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Research Paper

Potential Analysis of Snakehead Fish Albumin Extract (*Channa striata*) as Immunostimulant and Anti-Inflammatory in Indomethacin-Induced Inflammatory Bowel Disease (IBD) Rats

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Abstract
Inflammatory Bowel Disease (IBD) is a chronic gastrointestinal disorder driven by oxidative stress and immune dysregulation, often exacerbated by prolonged non-steroidal anti-inflammatory drug (NSAID) use. Conventional therapies provide symptomatic relief but are limited by severe adverse effects, necessitating safer alternatives. *Channa striata* (snakehead fish) is a rich source of albumin, essential amino acids, and bioactive compounds with potential immunomodulatory and anti-inflammatory effects. This study investigates the therapeutic efficacy of purified *Channa striata* albumin extract in an indomethacin-induced IBD rat model. Albumin was isolated via ammonium sulfate precipitation and characterized by SDS-PAGE, antioxidant activity (DPPH assay), and amino acid profiling. Rats were treated with *Channa striata* albumin (100-300 mg/kg BW) for 14 days post-indomethacin induction. Compared to controls and diclofenac-treated groups, the extract significantly reduced malondialdehyde (MDA) levels (up to 90.69%), improved jejunal histoarchitecture, and enhanced occludin expression. Immunohistochemistry showed a marked reduction in $CD4^+$T cell infiltration, indicating immunomodulatory activity. These findings establish *Channa striata* albumin as a potent natural antioxidant and immunomodulator, offering a safer alternative to NSAIDs for IBD management.

Keywords
Channa striata, Albumin, Oxidative Stress, Inflammation, Intestinal Barrier, Immunomodulation

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1. INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract (Saez et al., 2023), arising from a complex interplay between genetic susceptibility (Jarmakiewicz-Czaja et al., 2022), environmental factors (such as smoking, appendicitis, OCPs, diet, breastfeeding, infections/vaccinations, antibiotics, helminths, and childhood hygiene) (Masfria et al., 2023; Molodecky and Kaplan, 2010), and immune dysregulation (Ahluwalia et al., 2018). The increasing global prevalence of IBD poses a significant challenge due to its debilitating effects and the long-term dependency on pharmacological interventions, particularly non-steroidal anti-inflammatory drugs (NSAIDs) (Docherty et al., 2011; Yeshi et al., 2020). While NSAIDs are widely used to manage inflammation, their prolonged use is associated with severe adverse effects, including gastrointestinal mucosal damage (Tai and McAlindon, 2021), renal dysfunction, and cardiovascular risks (Koo et al., 2023), which significantly limit their therapeutic applicability. These limitations necessitate the exploration of alternative therapeutic agents with improved safety profiles and efficacy.

Natural bioactive compounds have gained increasing attention as promising alternatives due to their multitarget activity, safety, and efficacy in chronic inflammatory conditions. In particular, dietary peptides and amino acids from marine sources have been reported to modulate immune signaling, maintain epithelial barrier function, and attenuate oxidative stress in IBD models (Jiang et al., 2025; Mandal et al., 2025; Shahidi and Saeid, 2025; Yu et al., 2025). Snakehead fish (*Channa striata*) is among the most studied freshwater species known for its high albumin content and diverse bioactive constituents, including essential amino acids, unsaturated fatty acids, and trace minerals such as zinc (Zn), copper (Cu), and iron (Fe), which contribute to its immunomodulatory and anti-inflammatory

properties (Dwijayanti et al., 2016; Setiawan et al., 2024).

Recent studies have highlighted the therapeutic potential of natural bioactives in modulating inflammatory responses and preserving intestinal barrier function in IBD. For instance, marine peptides alleviate colitis symptoms by enhancing tight junction protein expression via Nrf2 activation (Guo et al., 2024; Jiang et al., 2023; Oliyaei et al., 2025). Others research reported that dietary fish-derived peptides reduced $CD4^+$T cell infiltration in DSS-induced colitis models through NF-κB pathway inhibition (Mamareli et al., 2021; Ren et al., 2021; Yu et al., 2017). In line with these findings, preclinical studies have also shown that *Channa striata* extract exhibits antioxidant activity and suppresses pro-inflammatory cytokines, reinforcing its potential as a natural candidate for IBD management (Mustafa et al., 2012). Moreover, bioactive peptides derived from fish proteins have been linked to improvements in mucosal integrity and reduced T cell-mediated inflammation, suggesting a mechanistic basis for their protective effects (Firmino et al., 2021). However, current research lacks a systematic investigation into purified albumin from *Channa striata*, particularly in relation to occludin regulation and immunomodulation in NSAID-induced IBD models (Aulanni'am et al., 2020). To the best of our knowledge, no study has yet assessed the specific effects of *Channa striata* albumin on jejunal tight junction integrity and $CD4^+$T cell dynamics under inflammatory stress.

To date, no study has systematically evaluated the therapeutic role of purified albumin extract from Channa striata in relation to critical IBD biomarkers such as occludin and $CD4^+$T cells in an NSAID-induced colitis model. This research addresses that gap by investigating the immunomodulatory and antioxidant effects of purified *Channa striata* albumin in an indomethacin-induced IBD rat model. Specifically, this study evaluates its potential to enhance occludin expression, reduce $CD4^+$T cell infiltration, lower malondialdehyde (MDA) levels, and restore jejunal histoarchitecture. The efficacy of *Channa striata* albumin extract is also compared with diclofenac sodium to assess its suitability as a safer alternative for IBD therapy.

By elucidating the mechanistic role of *Channa striata* albumin in intestinal inflammation, this study provides novel insights into its therapeutic application, offering a natural, bioactive-based strategy for managing IBD with minimal adverse effects. The findings of this study could contribute to the development of alternative therapeutic strategies that leverage bioactive compounds from natural sources to mitigate the adverse effects associated with conventional anti-inflammatory drugs.

2. EXPERIMENTAL SECTION

2.1 Materials

The following materials were used in this study: snakehead fish (*Channa striata*), male Wistar rats (125-265 g), and reagents of analytical grade. Albumin extraction and purification were performed using ammonium sulfate (Sigma-Aldrich), sodium phosphate dibasic (Na_2HPO_4, Merck), and potassium phosphate monobasic (KH_2PO_4, Merck). Indomethacin (Sigma-Aldrich) was used to induce inflammatory bowel disease (IBD) in experimental rats. Additional reagents included copper(II) sulfate hydrate (Sigma-Aldrich), potassium sodium tartrate (Sigma-Aldrich), sodium hydroxide (NaOH, Merck), DPPH (Sigma-Aldrich) and thiobarbituric acid (Sigma-Aldrich) for oxidative stress analysis. Trichloroacetic acid (Merck) was employed for protein precipitation, and sodium chloride (NaCl, Brauwn Pharmaceutical) was used for sample preparation. Histological and immunohistochemical analyses were conducted using hematoxylin-eosin (Ehrlich), 99% ethanol (Smart-Lab), and specific antibodies: anti-CD4 (GK1.5, sc-13573) and anti-occludin (E-5, sc-133256). All solutions were prepared using ultrapure distilled water.

2.2 Extraction, Purification, and Characterization of Snakehead Fish

2.2.1 Extraction and Purification of Snakehead Fish Albumin

Wild *Channa striata* specimens were collected, eviscerated, and thoroughly washed to remove impurities. The fish fillets were sliced and subjected to extraction at 50°C for 6 hours to obtain the crude albumin extract. The crude extract was then purified using a stepwise precipitation method with saturated ammonium sulfate (SAS) at varying concentrations and pH levels (Aulanni'am et al., 2020). The crude albumin extract underwent an initial centrifugation at 10,000 rpm for 30 minutes at 4°C to remove impurities. The supernatant was subjected to precipitation using SAS at concentrations of 70%, 80%, and 90% at pH 4.0. After the addition of SAS, the solution was homogenized and centrifuged under the same conditions. The resulting precipitate was separated, resuspended in phosphate buffer (pH 8), and subsequently dialyzed to remove excess salts. The dialyzed extract was then freeze-dried to obtain the purified albumin fraction (Nurilmala et al., 2021).

2.2.2 Molecular Weight Profile Analysis

The molecular weight of *Channa striata* albumin extract (SAS 70%, 80%, 90% pada pH 4.0) was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a standard technique for protein separation based on molecular weight (Fatma et al., 2023). The procedure was conducted using a 12.5% separating gel and a 12.5% stacking gel. Prior to electrophoresis, the albumin extract was mixed with reducing sample buffer (RSB) in a 1:1 ratio and heated in boiling water for 10 minutes to denature the proteins. A total of 15 μL of the treated albumin extract was loaded into individual wells of the polyacrylamide gel. The electrophoretic separation was performed at a constant voltage of 120 V for 60-80 minutes. Following electrophoresis, the gel was immersed in Coomassie Brilliant Blue dye solution for 1 hour to visualize protein bands. The gel was subsequently destained at intervals of 1.5 hours to enhance band clarity. Protein bands were documented using a Bio-Rad gel documentation system, and molecular weight analysis was performed using the Image Lab software.

2.2.3 Antioxidant Activity

A 0.1 mM stock solution of DPPH was freshly prepared by dissolving 3.94 mg of DPPH powder in 100 mL of methanol, followed by homogenization and storage in the dark at ambient temperature to minimize photodegradation. To prepare the test samples, 50 mg of *Channa striata* albumin extract-obtained via saturated ammonium sulfate (SAS) precipitation at 70%, 80%, and 90% saturation at pH 4.0 was dissolved in 50 mL of methanol until fully solubilized. From these stock solutions, aliquots of 20, 40, 60, 80, and 100 μL were taken and diluted with methanol to a final volume of 10 mL, corresponding to concentrations of 2, 4, 6, 8, and 10 ppm, respectively.

For the DPPH radical scavenging assay, 2 mL of each sample concentration was combined with 2 mL of the DPPH stock solution and subsequently brought to a total volume of 10 mL using methanol. The reaction mixtures were vortexed gently and incubated for 30 minutes in the dark at room temperature to ensure optimal interaction. The absorbance was recorded at 520 nm using a UV-Vis spectrophotometer. The radical inhibition capacity was expressed as a percentage of DPPH scavenged, calculated using Equation 1.

$$\%\text{Inhibition} = \frac{\text{Abs(blank)} - \text{Abs(sample)}}{\text{Abs(blank)}} \times 100 \qquad (1)$$

The antioxidant activity was expressed as the IC_{50} value, which represents the sample concentration required to inhibit 50% of DPPH radicals (Frezzini et al., 2019).

Analysis of Albumin Levels in *Channa striata* Extract The quantification of albumin concentration was conducted using the Biuret colorimetric assay. The Biuret reagent was prepared by dissolving 0.15 g of copper(II) sulfate hydrate and 0.6 g of potassium sodium tartrate in 50 mL of distilled water, followed by the addition of 30 mL of 10% (w/v) sodium hydroxide solution to complete the complexation. For calibration, a standard curve was generated using a bovine serum albumin (BSA) reference (Bianchi-Bosisio, 2005). A 1000 ppm BSA stock solution was prepared by dissolving 10 mg of BSA in 10 mL of distilled water, and serial dilutions were performed to yield working standards with final concentrations of 100, 150, 200, 250, and 300 ppm. Each standard was vortexed and incubated at ambient temperature for 30 minutes prior to spectrophotometric analysis at 520 nm.

Sample measurements were performed by mixing 2.5 mL of the *Channa striata* albumin extract with an equal volume of Biuret reagent, followed by incubation at room temperature for 30 minutes to allow for complete complex formation. Absorbance readings were taken at the maximum wavelength (520 nm), and albumin content was interpolated using the linear regression equation derived from the BSA calibration curve. A reagent blank composed of 2.5 mL distilled water and 2.5 mL Biuret reagent served as the baseline control.

2.3 Amino Acid Profile Analysis

The amino acid composition of *Channa striata* albumin extract was analyzed using Ultra Performance Liquid Chromatography (UPLC), following the standardized laboratory protocol of PT Saraswanti Indo Genetech. This method enables precise separation of amino acids based on structural and compositional differences (Asikin and Kusumaningrum, 2018).

For sample preparation, a single-point amino acid standard solution was prepared with an internal standard. The test sample was accurately weighed and placed in an appropriate reaction vessel, followed by hydrolysis with hydrochloric acid (HCl) to release free amino acids. The hydrolyzed solution was then transferred to a 50 mL volumetric flask, diluted to volume with demineralized water, and homogenized. The sample was subsequently filtered through a 0.2 μm syringe filter to remove particulates, and the internal standard was added to ensure accurate quantification. The derivatized sample was injected into the UPLC system for analysis. Chromatographic separation was performed using a C18 analytical column with a gradient pump system. The mobile phase consisted of Eluent AccQ-Tag Ultra and demineralized water. The column was maintained at a temperature of 49°C, and detection was carried out using a photodiode array (PDA) detector.

2.4 In Vivo Anti-Inflammatory Assays

2.4.1 Experimental Design

Male Wistar rats (1-2 months old) weighing 125-265 g were housed under controlled laboratory conditions with a 12-hour light/dark cycle. The animals were provided ad libitum access to standard chow and water. Prior to the experiment, all rats underwent a one-week acclimatization period. The study was conducted following ethical guidelines and approved by the Ethics Committee of Brawijaya University (Approval No. 131-KEP-UB).

The rats were randomly assigned into six experimental groups, each consisting of five animals (n = 5 per group). Group P0 served as the negative control and comprised healthy rats that did not receive any treatment. Group P1 functioned as the positive control and included rats induced with IBD without any subsequent treatment. Groups P2, P3, and P4 consisted of IBD-induced rats that were orally administered *Channa striata* albumin extract at doses of 100 mg/kg BW, 200 mg/kg BW, and 300 mg/kg BW, respectively. Group P5 included IBD-induced rats treated with diclofenac sodium at a dose of 2 mg/kg BW as the standard drug control.

IBD was induced using indomethacin at a dose of 15 mg/kg BW. Treatments with *Channa striata* albumin extract and diclofenac sodium were administered orally for 14 consecutive days. At the end of the treatment period, the animals were euthanized following ethical protocols. The jejunum was carefully excised, collected, and preserved in 10% neutral buffered formalin for histopathological and immunohistochemical analysis.

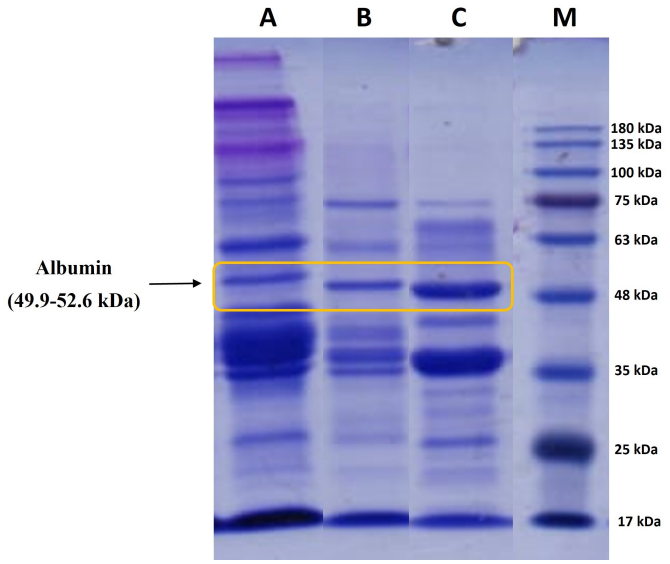

Figure 1. Electrophoregram of Pure Extract of *Channa striata* Albumin Extract: (A) SAS 70% pH 4.0, (B) SAS 80% pH 4.0, (C) SAS 90% pH 4.0, (M) Protein Marker

2.4.2 Analysis of Malondialdehyde (MDA) Levels

Malondialdehyde (MDA) is a cytotoxic byproduct of lipid peroxidation induced by oxidative stress. MDA levels were quantified using the thiobarbituric acid (TBA) assay, which relies on the reaction between MDA and TBA to form a pink chromogenic complex, measurable via UV-Vis spectrophotometry (Safithri et al., 2022). A calibration curve for malondialdehyde (MDA) quantification was established using standard solutions with concentrations ranging from 0.1 to 10 mM. Each standard was prepared by mixing 1 mL of the respective MDA solution with 2 mL of thiobarbituric acid–trichloroacetic acid (TBA–TCA) reagent, followed by homogenization and incubation at 95°C for 30 minutes to facilitate chromogen formation. After incubation, the mixtures were cooled to room temperature, and absorbance was recorded at the maximum absorbance wavelength.

For tissue sample analysis, 1 g of jejunum was carefully minced on an ice-cooled surface using a pre-chilled mortar and pestle to prevent thermal degradation. The homogenization was carried out in 1 mL of physiological saline (0.9% NaCl) to extract lipid peroxidation products. The resulting homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C, and the supernatant was collected for analysis. A 1 mL aliquot of the supernatant was then combined with 2 mL of TBA–TCA reagent, homogenized, and subjected to thermal incubation at 95°C for 30 minutes. Following cooling to ambient temperature, absorbance was measured at 532 nm using a Shimadzu UV-1800 UV-Vis spectrophotometer.

2.4.3 Histopathological Observation

Histopathological analysis of jejunum tissue was performed using hematoxylin and eosin (H&E) staining. Tissue samples were initially deparaffinized by immersing the formalin-fixed sections in absolute xylene for 5 minutes, repeated twice. This was followed by a graded xylene-ethanol series for progressive rehydration: xylene:absolute ethanol at ratios of 3:1, 1:1, and 1:3, each for 5 minutes (Metgud et al., 2013).

Rehydration was further performed by sequential immersion in decreasing ethanol concentrations (absolute ethanol, 95%, 90%, 80%, and 70%) for 5 minutes at each step, followed by rinsing in distilled water for 5 minutes. The tissue sections were then stained with hematoxylin for 10 minutes to ensure optimal nuclear staining. After staining, slides were rinsed under running water for 30 minutes, followed by a brief rinse with distilled water. Eosin staining was subsequently performed for 5 minutes, after which slides were immersed in distilled water to remove excess stain. Dehydration was conducted by sequential immersion in increasing ethanol concentrations (80%, 90%, 95%, and absolute ethanol). Finally, the clearing process was carried out by immersing the tissue sections in xylene for 5 minutes, followed by drying and mounting with Entellan before covering with a glass coverslip.

2.4.4 Immunohistochemistry

Immunohistochemical (IHC) analysis was performed to assess the expression of target proteins in jejunum tissue samples. The procedure consisted of two main stages: deparaffinization and staining. For deparaffinization, tissue slides were immersed in xylene for 15 minutes, repeated twice, followed by absolute ethanol for 15 minutes, also repeated twice. The slides were then sequentially immersed in 80% ethanol for 15 minutes and 70% ethanol for 15 minutes, followed by rinsing with distilled water to remove residual solvents (Muniz Partida and Walters, 2023).

Immunohistochemical staining was performed using the Star Trek Universal HRP Detection Kit (Biocare Medical, ST-UHRP700L10-KIT). The slides were first incubated with 3% hydrogen peroxide blocking solution for 40 minutes at room temperature to inhibit endogenous peroxidase activity. This was followed by incubation with blocking serum (Sniper Background) for 30 minutes to reduce non-specific binding. After three washes with phosphate-buffered saline (PBS), the slides were incubated with primary antibodies: anti-CD4 (GK1.5, sc-13573) and anti-occludin (E-5, sc-133256) for 1 hour at room temperature. The slides were then washed three times with PBS before incubation with the secondary antibody (Trekkie Universal Link) for 1 hour at room temperature, followed by another three PBS washes.

To enhance signal detection, the slides were incubated with Trekavidin-HRP Label for 40 minutes at room temperature, washed three times with PBS, and rinsed with distilled water. Visualization was achieved by incubating the slides with Betazoid DAB substrate for 15 minutes, followed by rinsing with distilled water until the DAB signal was clear. Counterstaining was performed using Mayer's hematoxylin, followed by rinsing with distilled water until the stain was fully developed. The slides were then air-dried at room temperature overnight be-

fore mounting with an adhesive medium and covering with a glass coverslip. The prepared slides were left to dry at room temperature for another 24 hours to ensure complete fixation. Immunohistochemical staining results were observed under a light microscope for qualitative and quantitative analysis.

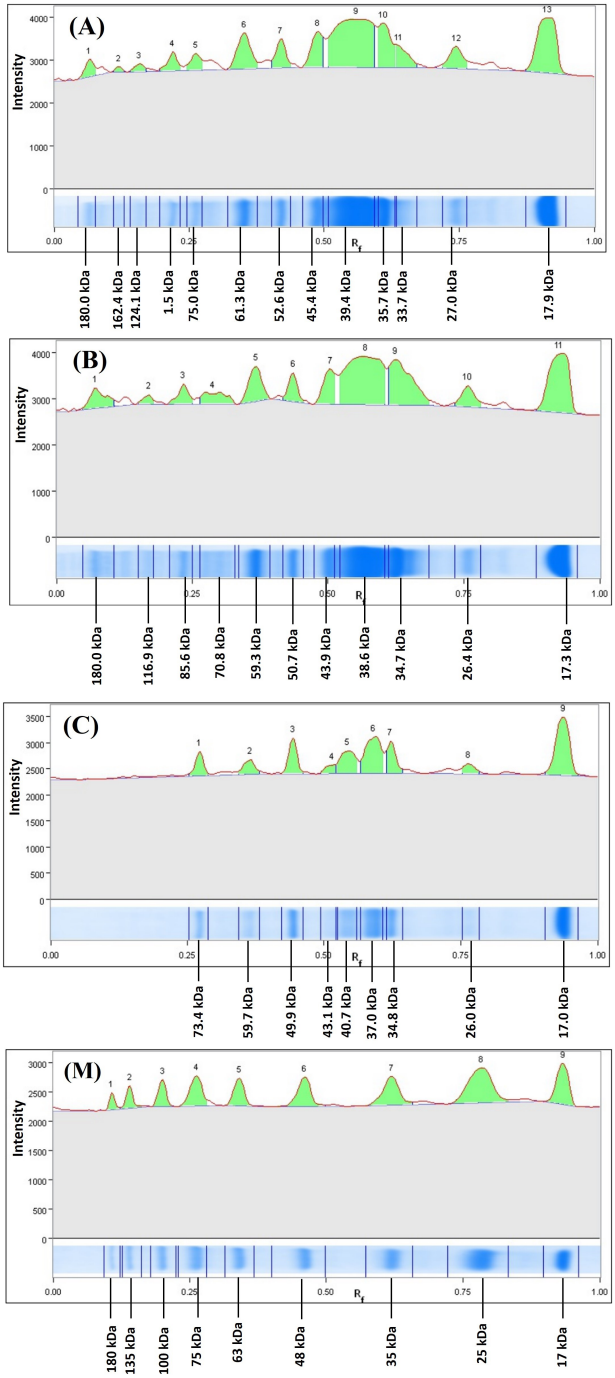

Figure 2. SDS-PAGE Molecular Weight Profile of Purified *Channa striata* Albumin: (A) SAS 70% pH 4.0, (B) SAS 80% pH 4.0, (C) SAS 90% pH 4.0, (M) Protein Marker

3. RESULTS AND DISCUSSION

3.1 Characterization of *Channa striata* Albumin Extract

3.1.1 Molecular Weight Profiling

Electrophoretic techniques, particularly sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), serve as a fundamental tool for assessing protein molecular weight distribution and purity. In this study, SDS-PAGE was utilized to elucidate the molecular weight profile of purified *Channa striata* albumin following fractionation with saturated ammonium sulfate (SAS). The electrophoretic patterns (Figure 1) revealed distinct protein bands, indicative of differential solubility and selective precipitation effects induced by SAS at varying concentrations.

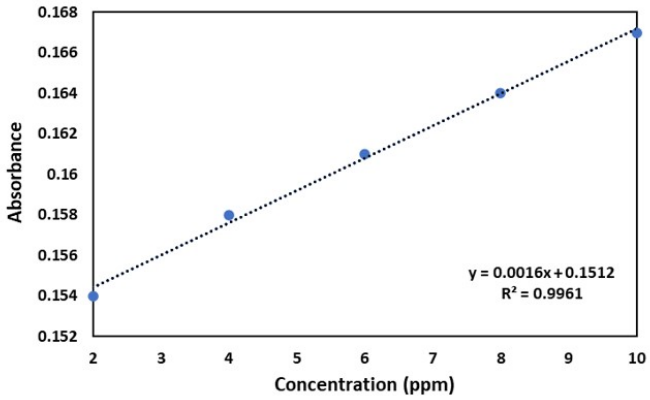

Figure 3. DPPH Standard Curve

A comparative analysis of the protein band intensities demonstrates that increasing SAS concentrations modulated albumin recovery, as evidenced by variations in band thickness and intensity. More intense bands correspond to higher protein concentrations, while fainter bands suggest reduced albumin retention. This observation aligns with protein solubility dynamics, wherein ammonium sulfate promotes selective precipitation by reducing protein hydration layers and altering intermolecular interactions (Magdeldin, 2012). Notably, the number of detectable protein fractions decreased with increasing SAS concentration, with Band A exhibiting 12 fractions, Band B 11 fractions, and Band C 9 fractions, suggesting that protein aggregation or differential solubility may govern albumin fractionation under these conditions. These findings corroborate prior studies demonstrating that precipitation efficiency is influenced by both protein conformation and solution parameters.

Molecular weight analysis (Figure 2) further revealed a distribution of protein fractions ranging from 17.0 kDa to 180.0 kDa, indicative of a heterogeneous protein composition. Albumin, a key biomolecule in *Channa striata*, was detected at distinct molecular weights across the purification conditions: 52.6 kDa in Band A (70% SAS, pH 4.0), 50.7 kDa in Band B (80% SAS, pH 4.0), and 49.9 kDa in Band C (90% SAS, pH 4.0). The observed molecular weight shift suggests that albu-

Table 1. IC_{50} Values of Antioxidant Activity in *Channa striata* Albumin Extract

Treatment	IC_{50} (ppm)
SAS 70% pH 4.0	2.75
SAS 80% pH 4.0	37.21
SAS 90% pH 4.0	38.81

min undergoes subtle structural modifications or differential precipitation dynamics in response to increasing SAS concentration. These variations may arise from alterations in tertiary structure stability, differential binding interactions, or minor proteolytic events during the purification process. The findings underscore the importance of optimizing SAS fractionation conditions to maximize albumin recovery while minimizing co-precipitation of non-target proteins.

3.1.2 Antioxidant Activity Test

The antioxidant activity of *Channa striata* albumin extract, purified using SAS at varying concentrations (70%, 80%, and 90%) at pH 4.0, was evaluated through IC_{50} values, as summarized in Table 1. Based on the linear regression equation $Y = -0.0016x + 0.1512$ from the DPPH standard curve (Figure 3), The lowest IC_{50} value was observed in SAS 70% (2.75 ppm), followed by SAS 80% (37.21 ppm) and SAS 90% (38.81 ppm). These findings indicate a significant decline in antioxidant capacity with increasing SAS concentration, suggesting that excessive ammonium sulfate precipitation may lead to structural modifications or partial denaturation of bioactive proteins, thereby reducing their radical scavenging efficiency.

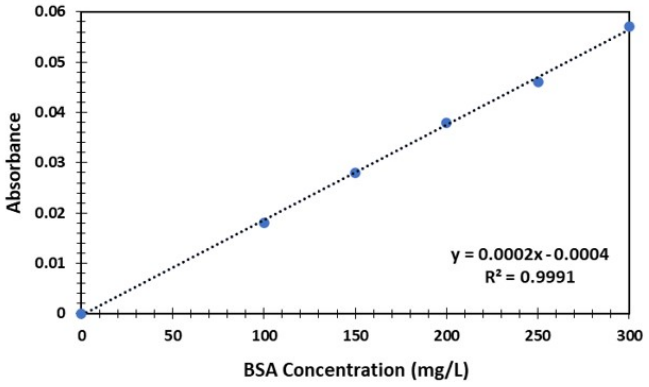

Figure 4. Bovine Serum Albumin (BSA) Standard Curve

The inverse correlation between SAS concentration and antioxidant activity is consistent with prior studies demonstrating that excessive ammonium sulfate exposure can induce protein aggregation and precipitation, altering tertiary and quaternary structures critical for redox interactions (Surjanto et al., 2019). The superior antioxidant activity of SAS 70% can be attributed to its optimal retention of albumin and associated bioactive peptides, which serve as potent radical scavengers by donating hydrogen atoms or electrons to stabilize DPPH radicals.

The reliability of the antioxidant activity results is underpinned by the rigorous validation of the analytical method employed. Sensitivity was confirmed through determination of the detection limit (LOD) and quantification limit (LOQ), while accuracy was assessed via recovery analysis. Analytical protocols exhibiting low LOD and LOQ values, coupled with recovery rates within the acceptable range of 90-110%, are indicative of high sensitivity and precision. As shown in Figure 3, the method demonstrated excellent analytical performance, with an LOD of 1.19 ppm, LOQ of 3.61 ppm, and recovery values ranging from 96% to 104%, validating the robustness and reproducibility of the assay.

Table 2. Amino Acid Profile of Pure Extract of *Channa striata* Albumin Extract

Types of Amino Acid	Symbol	Content (mg/kg)
Alanine	Ala	395.70
Arginine	Arg	290.88
Aspartic Acid	Asp	382.96
Glycine	Gly	831.17
Glutamic Acid	Glu	553.88
Histidine	His	78.73
Isoleucine	Ile	173.63
Leucine	Leu	274.18
Lycine	Lys	377.97
Valin	Val	202.62
Phenylalanine	Phe	216.54
Proline	Pro	459.35
Serin	Ser	206.83
Treonin	Thr	199.68
Tyrosine	Tyr	100.96

These findings are consistent with previous reports indicating that excessive ammonium sulfate precipitation adversely affects antioxidant efficacy due to the loss of functional protein domains (Tarmizi et al., 2023). Previous research obtained that IC_{50} of 1860 ppm at 65% ammonium sulfate saturation. At 20-40% ammonium sulfate saturation, there was an increase in free radical scavenging activity of up to 64% at 100 ppm (IC_{50} ~60 ppm). Previous studies have reported markedly lower antioxidant efficacy at intermediate saturation levels. For instance, Wu et al. (2016) observed an IC_{50} of 1860 ppm at 65% ammonium sulfate saturation, indicating minimal bioactivity retention. Conversely, at lower saturation levels (20-40%), Patel et al. (2018) documented enhanced radical scavenging efficiency, with up to 64% inhibition at 100 ppm, corresponding to an estimated IC_{50} of approximately 60 ppm, albeit with limited purification specificity. Given its significantly lower IC_{50}, the SAS 70% extract was selected for subsequent analyses, as it exhibited a highly potent radical scavenging effect, clas-

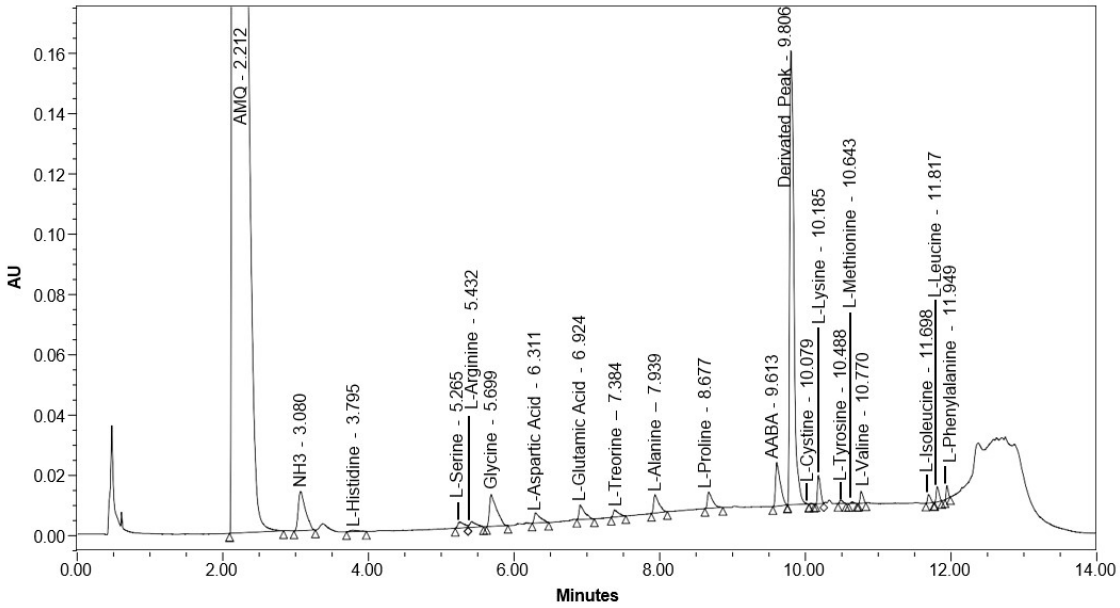

Figure 5. UPLC Chromatogram of Pure of *Channa striata* Albumin Extract

sifying it as a "very strong" antioxidant according to the IC_{50} classification standard (IC_{50} < 50 ppm).

Furthermore, the sharp increase in antioxidant activity at 70% ammonium sulfate saturation, followed by a steep decline at higher concentrations, underscores the critical physicochemical interplay between protein solubility, structure, and functionality under salting-out conditions. Ammonium sulfate precipitation operates by exploiting differences in protein surface hydrophobicity and solubility; thus, each saturation level selectively enriches distinct protein populations based on their solubility profile (Koteshwara et al., 2021).

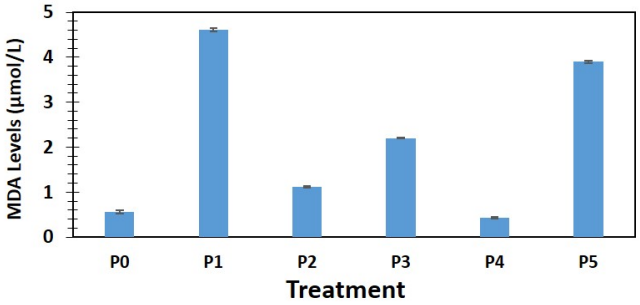

Figure 6. Comparison of Average MDA Levels Between Treatments. P0 (Negative Control/Healthy); P1 (Positive Control/IBD); P2 (IBD with 100 mg/kg BW Albumin Extract Therapy); P3 (IBD with 200 mg/kg BW Albumin Extract Therapy); P4 (IBD with 300 mg/kg BW Albumin Extract Therapy); P5 (IBD with 2 mg/kg BW Sodium Diclofenac Therapy)

At concentrations below 70%, precipitation of the target albumin and its associated bioactive peptides may be incomplete, resulting in co-elution with hydrophilic impurities or less-active proteins. This leads to a dilution of specific antioxidant activity and subsequently higher IC_{50} values. Such outcomes have been corroborated in previous studies (Patel et al., 2018), where 20–40% saturation levels produced less potent antioxidant fractions (IC_{50} ~60 ppm), likely due to low enrichment specificity.

In contrast, the 70% saturation level appears to represent an optimal threshold-precipitating antioxidant-active albumin fractions while preserving their native or near-native conformations. This structural integrity is essential for redox functionality, as it retains critical thiol groups, aromatic residues, and electron-donating peptide motifs that are directly involved in DPPH radical neutralization. The moderate ionic strength at this concentration may further help stabilize protein tertiary structures while minimizing undesired inter-chain disulfide crosslinking or aggregation (Kawakami et al., 2006).

The region between 70% and 80% saturation acts as a critical transitional zone. Even a modest increase beyond 70% likely elevates ionic strength and protein crowding, leading to subtle yet functionally significant conformational changes. These may include partial unfolding or aggregation that obstruct redox-active sites, diminish accessibility of reactive residues, and compromise overall radical scavenging performance (Schweitzer-Stenner, 2025). This mechanistic hypothesis is supported by literature (Surjanto et al., 2019; Tarmizi et al., 2023) that emphasizes the detrimental impact of over-saturation on protein functionality due to denaturation or irreversible aggregation.

Beyond 80%, the saturation further intensifies these structural disruptions. The significant increase in IC_{50} at 80% and 90% SAS thus reflects not only a quantitative decrease in bioactive protein but also a qualitative loss in biochemical efficacy.

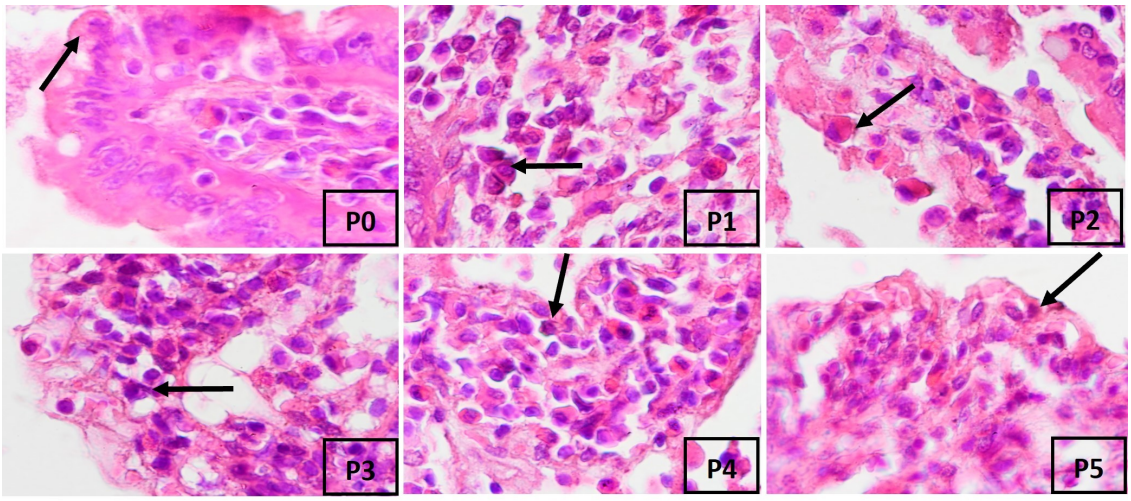

Figure 7. Histopathological Examination of Rat Jejunum Using Hematoxylin-eosin (HE) Staining at 1000× Magnification. P0: Negative Control (Healthy); P1: Positive Control (IBD-induced); P2: IBD with 100 mg/kg BW *Channa striata* Albumin Extract Therapy; P3: IBD with 200 mg/kg BW Albumin Extract Therapy; P4: IBD with 300 mg/kg BW Albumin Extract Therapy; P5: IBD with 2 mg/kg BW Sodium Diclofenac Therapy. The Images Illustrate Structural Changes in Jejunal Cells Across Different Treatment Groups. Arrows Indicate Notable Histopathological Alterations

Importantly, this interpretation is reinforced by rigorous method validation. The DPPH assay employed demonstrated high analytical reliability, as evidenced by low LOD (1.19 ppm), LOQ (3.61 ppm), and recovery values within the acceptable range (96-104%). These parameters confirm that the observed variations in IC_{50} truly reflect biochemical differences among the extracts rather than methodological artifacts.

Collectively, these findings illuminate the non-linear and highly sensitive nature of protein precipitation as it relates to antioxidant activity. The results affirm that 70% SAS saturation is not merely a procedural parameter but a biochemical inflection point that governs the balance between protein enrichment and structural preservation. This insight highlights the necessity of optimizing precipitation strategies in bioactive protein extraction workflows to ensure maximum functional integrity and therapeutic potential, particularly in the development of antioxidant agents derived from sustainable marine resources such as *Channa striata*.

3.1.3 Albumin levels

Albumin is a globular protein soluble in water, saline, and acidic solutions. The albumin content in *Channa striata* is a key indicator of its quality as a raw material for health supplements or functional foods. The results of purification of snakehead fish albumin extract with multistage SAS treatment, obtained the best SAS concentration and pH based on antioxidant activity is albumin extract at 70% concentration and pH 4.0.

Based on the linear regression equation $Y = 0.0002x - 0.0004$ from the BSA standard curve (Figure 4), the albumin content was obtained as 33.15 ± 0.002 (mg/L). Albumin and fatty acid compounds contained in snakehead fish have activity as chronic inflammation inhibitors (Somchit et al., 2004). Both compounds can reduce pro-inflammatory cytokine production, inhibit cyclooxygenase activity, and suppress nitric oxide (NO) formation. The immunostimulatory activity of albumin through the expression level of $CD4^+$T cells because it has the ability to increase the proliferation of immunocompetent cells (Dwijayanti et al., 2016). Albumin is a source of animal anti-oxidants that function as radical binders so that they play a role in the process of cleaning and capturing reactive oxygen species (ROS). *Channa striata* extract contains abundant albumin which is able to work as trapping oxidants and scavenging free radicals as well as its ability to improve the body's immune function.

The reliability of the measured albumin concentrations is supported by the rigorous validation of the analytical method employed. Sensitivity and accuracy were evaluated through detection limit (LOD), quantification limit (LOQ), and recovery analyses. A method is considered analytically robust when it demonstrates low LOD and LOQ values alongside recovery rates within the acceptable range of 90-110%. As shown in Figure 4, the method exhibited excellent performance, with an LOD of 0.55 mg/L, an LOQ of 1.68 mg/L, and a recovery rate of 98%, confirming its high sensitivity and accuracy for quantitative albumin determination.

3.1.4 Amino Acid Profile and Its Functional Implications in Metabolic and Immunological Pathways

Amino acids serve as the fundamental building blocks of proteins, playing essential roles in metabolic regulation, physiological processes, and immune responses. They are classified into two groups: essential and non-essential amino acids (Wu, 2010). Essential amino acids, including histidine, isoleucine, leucine, lysine, valine, phenylalanine, and threonine, cannot be

Table 3. MDA Levels in The Jejunum of Rats Exposed to Indomethacin and After Being Treated with Pure *Channa striata* Albumin Extract

Treatment Group	Average MDA Level (μmol/L)	MDA Level (%)	
		Increase	Decrease
P0	0.559 ± 0.034^{a}	-	-
P1	4.610 ± 0.033^{b}	724.69*	-
P2	1.119 ± 0.017^{c}	-	75.73**
P3	2.203 ± 0.016^{d}	-	52.21**
P4	0.429 ± 0.025^{e}	-	90.69**
P5	3.898 ± 0.033^{f}	-	15.44**

Note: Data are presented as mean ± standard deviation. Different superscript letters indicate statistically significant differences among groups ($p < 0.05$). Percent changes are calculated relative to the respective control groups. *Compared to P0 (negative control/healthy). **Compared to P1 (positive control/IBD).

synthesized endogenously and must be obtained from dietary protein sources. In contrast, non-essential amino acids such as alanine, arginine, aspartic acid, glycine, glutamic acid, proline, serine, and tyrosine can be endogenously synthesized by the human body.

The amino acid profile analysis of pure extract of snakehead fish abumin, as presented in Figure 5 and Table 2, reveals the presence of 15 distinct amino acids, comprising both essential and non-essential types. Notably, high concentrations of glycine, glutamic acid, proline, alanine, aspartic acid, and lysine were observed. These amino acids are known to be crucial in maintaining metabolic homeostasis, modulating oxidant-antioxidant balance, and enhancing immune responses (Hissen et al., 2023).

Recent studies have demonstrated that amino acids play a pivotal role in intestinal health, particularly in inflammatory bowel diseases (IBD). Their protective mechanisms involve the regulation of apoptosis and proliferation of intestinal epithelial cells (IECs), the modulation of tight junction protein (TJP) expression, and the attenuation of intestinal inflammation and oxidative stress through inhibition of the NF-κB signaling pathway and activation of the nuclear erythroid-related factor 2 (Nrf2) pathway (Zhang et al., 2024; Zhou et al., 2018). These findings underscore the therapeutic potential of amino acids in gastrointestinal disorders and immune regulation.

Among the essential amino acids detected, lysine, leucine, and phenylalanine were found in the highest concentrations. Phenylalanine has been reported to exhibit anti-inflammatory effects in IBD by suppressing TNF-α production and enhancing immune responses (Li et al., 2007). Additionally, the antioxidant and anti-inflammatory characteristics of phenylalanine, particularly when complexed with chromium, confer protective effects against IBD in indomethacin-induced models (Nagarjun et al., 2017).

Lysine, a distinguishing amino acid in fish-derived proteins, plays a critical role in digestion and the regulation of amino acid transporter expression in the gut. The bioactive form, poly-L-lysine (PL), has been demonstrated to reduce IL-8 production in IECs by modulating calcium-sensing receptors (CaSR), thereby suppressing the expression of pro-inflammatory cytokines (Mine and Zhang, 2015). Furthermore, glucose-lysine Maillard reaction products (Glc-LysMRPs) have been shown to improve DSS-induced colitis by enhancing glutathione levels, boosting antioxidant defenses, and downregulating NF-κB-mediated inflammatory responses, highlighting their potential in IBD prevention and therapy (Hong et al., 2017; Oh et al., 2017).

Leucine, an essential branched-chain amino acid (BCAA) along with isoleucine and valine, is well known for its role in protein synthesis and energy metabolism. In addition to its anabolic properties, BCAAs have been implicated in enhancing intestinal immune defense by maintaining morphological integrity and increasing immunoglobulin production (Ren et al., 2015). These findings support the functional importance of leucine in gut health and systemic immunity.

3.2 In Vivo Anti-Inflammatory Assays

3.2.1 Malondialdehyde (MDA) Levels

In rats induced with indomethacin, oxidative stress leads to significant cellular damage, as indicated by increased malondialdehyde (MDA) levels. Oxidative stress is a pathological condition caused by excessive free radicals, leading to lipid peroxidation (Prahastuti et al., 2025). MDA levels (Figure 6) in the jejunum were measured using the thiobarbituric acid (TBA) test at 532 nm. The results show that IBD-induced rats treated with *Channa striata* albumin extract exhibited a reduction in MDA levels (Table 3).

Table 3 indicates that MDA levels in the jejunum of negative control rats (0.559 ± 0.034 μmol/L) were significantly lower than those in positive control/IBD rats (4.610 ± 0.033 μmol/L), confirming that indomethacin induces oxidative stress by increasing free radical production. Compared to negative controls, indomethacin administration (15 mg/kg BW) led to a 724.69% increase in MDA levels. Indomethacin at this dose over 24 hours causes acute toxicity and elevates MDA levels.

Indomethacin-induced oxidative stress is driven by reactive

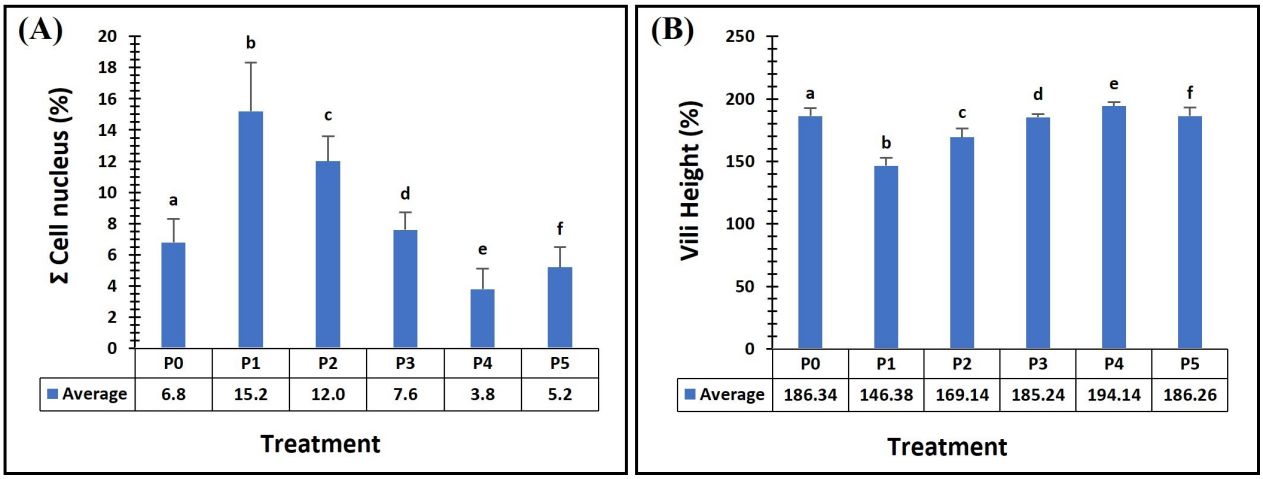

Figure 8. Comparative Analysis of (A) the Σ of Cell Nuclei and (B) Jejunum Villus Height in Mice Across Different Treatment Groups. Data are Presented as Mean Values for Each Treatment Condition. Different Superscript Letters Indicate Statistically Significant Differences Between Groups ($p < 0.05$)

oxygen species (ROS) generation, including superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), primarily due to mitochondrial electron leakage at complexes I and III. Furthermore, the oxidation of indomethacin metabolites, such as dimethoxybenzoindole (DMBI) to iminoquinone, contributes to ROS production, while macrophage and neutrophil activation exacerbates ROS and reactive nitrogen species (RNS) formation.

MDA, as the end product of lipid peroxidation, indirectly reflects free radical levels. Excessive free radicals cause oxidative stress unless neutralized by antioxidants (Rasool et al., 2015). Albumin functions as an antioxidant by scavenging free radicals and preventing lipid peroxidation. *Channa striata* extract, rich in albumin, effectively acts as an oxidant trap, reducing oxidative stress and supporting immune function. The strong binding between albumin and polyunsaturated fatty acids (PUFA) reduces lipid peroxidation and protects against oxidative damage (Roche et al., 2008; Taverna et al., 2013). These findings suggest that *Channa striata* albumin extract holds promise as a therapeutic agent in mitigating indomethacin-induced oxidative stress and managing inflammatory bowel disease (IBD).

3.2.2 Histological Analysis of the Rat Jejunum

Histopathological examination using hematoxylin-eosin (HE) staining provides critical insights into structural alterations in the jejunal mucosa across different treatment groups (Figure 7). In the negative control group (P0), the jejunal villi exhibited a well-preserved, elongated, and densely packed structure with intact mucosal architecture, indicative of normal tissue morphology. Conversely, in the indomethacin-induced inflammatory bowel disease (IBD) group (P1), substantial mucosal degradation was evident, characterized by disrupted villi architecture, epithelial erosion, and interstitial vacuolation. These morphological disruptions can be attributed to oxidative stress triggered by indomethacin, which promotes excessive reactive oxygen species (ROS) production, leading to phagocyte activation, lipid peroxidation, and subsequent structural disintegration of the intestinal epithelium. The oxidative damage mechanisms align with previous findings that highlight ROS-mediated nucleic acid oxidation and protein dysfunction as key contributors to tissue injury in inflammatory conditions (Cho et al., 2007; Silva et al., 2008).

Administration of *Channa striata* albumin extract at varying concentrations (P2, P3, P4) demonstrated progressive histological improvements compared to the IBD-induced group (P1). The most pronounced tissue recovery was observed in the P4 group (300 mg/kg BW), where villi exhibited improved elongation and epithelial integrity, suggesting effective mucosal regeneration. This therapeutic effect is likely attributed to the bioactive constituents of *Channa striata*, particularly its antioxidative and anti-inflammatory properties, which counteract oxidative stress, mitigate inflammatory damage, and facilitate epithelial cell proliferation. The observed histopathological restoration corroborates prior studies that have demonstrated the regenerative potential of bioactive peptides and albumin in modulating intestinal homeostasis and promoting mucosal healing.

The P5 group, treated with sodium diclofenac (2 mg/kg BW), exhibited partial restoration of villi morphology, although the extent of recovery was comparatively lower than that observed in the P4 group. While sodium diclofenac possesses anti-inflammatory properties, its impact on tissue regeneration appears less pronounced than that of *Channa striata* albumin extract, likely due to its mechanism of action focusing on cyclooxygenase inhibition rather than direct mucosal repair.

Quantitative histological analysis (Figure 8) further supports these observations, revealing significant intergroup varia-

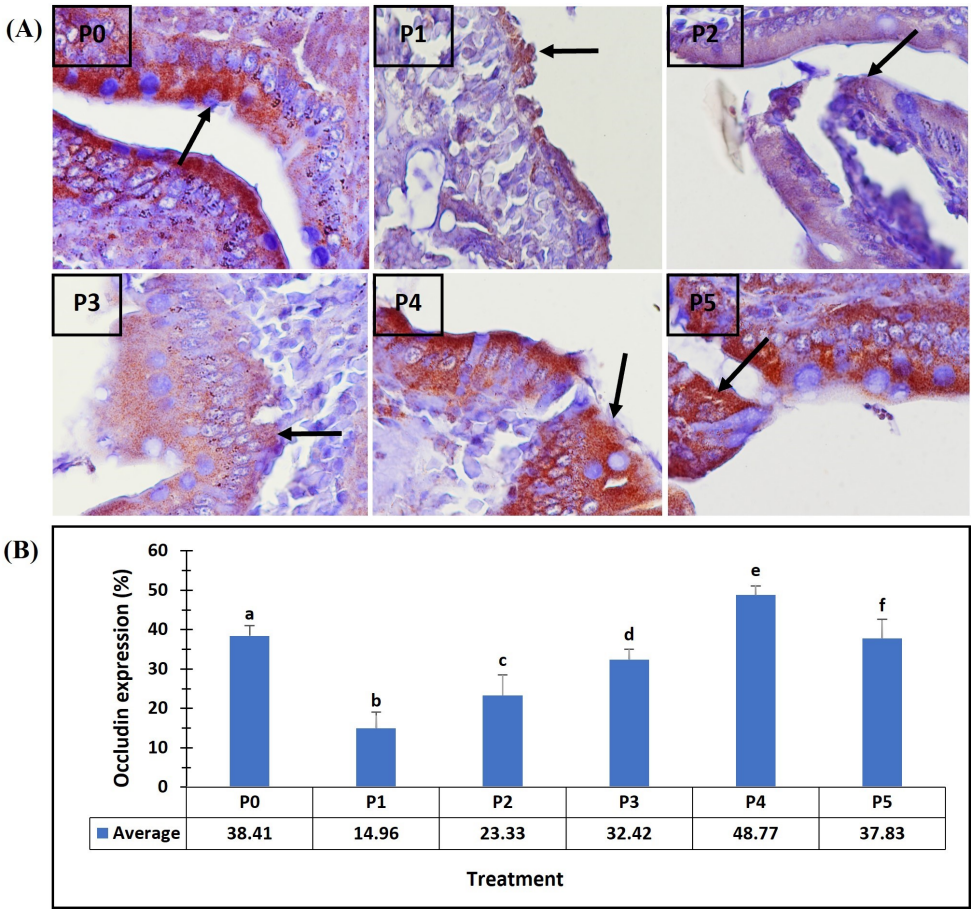

Figure 9. (A) Immunohistochemical Analysis of Occludin Expression in the Rat Jejunum at 1000× Magnification. (B) Comparative Analysis of The Mean Occludin Expression Across Treatment Groups. P0: Negative Control (Healthy); P1: Positive Control (IBD-Induced); P2: IBD with 100 mg/kg BW *Channa striata* Albumin Extract Therapy; P3: IBD with 200 mg/kg BW Albumin Extract Therapy; P4: IBD With 300 mg/kg BW Albumin Extract Therapy; P5: IBD with 2 mg/kg BW Sodium Diclofenac Therapy. Different Superscript Letters Indicate Statistically Significant Differences Between Groups ($p < 0.05$)

tions in percentage of Σ cells and villi height ($p < 0.05$). The P4 treatment group demonstrated the most substantial suppression of villi degradation and cell damage, with nuclear cell counts and villi height approaching levels observed in the negative control (P0). These findings highlight the superior efficacy of *Channa striata* albumin extract in mitigating indomethacin-induced jejunal injury through its multifaceted role in oxidative stress attenuation, inflammatory modulation, and epithelial regeneration. The overall results suggest a dose-dependent therapeutic effect, with the highest tested dose (300 mg/kg BW) exhibiting optimal histoprotective benefits. These findings underscore the potential of *Channa striata* albumin extract as a natural therapeutic intervention for intestinal inflammation, warranting further investigation into its molecular mechanisms and clinical applicability in IBD management.

3.2.3 Immunohistochemical Analysis of Occludin Expression in The Jejunum

Intestinal barrier integrity is crucial in preventing the translocation of harmful luminal contents, and its disruption is a hallmark of inflammatory bowel disease (IBD). Tight junction proteins, including occludin, are key regulators of paracellular permeability, and their dysregulation contributes to barrier dysfunction in IBD. The immunohistochemical analysis (Figure 9A) highlights occludin localization in the villi, crypts, and mucosa of the rat jejunum, with significant treatment-dependent variations.

Quantitative analysis (Figure 9B) revealed a statistically significant improvement in occludin expression following therapy with *Channa striata* albumin extract and sodium diclofenac ($p < 0.05$, One-Way ANOVA). The highest occludin expression was observed in group P4 (300 mg/kg BW *Channa striata* albumin extract), suggesting a potent restorative effect on tight junction integrity. In contrast, P1 (IBD-induced group) ex-

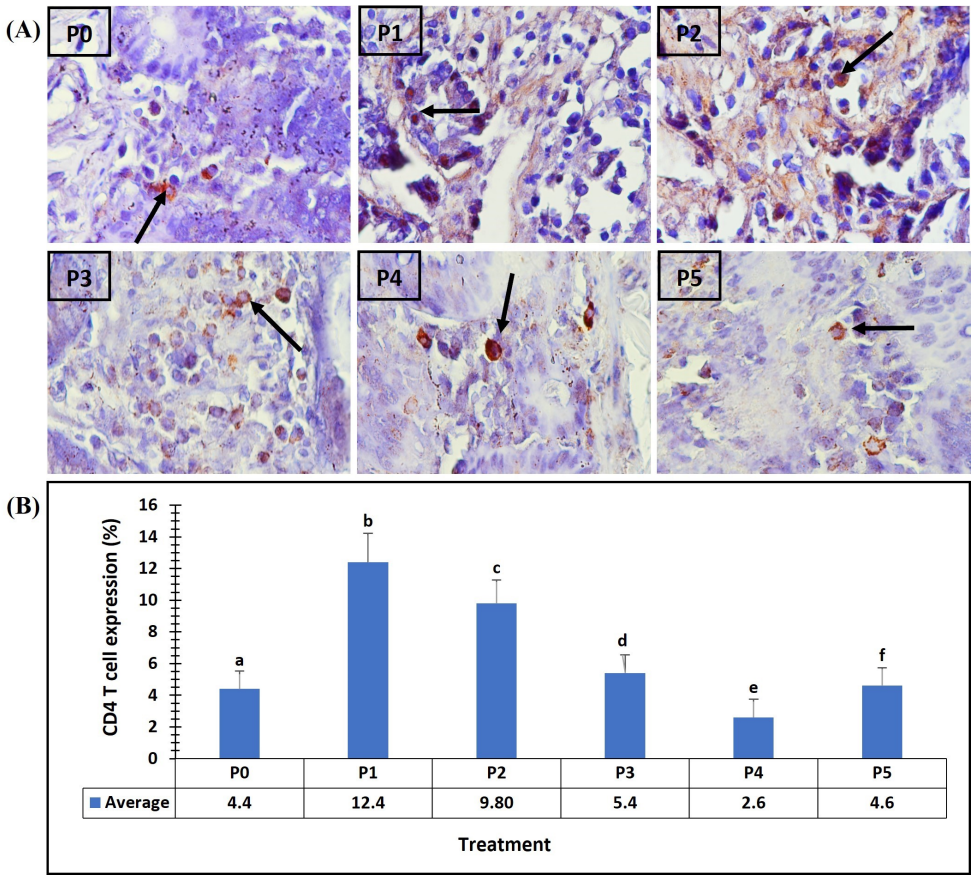

Figure 10. CD4$^+$T Cell Expression in The Jejunum: (A) Representative Immunohistochemical Staining of CD4$^+$T Cells in The Jejunal Tissue of Mice Under Different Treatment Conditions, Captured at 1000× Magnification. Arrows Indicate Positively Stained CD4$^+$T Cells. (B) Quantitative Comparison of CD4$^+$T Cell Expression Across Treatment Groups. P0: Negative Control (healthy); P1: Positive Control (IBD-induced); P2: IBD with 100 mg/kg BW *Channa striata* Elbumin Extract Therapy; P3: IBD with 200 mg/kg BW Albumin Extract Therapy; P4: IBD with 300 mg/kg BW Albumin Extract Therapy; P5: IBD with 2 mg/kg BW Sodium Diclofenac Therapy. Different Superscript Letters Denote Statistically Significant Differences Between Groups ($p < 0.05$)

hibited a marked 61.05% reduction in occludin levels relative to the healthy control (P0), consistent with previous findings that correlate IBD pathogenesis with impaired tight junction protein expression (Górecka et al., 2024).

The loss of occludin in IBD is linked to oxidative stress and pro-inflammatory cytokine activity, which compromise epithelial integrity (Al-Sadi et al., 2011). Indomethacin exposure exacerbates reactive oxygen species (ROS) production, further damaging the mucosal barrier. Treatment with *Channa striata* albumin extract effectively mitigated these effects, likely due to its potent antioxidant properties. Albumin serves as a free radical scavenger through multiple binding sites, reducing oxidative damage and lipid peroxidation (Roche et al., 2008). This mechanism supports the observed increase in occludin expression, restoring epithelial integrity and counteracting IBD-induced barrier dysfunction. Overall, these findings highlight the therapeutic potential of *Channa striata* albumin in enhancing intestinal barrier function via occludin regulation, positioning it as a promising candidate for IBD management.

3.2.4 Immunohistochemistry of CD4$^+$T Cell Expression in The Rat Jejunum

Inflammatory Bowel Disease (IBD) is a chronic immune-mediated disorder of the gastrointestinal tract, characterized by alternating phases of relapse and remission (Burisch et al., 2017; Liu and Stappenbeck, 2016; Peloquin et al., 2016; Rozirwan et al., 2024; Torres et al., 2017). The disease pathogenesis is multifactorial, involving genetic susceptibility, environmental triggers, microbial dysbiosis, and immune dysregulation, all contributing to impaired intestinal barrier function and tissue damage (Choy et al., 2017; De Souza et al., 2017; Torres et al., 2017). Central to this process is the dysregulation of CD4$^+$T cells, which play a pivotal role in orchestrating the inflammatory milieu, modulating antibody production, and regulating both innate and adaptive immune responses (Elahi and Horton, 2012; Sakaguchi et al., 2010). Figure 8A highlights the

immunohistochemical localization of $CD4^+$T cells within the villi, crypts, and mucosa of the jejunum.

Quantitative analysis using One-Way ANOVA revealed a significant reduction ($p < 0.05$) in $CD4^+$T cell expression following therapy with *Channa striata* albumin extract at doses of 100, 200, and 300 mg/kg BW, as well as with sodium diclofenac at 2 mg/kg BW (Figure 8B). The highest $CD4^+$T cell expression was observed in the IBD-induced group (P1), demonstrating an increase of 181.82% relative to the healthy control (P0), consistent with prior reports linking IBD with excessive $CD4^+$T cell infiltration in inflamed mucosa (Basso et al., 2018). Notably, the P4 group (300 mg/kg BW *Channa striata* albumin therapy) exhibited the lowest $CD4^+$T cell levels among treatment groups, suggesting a pronounced immunomodulatory effect.

The inflammatory microenvironment in IBD is known to drive dynamic shifts in $CD4^+$T cell populations, favoring the expansion of regulatory T cells (Tregs) and proinflammatory effector subsets. At the molecular level, epithelial inflammation triggers T cell activation, heightens interferon-gamma (IFN-γ) responses, and promotes effector Treg differentiation (Lutter et al., 2023). Under homeostatic conditions, the intestinal mucosa contains dispersed intraepithelial lymphocytes and innate lymphoid cells, with relatively low $CD4^+$T cell numbers (Allaire et al., 2018). However, IBD pathology is characterized by the accumulation of activated $CD4^+$T cells within the epithelium (Basso et al., 2018), alongside an altered immune phenotype marked by enhanced activation (Müller et al., 1998; Rabe et al., 2019; Schreiber et al., 1991) and shifts in functional T cell subsets (Corridoni et al., 2020).

Therapeutic intervention with *Channa striata* albumin extract appears to modulate immune homeostasis by attenuating $CD4^+$T cell expansion. The bioactive amino acids in the extract contribute to metabolic regulation, redox homeostasis, and immune function (Hissen et al., 2023). Amino acids serve as essential substrates for immune cell proliferation and antibody synthesis, and their deficiency compromises immunocompetence. Supplementation with bioavailable amino acids acts as an immunostimulant, in part through antioxidant-mediated mechanisms (Carvalho et al., 2023). Antioxidants facilitate the scavenging of reactive oxygen species (ROS), mitigating oxidative stress-induced immune dysregulation. This restoration of redox balance likely contributes to the observed normalization of $CD4^+$T cell populations, underscoring the therapeutic potential of *Channa striata* albumin in IBD management.

4. CONCLUSIONS

This study demonstrates the significant therapeutic potential of purified *Channa striata* albumin extract in mitigating indomethacin-induced Inflammatory Bowel Disease (IBD). The extract effectively reduced MDA levels, enhanced occludin expression to restore intestinal barrier integrity, and suppressed $CD4^+$T cell infiltration, highlighting its dual role as an antioxidant and immunomodulator. Histopathological analysis revealed superior preservation of jejunal villus structure compared to diclofenac sodium, which is commonly associated with gastrointestinal toxicity. A key advantage of *Channa striata* albumin extract lies in its ability to attenuate inflammation while simultaneously protecting intestinal mucosa, distinguishing it from conventional NSAIDs. Compared with diclofenac sodium, *Channa striata* demonstrated superior efficacy in reducing oxidative stress and inflammation, without inducing NSAID-related toxicity. These findings establish *Channa striata* albumin extract as a promising bioactive-based therapeutic strategy for IBD management. Further investigations are warranted to elucidate its precise molecular mechanisms and clinical applicability.

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