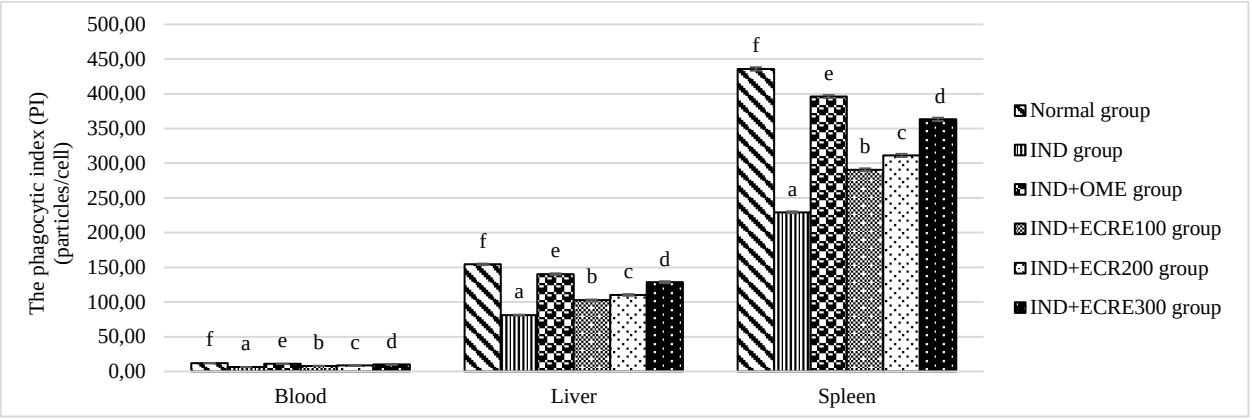

Figure 6. Effect of EEOC on phagocytic ratio (PI). Results are presented as Mean ± SD, with the letters a - f denoting significant differences between groups ($p < 0.05$).

Figures 5 and 6 present the effects of EEOC on the phagocytic ratio (PR) and phagocytic index (PI) in mice with IND-induced gastric inflammation. In the normal group, the PR was highest in the spleen at 68.18% and lowest in the liver at 54.27% ($p < 0.05$). The IND group exhibited a significant decline in PR across all organs, with values dropping to 34.27% for blood, 103.11% for liver, and 129.54% for spleen ($p < 0.05$), indicating impaired phagocytic activity. Treatment with EEOC led to partial recovery, with the IND+ECRE100 group showing a PR of 43.41% in blood and 102.27% in the spleen ($p < 0.05$). The IND+ECR200 group had a PR of 46.51% in blood and 95.45% in the spleen ($p < 0.05$), while the IND+ECR300 group showed a PR of 54.26% in blood, although it significantly decreased to 17.01% in the liver ($p < 0.05$). For the PI, the normal group had values of 12.06 particles/cell in blood, 154.32 particles/cell in the liver, and 435.81 particles/cell in the spleen ($p < 0.05$), reflecting robust phagocytic activity. In contrast, the IND group recorded lower PI values of 6.35 particles/cell in blood, 81.22 particles/cell in the liver, and 229.37 particles/cell in the spleen ($p < 0.05$), highlighting diminished phagocytic function. EEOC treatment improved the PI, with the IND+ECR300 group showing values of 10.05 particles/cell in blood, 128.61 particles/cell in liver, and 363.18 particles/cell in spleen ($p < 0.05$). EEOC significantly and dose-dependently improved PR and PI, indicating enhanced immune function and phagocytic efficiency. The EEOC300 group exhibited the strongest immunomodulatory effects, though liver-specific PR reduction suggests potential tissue-dependent variations in phagocytic regulation.

The immunomodulatory effects of EEOC were evaluated by examining changes in WBC counts, NBT positivity, TI levels, PR, and PI in mice with IND-induced gastric inflammation. The significant increase in WBC and NBT counts in the IND group indicates a pronounced inflammatory response linked to heightened immune activity, which can be detrimental in chronic inflammation and is associated with increased reactive oxygen species (ROS) production. This response primarily results from IND's inhibition of COX enzymes, leading to decreased synthesis of PGE [35]. The reduction in anti-inflammatory signals subsequently elevates pro-inflammatory cytokine levels, stimulating the bone marrow to produce more WBCs and raising their counts in circulation [36]. Conversely, treatment with EEOC resulted in decreased WBC and NBT counts, suggesting effective modulation of the immune response. By preventing excessive inflammation and promoting a return to homeostasis, EEOC helps downregulate oxidative stress and modulate phagocyte activity, potentially minimizing tissue damage associated with chronic inflammation. This modulation occurs through the inhibition of pro-inflammatory mediators, regulation of immune cell activity, and improvement of endothelial function, which together reduce leukocyte migration [17]. Additionally, the significant drop in TI levels in the IND group indicates impaired immune function, while EEOC treatment led to a partial restoration of these levels, suggesting enhanced humoral immunity and overall immune function. The substantial decline in both PR and PI in the IND group reflects reduced phagocytic activity during inflammation. In contrast, EEOC treatment resulted in varying improvements in PR and PI, indicating enhanced phagocytic function and restored efficiency [37]. Previous studies support these findings; for instance, research by Tran and Le (2024a) demonstrated that extracts of *Caryota urens* fruit, rich in flavonoids and polyphenols, exhibit anti-inflammatory and antioxidant properties that help regulate immune responses [17]. Similarly, Tran and Le (2024b) also found that extracts of *Gardenia*

stenophylla fruit can restore immune homeostasis by downregulating excessive WBC production [18]. Collectively, these studies reinforce the notion that natural products, like EEOC, are effective therapeutic agents in managing inflammation and regulating immune function in preclinical models. Thus, the results from this study on EEOC further highlight the potential of plant extracts to regulate immune responses and provide therapeutic benefits in inflammatory conditions, emphasizing the need for further exploration of such extracts in developing novel anti-inflammatory therapies.

4. CONCLUSION

The ethanol extract from *Oldenlandia capitellata* (EEOC) demonstrates strong antioxidant and immunomodulatory effects in a mouse model of indomethacin-induced gastric inflammation. EEOC effectively reduces oxidative stress by decreasing malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels while enhancing key antioxidant defenses, including glutathione (GSH), total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, EEOC modulates immune responses by lowering white blood cell (WBC) count and nitroblue tetrazolium (NBT) positivity while restoring total immunoglobulin (TI) levels, phagocytic ratio (PR), and phagocytic index (PI). These findings highlight EEOC's therapeutic potential in mitigating oxidative stress and regulating immune function, making it a promising candidate for managing gastric inflammation and related disorders.

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TÁC DỤNG CHỐNG OXY HÓA VÀ ĐIỀU HÒA MIỄN DỊCH CỦA CHIẾT XUẤT ETHANOL TỪ LÁ KUNTZE OLDENLANDIA CAPITELLATA TRONG VIÊM DẠ DÀY DO INDOMETHACIN GÂY RA Ở CHUỘT

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Tóm tắt. Mục tiêu: Nghiên cứu này đánh giá tác dụng chống oxy hóa và điều hòa miễn dịch của chiết xuất ethanol từ Oldenlandia capitellata (EEOC) trong mô hình chuột bị viêm dạ dày do indomethacin (IND) gây ra. Phương pháp: Chuột được điều trị bằng IND (45 mg / kg), omeprazole (20 mg / kg) hoặc EEOC (100, 200 và 300 mg / kg). Các dấu ấn sinh học chính của stress oxy hóa và đáp ứng miễn dịch, bao gồm malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glutathione (GSH), tổng khả năng chống oxy hóa (TAC), catalase (CAT), superoxide dismutase (SOD), số lượng bạch cầu (WBC), khử Nitroblue tetrazolium (NBT), globulin miễn dịch toàn phần (TI) và hoạt động thực bào (PA), đã được đánh giá. Kết quả: Nhóm IND cho thấy mức MDA tăng (3,57 ± 0,03 nmol / mg protein) và H_2O_2 (2,15 ± 0,04 nmol / g mô) ($p < 0,05$). Điều trị EEOC, đặc biệt là ở mức 300 mg / kg, làm giảm đáng kể các dấu hiệu stress oxy hóa (MDA: 2,26 ± 0,01 nmol / mg, H_2O_2: 1,36 ± 0,02 nmol / g, $p < 0,05$) và phục hồi các hoạt động của enzyme chống oxy hóa. EEOC cũng điều chỉnh phản ứng miễn dịch bằng cách giảm số lượng WBC, NBT, TI và PA đồng thời cải thiện hoạt động thực bào. Kết luận: EEOC giảm thiểu stress oxy hóa và điều chỉnh phản ứng miễn dịch trong viêm dạ dày, cho thấy tiềm năng của nó như một tác nhân điều trị cho các tình trạng liên quan đến stress oxy hóa.

Từ khóa: Oldenlandia capitellata K., Chiết xuất etanol, Hoạt động chống oxy hóa, Điều hòa miễn dịch, Bảo vệ dạ dày, Liệu pháp thực vật.

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