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University of Eldoret, Kenya, Mount Kenya University, Kenya, Chukwuemeka Odumegwu Ojukwu University, Nigeria, Kyambogo University, Uganda and University of Makeni, Sierra Leone

Histopathological Restoration and Immunomodulatory Effects of Combined *Plectranthus comosus* and *Allium sativum* Extracts on Gastrointestinal Tissue in *Salmonella typhi*–Infected Wistar Rats

*** Katana Lydia Kadzo, Wanga James, & Rono Salinah**
Department of Biological Sciences, School of Sciences, University of Eldoret, P.O Box 1125-30100 Eldoret, Kenya

***Correspondence:** katanalydiakadzo@gmail.com

Abstract

Typhoid fever remains a major public health concern in endemic regions, with gastrointestinal inflammation and mucosal damage contributing significantly to disease severity and complications (Stanaway et al., 2021; Marchello et al., 2022). This study evaluated the in vivo therapeutic effectiveness of combined aqueous extracts of Plectranthus comosus and Allium sativum on intestinal histopathology and inflammatory responses in Salmonella typhi–infected rats. Male Wistar rats were orally infected with S. typhi and treated with combined plant extracts for 14 days at doses of 100, 150, and 200 mg/kg. Ciprofloxacin (10 mg/kg) served as a positive control. Histopathological assessment revealed severe villus atrophy, crypt hyperplasia, inflammatory infiltration, mucosal lesions, and necrosis in untreated infected rats. In contrast, extract-treated groups demonstrated dose-dependent restoration of intestinal architecture, with the 200 mg/kg dose producing near-normal villus height (470.0 ± 9.5 µm) and crypt depth (155.0 ± 5.2 µm), comparable to ciprofloxacin. Immunohistochemical analysis showed significant reductions in TNF-α and IL-6 staining intensities at the highest extract dose (TNF-α: 1.5 ± 0.1; IL-6: 1.6 ± 0.2), relative to untreated infected controls (TNF-α: 4.5 ± 0.2; IL-6: 4.8 ± 0.3). These findings were corroborated by qPCR analysis, which demonstrated marked downregulation of TNF-α and IL-6 mRNA expression. Overall, combined aqueous P. comosus and A. sativum extract therapy significantly attenuated intestinal inflammation and promoted mucosal recovery in S. typhi–infected rats, highlighting its potential as a complementary therapeutic strategy for typhoid-associated gastrointestinal pathology.

Keywords: *Salmonella typhi*; *Plectranthus comosus*; *Allium sativum*; histopathology; gastrointestinal inflammation

Introduction

Typhoid fever remains a major cause of morbidity and mortality in low- and middle-income countries, particularly in sub-Saharan Africa, where inadequate sanitation and limited access to clean water facilitate sustained transmission. The disease is caused by *Salmonella typhi*, an intracellular pathogen that primarily targets the gastrointestinal tract, leading to mucosal damage, systemic inflammation, and potentially life-threatening complications if untreated (Stanaway *et al*., 2021; Marchello *et al*., 2022). Despite the availability of antibiotics, the emergence and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *S. typhi* strains have significantly compromised treatment efficacy, underscoring the need for alternative or adjunct therapeutic strategies (Browne *et al*., 2021; Klemm *et al*., 2021; Hooda *et al*., 2023).

In vivo infection with *S. typhi* is characterized by profound disruption of intestinal architecture, including villus blunting, crypt hyperplasia, epithelial erosion, and infiltration of inflammatory cells into the lamina propria (Everest *et al*., 2020). These pathological changes are largely driven by an exaggerated host immune response, particularly the overproduction of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which can contribute to epithelial barrier dysfunction and delayed tissue repair (Fàbrega & Vila, 2019; Behnsen *et al*., 2020). Therefore, effective therapeutic interventions should not only reduce bacterial burden but also address infection-associated inflammation and tissue injury.

Medicinal plants have gained renewed scientific attention as potential sources of bioactive compounds with antimicrobial, anti-inflammatory, and immunomodulatory activities (Abdulrahman *et al.*, 2021; Salehi *et al.*, 2019). Plant-derived preparations may act through multiple biological pathways and therefore warrant investigation as complementary approaches to conventional antimicrobial therapy. Among medicinal plants used traditionally for infectious and inflammatory conditions, *Plectranthus comosus* Sims and *Allium sativum* L. have attracted interest because of their reported antimicrobial and anti-inflammatory properties.

P. comosus is reported to contain diterpenoids, phenolic acids, and flavonoids with antibacterial and anti-inflammatory activities (Kiprono *et al*., 2021; Mwangi *et al*., 2022). Preclinical studies suggest that these compounds can attenuate inflammatory cell infiltration and promote epithelial regeneration, although *in vivo* evidence in typhoid-specific gastrointestinal injury remains limited. *A. sativum*, commonly known as garlic, contains sulfur-containing compounds such as allicin, diallyl sulfides, and ajoene, which have been shown to suppress pro-inflammatory cytokine production, enhance mucosal immunity, and protect intestinal integrity during bacterial infections (Arreola *et al*., 2021; Farhana *et al*., 2021). A

previous study by Katana *et al*. (2025) also demonstrated *in vitro* antimicrobial activity of extracts of *P. comosus* and *A. sativum* against *S. typhi*.

Recent research has highlighted the potential benefits of combined aqueous plant extract therapy, where synergistic interactions between bioactive compounds may enhance therapeutic effectiveness while minimizing toxicity (Mwangi *et al*., 2020; Sultana & Asif, 2020). However, most available studies focus on *in vitro* antimicrobial screening (Jajere, 2019), with comparatively few investigations examining *in vivo* histopathological recovery and cytokine modulation following combined aqueous plant extract treatment in *S. typhi* infection. Moreover, there is a lack of dose-response data linking intestinal tissue restoration with changes in TNF-α and IL-6 inflammatory markers. Therefore, a critical knowledge gap exists regarding the *in vivo* therapeutic potential of combined aqueous extracts of *P. comosus* and *A. sativum* in restoring gastrointestinal architecture and modulating inflammatory responses during *S. typhi* infection. Addressing this gap is essential for validating these plants as viable complementary therapies for typhoid fever.

This study was designed to evaluate the dose-dependent effects of combined aqueous *P. comosus and A. sativum* extracts on gastrointestinal histopathology and inflammatory marker expression in *S. typhi*–infected Wistar rats. Specifically, the study assessed villus height, crypt depth, mucosal damage, inflammatory cell infiltration, and necrosis, alongside TNF-α and IL-6 expression at the protein and mRNA levels. By integrating histological and molecular endpoints, this study provides comprehensive insight into the therapeutic and immunomodulatory potential of these medicinal plants in experimental typhoid infection.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (REF: FVM BAUEC/2024/554). All procedures involving animals were conducted in accordance with internationally accepted guidelines for laboratory animal care and use.

Plant Materials and Extract Preparation

Plant materials and extract preparation were conducted in accordance with standard phytochemical extraction procedures as described by Harborne (1998), Sofowora (2008) and Simon *et al*., (2016) with slight modifications. Fresh leaves of *Plectranthus comosus* Sims (Lamiaceae) and bulbs of *Allium sativum* L. (Amaryllidaceae) were collected from Njoro, Nakuru County, Kenya. The plant materials were authenticated by Mr. Denis Onyango at the University of Eldoret Herbarium, Uasin Gishu County, Kenya, where voucher specimens were deposited (Muh/As/03/91 Lk/Uoe/021/25 for *Allium sativum* and Muh/Plc/013/88 Lk/Uoe/02/25 for *Plectranthus comosus*).

Plant Processing and Aqueous Preparation

The plant materials were thoroughly washed with clean water to remove soil and other surface contaminants. The leaves of *P. comosus* and bulbs of *A. sativum* were then air-dried at an ambient temperature of 25 ± 2°C for two weeks, away from direct sunlight to preserve phytochemicals. The dried plant materials were subsequently ground into fine powders using an electric grinder. The powdered samples were stored in clean, labelled, airtight containers until extraction.

Aqueous extracts were prepared by macerating the powdered plant material in distilled water at a ratio of 1:10 (w/v). The mixture was extracted three consecutive times over 72 hours at room temperature with occasional stirring. The extracts were filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator and subsequently freeze-dried (Edwards freeze dryer Modulyo) to obtain dry extracts. The dried extracts were stored in airtight containers at 4°C until use.

Bacterial Strain and Inoculum Preparation

A strain of *Salmonella enterica subsp. enterica serovar Typhi* (ATCC 19430, strain Ty2) was obtained from Biotechnology Laboratory in the Department of Biological Sciences, Egerton University, Nakuru County, and originally sourced from the Center of Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI).

The test bacteria were sub-cultured on nutrient agar and maintained under standard laboratory conditions according to Anderson *et al*., (2020), in Safe Foods Reference Laboratory (SAF-LAB-PROJECT), Egerton University, Kenya. Cultures were grown on *Salmonella-Shigella* (SS) Agar, which selectively isolates *Salmonella* species by inhibiting gram-positive bacteria and allowing the growth of non-lactose fermenting colonies (Lee *et al*., 2010). For inoculation, a fresh culture was

prepared by transferring a loopful of bacteria into nutrient broth and incubating at 37°C for 21 ± 3 hours. The bacterial suspension was standardized to approximately 1×10^8 CFU/mL using the McFarland standard.

Experimental Animals and Study Design

The experimental design and treatment protocol were conducted in accordance with established guidelines for laboratory animal studies as described by OECD (2008) with slight modifications. Fifty-four healthy male Wistar rats (10 ± 2 weeks old; 225 ± 25 g) were obtained from the Faculty of Veterinary Medicine, University of Nairobi. The animals were acclimatized under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 50 ± 5%, and a 12-hour light/dark cycle), with free access to standard pellet feed (Unga Feeds Ltd., Kenya) and water *ad libitum*. The animals were randomly assigned into six experimental groups (n = 9 per group): Group 1 (G1): uninfected, untreated (reference group); Group 2 (G2): Infected, untreated (negative control); Group 3 (G3): infected, treated with ciprofloxacin at 10 mg/kg body weight (positive control), and Groups 4–6 (G4-G6): infected, treated with combined aqueous extracts of *P. comosus* and *A. sativum* (1:1 v/v) at doses of 100, 150, and 200 mg/kg body weight, respectively.

Induction of Infection and Treatment

Infection was induced in Groups 2–6 by oral administration of 1 mL of *Salmonella typhi* suspension containing 1×10^8 CFU/mL. Successful infection was confirmed 48 hours post-inoculation by fecal bacterial count, and only animals with counts $\geq 1 \times 10^6$ CFU/g were included in the study. Treatments were administered orally once daily for 14 consecutive days. Throughout the experimental period, animals were monitored for clinical signs and general health status, and all procedures were conducted in compliance with ethical standards for animal care and use.

Sample Collection and Tissue Storage

Sample collection was conducted in accordance with standard laboratory animal procedures as described by Festing and Altman (2002) with slight modifications. At the end of the experimental treatment period, animals were humanely euthanized under appropriate anesthesia to minimize pain and distress. Gastrointestinal tissues (small intestine) were carefully excised, rinsed gently with cold phosphate-buffered saline (PBS) to remove residual luminal contents, and blotted dry. The tissues were then sectioned into portions for histopathology and molecular analyses: one portion was fixed in 10% neutral-buffered formalin for histopathological and immunohistochemical evaluation, while the portion designated for molecular analysis was immediately placed in sterile, pre-labelled cryovial and snap-frozen in liquid nitrogen to rapidly preserve tissue RNA and minimize RNA degradation. The frozen tissues were maintained in liquid nitrogen during immediate transfer and subsequently stored in a −80°C freezer for long-term storage until RNA extraction and subsequent quantitative real-time PCR (qPCR) analysis. All sample handling procedures were carried out under sterile conditions to prevent contamination and preserve tissue integrity

Histopathological Examination

Gastrointestinal tissue samples (small intestine) were collected immediately after euthanasia, rinsed with phosphate-buffered saline (PBS), and fixed in 10% neutral-buffered formalin for 24–48 hours. Tissue processing, embedding, sectioning, and staining were performed according to standard histological procedures as described by Bancroft and Gamble (2008). Briefly, fixed tissues were dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome, mounted on glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope at magnifications of ×100 and ×400, and photomicrographs were captured for analysis. Morphometric measurements of villus height (from the villus tip to the villus–crypt junction) and crypt depth (from the base of the crypt to the villus–crypt junction) were performed using calibrated image analysis software. Histopathological changes, including inflammatory cell infiltration, mucosal damage, epithelial disruption, lesion formation, and necrosis, were evaluated using a semi-quantitative scoring system (0–3 scale: 0 = normal, 1 = mild, 2 = moderate, 3 = severe), with assessments conducted in a blinded manner to minimize observer bias.

Immunohistochemical Analysis

Immunohistochemical analysis of gastrointestinal tissue sections was performed following standard protocols as described by Ramos-Vara (2005) with slight modifications. Briefly, paraffin-embedded tissue sections (5 µm) were deparaffinized in xylene and rehydrated through graded ethanol to distilled water. Antigen retrieval was carried out using heat-induced epitope retrieval in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked by incubating sections in 3% hydrogen peroxide, followed by blocking of non-specific binding sites using normal serum. The sections were then incubated overnight at 4°C with primary antibodies against tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). After washing, sections

were incubated with appropriate biotinylated secondary antibodies, followed by streptavidin–horseradish peroxidase conjugate. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, and sections were counterstained with hematoxylin. Stained sections were examined under a light microscope, and photomicrographs were captured for analysis. The intensity of immunostaining was evaluated semi-quantitatively using a scoring system (0–4 scale: 0 = no staining, 1 = weak, 2 = moderate, 3 = strong, 4 = very strong).

Quantitative Real-Time PCR (qPCR)

Quantitative real-time polymerase chain reaction (qPCR) analysis was performed to determine the relative mRNA expression levels of pro-inflammatory cytokines TNF-α and IL-6 in gastrointestinal tissues, following standard protocols as described by Livak and Schmittgen (2001) with slight modifications. Total RNA was extracted from tissue samples using a commercial RNA extraction kit according to the manufacturer's instructions, and RNA purity and concentration were assessed spectrophotometrically using the A260/A280 ratio. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit. qPCR amplification was carried out using SYBR Green chemistry in a real-time PCR system under optimized cycling conditions. Specific primers for TNF-α, IL-6, and the housekeeping gene β-actin were used for amplification. Each reaction was performed in triplicate to ensure reproducibility. The relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method, with β-actin serving as the internal control for normalization. Results were expressed as fold change relative to the uninfected control group.

Data Analysis

Data were analyzed using IBM SPSS statistics 20.0. Results are presented as mean ± standard deviation (SD). Differences among groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$. All experiments were performed in triplicate.

Results

Histopathological Changes in the Gastrointestinal Mucosa of Rats following Treatment with Combined Aqueous Extracts of *Plectranthus comosus* and *Allium sativum*

Histopathological examination of gastrointestinal tissues revealed marked differences among the experimental groups (Table 1; Figure 1). Quantitative morphometric analysis (Table 1) demonstrated significant differences in villus height and crypt depth across experimental groups ($p < 0.05$). The uninfected, untreated group (G1) exhibited normal intestinal architecture characterized by well-organized mucosal layers, intact epithelial lining, tall villi, shallow crypts, with no evidence of inflammatory cell infiltration, lesions, mucosal damage, or necrosis. These findings represent the baseline structural integrity of healthy gastrointestinal tissue.

In contrast, rats infected and untreated (G2) showed severe histopathological alterations. These included a significant reduction in villus height, pronounced crypt hyperplasia, severe inflammatory cell infiltration, presence of mucosal lesions, extensive mucosal damage, and evidence of necrosis. Such pathological changes are consistent with *S typhi*-induced enteritis, which is known to compromise epithelial integrity and trigger excessive inflammatory responses in the gastrointestinal tract (Stanaway *et al*., 2021; Klemm *et al*., 2021).

Ciprofloxacin treatment (G3) resulted in marked improvement in intestinal morphology, with restoration of villus height (450 ± 9.0 µm) and a reduction in crypt depth (160 ± 5.5 µm). These values were not significantly different from those of the uninfected control ($p > 0.05$), suggesting effective reversal of infection-induced damage, minimal inflammatory infiltration, and absence of lesions or necrosis. This confirms the efficacy of ciprofloxacin in controlling bacterial infection and limiting intestinal damage.

Treatment with combined aqueous extracts demonstrated a dose-dependent histopathological recovery. At 100 mg/kg (G4), moderate inflammatory infiltration and partial villus restoration were observed, accompanied by reduced mucosal damage and absence of necrosis. Villus height increased to 300 ± 7.8 µm, representing a 50% improvement relative to the infected group, while crypt depth decreased to 250 ± 6.5 µm. Increasing the dose to 150 mg/kg (G5), resulted in further structural improvement, characterized by increased villus height (400 ± 8.2 µm), reduced crypt depth (180 ± 5.5 µm) indicating substantial recovery of intestinal structure, and mild inflammatory infiltration. Notably, rats treated with the highest dose of 200 mg/kg (G6) exhibited near-normal intestinal architecture. Villus height (470 ± 9.5 µm) and crypt depth (155 ± 5.2 µm) were comparable to the ciprofloxacin-treated group, minimal inflammatory infiltration, absence of lesions, and no evidence of necrosis. These findings indicate a strong tissue-protective and restorative effect of the combined plant extracts at higher doses.

Table 1: Histopathological changes in the GI Mucosa

Group	Villus Height (µm)	Crypt Depth (µm)	Inflammatory Infiltration	Lesions	Mucosal Damage	Necrosis
G1	500 ± 10.2^{a}	150 ± 5.0^{a}	0	No	0	No
G2	200 ± 8.5^{c}	300 ± 7.0^{c}	3	Yes	3	Yes
G3	450 ± 9.0^{ab}	160 ± 5.5^{ab}	1	No	1	No
G4	300 ± 7.8^{c}	250 ± 6.5^{b}	2	Yes	2	No
G5	400 ± 8.2^{b}	180 ± 5.5^{b}	1	No	1	No
G6	470 ± 9.5^{a}	155 ± 5.2^{a}	1	No	1	No

Values are mean ± SD. Means within the same column followed by different superscript letters differ significantly at $p < 0.05$ according to one-way ANOVA followed by Tukey's post hoc test. G1 = uninfected untreated; G2 = infected untreated; G3 = infected + ciprofloxacin (10 mg/kg); G4 = infected + combined P. comosus and A. sativum extracts (100 mg/kg); G5 = infected + combined extracts (150 mg/kg); G6 = infected + combined extracts (200 mg/kg).

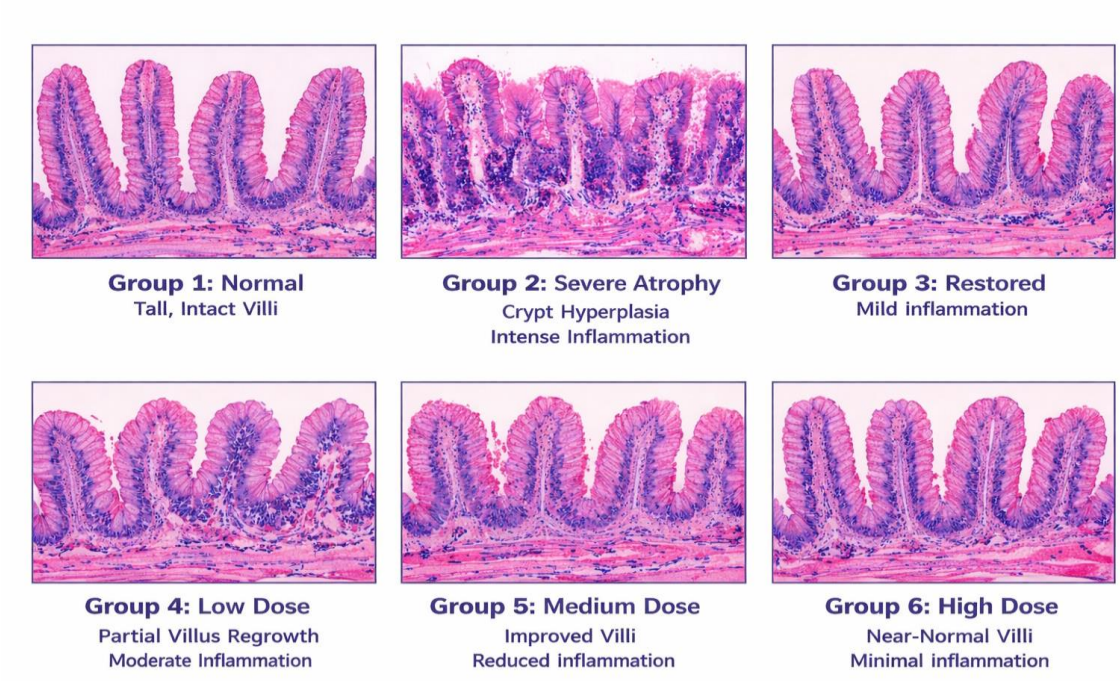

***Figure 1**: Intestinal sections (H&E, 400X) show: Normal villus architecture with intact epithelial lining and no inflammation (G1); Severe villus atrophy with crypt hyperplasia and heavy inflammatory infiltration (G2); Partial villus restoration with mild inflammation (G3); Moderate villus regrowth and reduced inflammation (G4); Improved villus structure with minimal inflammation (G5); Near-normal villi comparable to control tissue (G6).*

Expression Levels of Inflammatory Markers

Immunohistochemical Expression of TNF-α and IL-6

Immunohistochemical analysis revealed significant differences in the expression of TNF-α and IL-6 among the experimental groups (Table 2). The uninfected, untreated group (G1) exhibited low baseline staining intensities for TNF-α (1.0 ± 0.1^{a}) and IL-6 (1.0 ± 0.1^{a}), indicating normal physiological levels of cytokine expression. In contrast, the infected, untreated group (G2) showed significantly elevated staining intensities for TNF-α (4.5 ± 0.2^{c}) and IL-6 (4.8 ± 0.3^{c}), indicating severe inflammatory activation following *S. typhi* infection. Treatment with ciprofloxacin (G3) significantly reduced TNF-α (2.0 ±

0.2^{b}) and IL-6 (2.1 ± 0.3^{b}) staining intensity, confirming effective suppression of infection-induced inflammation. Similarly, rats treated with combined aqueous extracts of *P. comosus* and *A. sativum* demonstrated dose-dependent attenuation of cytokine expression. At 100 mg/kg (G4), moderate staining intensities were observed for TNF-α (2.5 ± 0.2^{b}) and IL-6 (2.7 ± 0.3^{b}). Increasing the dose to 150 mg/kg (G5) resulted in further reductions to 2.0 ± 0.2^{b} (TNF-α) and 2.2 ± 0.3^{b} (IL-6). At the highest dose of 200 mg/kg (G6), staining intensities for TNF-α (1.5 ± 0.1^{a}) and IL-6 (1.6 ± 0.2^{a}) were not significantly different from those of the uninfected control group ($p > 0.05$), indicating near-complete normalization of cytokine expression.

Table 2: Immunohistochemical Staining Intensity

Group	Treatment	TNF-α	IL-6
G1	Uninfected	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}
G2	Infected	4.5 ± 0.2^{c}	4.8 ± 0.3^{c}
G3	Ciprofloxacin	2.0 ± 0.2^{b}	2.1 ± 0.3^{b}
G4	100mg/kg	2.5 ± 0.2^{b}	2.7 ± 0.3^{b}
G5	150mg/kg	2.0 ± 0.2^{b}	2.2 ± 0.3^{b}
G6	200mg/kg	1.5 ± 0.1^{a}	1.6 ± 0.2^{a}

Values are expressed as mean ± SD. Means within the same column followed by different superscript letters (a-c) are significantly different at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. *G1 = uninfected untreated; G2 = infected untreated; G3 = infected + ciprofloxacin (10 mg/kg); G4 = infected + combined extracts (100 mg/kg); G5 = infected + combined extracts (150 mg/kg); G6 = infected + combined extracts (200 mg/kg).*

Relative mRNA Expression of TNF-α and IL-6 (qPCR Analysis)

Quantitative PCR analysis (Table 3) corroborated the overall pattern observed in the immunohistochemical findings. Relative expression in the uninfected, untreated group (G1) exhibited low baseline staining intensities for both TNF-α (1.0 ± 0.0^{a}) and IL-6 (1.0 ± 0.0^{a}), consistent with minimal mRNA expression levels (1.0-fold), indicating normal physiological conditions. In contrast, the infected, untreated group (G2) showed markedly elevated staining intensities for TNF-α (4.5 ± 0.2^{c}) and IL-6 (4.8 ± 0.3^{c}), corresponding to significantly upregulated gene expression levels of 8.0 ± 0.5^{d} and 9.5 ± 0.6^{d}, respectively. This substantial increase reflects strong activation of pro-inflammatory pathways following *S. typhi* infection. Treatment with ciprofloxacin (G3) resulted in a pronounced reduction in cytokine expression, with staining intensities of 2.0 ± 0.2^{b} (TNF-α) and 2.1 ± 0.3^{b} (IL-6), which aligned with moderate mRNA expression levels (3.0 ± 0.3^{b}) and 3.2 ± 0.4^{b}, respectively). Similarly, administration of combined aqueous extracts of *P. comosus* and *A. sativum* demonstrated dose-dependent attenuation of cytokine expression. At 100 mg/kg (G4), relatively higher staining intensities (2.5 ± 0.2^{b} for TNF-α 2.7 ± 0.3^{b} for IL-6) corresponded with elevated gene expression levels (4.0 ± 0.4^{c} and 4.5 ± 0.5^{c}), indicating partial suppression of inflammation. Increasing the dose to 150 mg/kg (G5) resulted in further reductions in staining (2.0 ± 0.2^{b} and 2.2 ± 0.3^{b}) and mRNA expression (2.5 ± 0.3^{b} and 2.8 ± 0.3^{b}) reflecting enhanced anti-inflammatory activity. Notably, the highest dose (200 mg/kg, G6) produced minimal staining intensities for TNF-α (1.5 ± 0.1^{a}) and IL-6 (1.6 ± 0.2^{a}), which were not significantly different from the uninfected control group ($p > 0.05$). This observation is consistent with gene expression levels approaching baseline (1.5 ± 0.2^{a} and 1.6 ± 0.2^{a}), suggesting substantial attenuation of the inflammatory response. Overall, the strong concordance between immunohistochemical staining intensity and relative mRNA expression levels confirms that the combined plant extracts exert a dose-dependent immunomodulatory effect at both the protein and transcript levels within the same column followed by different superscript letters differ significantly at $p < 0.05$.

Table 3: Relative Cytokine mRNA Expression ($2^{-}\Delta\Delta Ct$)

Group	Treatment	TNF-α	IL-6
G1	Uninfected	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
G2	Infected untreated	8.0 ± 0.5^{d}	9.5 ± 0.6^{d}
G3	Ciprofloxacin	3.0 ± 0.3^{b}	3.2 ± 0.4^{b}
G4	100mg/kg	4.0 ± 0.4^{c}	4.5 ± 0.5^{c}
G5	150mg/kg	2.5 ± 0.3^{b}	2.8 ± 0.3^{b}
G6	200mg/kg	1.5 ± 0.2^{a}	1.6 ± 0.2^{a}

Values are mean ± SD. Means within the same column followed by different superscript letters (a-d) are significantly different at $p < 0.05$ using one-way ANOVA followed by Tukey's post hoc test. G1 = uninfected untreated; G2 = infected untreated; G3 = infected + ciprofloxacin (10 mg/kg); G4 = infected + combined P. comosus and A. sativum extracts (100 mg/kg); G5 = infected + combined extracts (150 mg/kg); G6 = infected + combined extracts (200 mg/kg).

Discussion

The present study demonstrates that *Salmonella typhi* infection induces severe gastrointestinal pathology and pronounced inflammatory responses, as evidenced by structural damage, elevated cytokine expression, and increased gene transcription levels. Histopathological findings revealed significant villus atrophy, crypt hyperplasia, mucosal damage, and necrosis in infected untreated rats, consistent with previous reports describing enteric fever–associated intestinal injury driven by excessive immune activation and inflammatory signaling (Stanaway *et al*., 2019; Marchello *et al*., 2022). The observed reduction in villus height and increase in crypt depth reflect impaired absorptive capacity and compensatory epithelial proliferation in response to mucosal injury.

Importantly, treatment with combined aqueous extracts *of Plectranthus comosus* and *Allium sativum* resulted in significant, dose-dependent restoration of intestinal architecture. At the highest dose (200 mg/kg), morphometric parameters approached those of the uninfected control group, suggesting effective regeneration of the intestinal epithelium. This restorative effect may be attributed to the ability of phytochemicals such as flavonoids and phenolic compounds to enhance epithelial cell proliferation, promote antioxidant defense, and stabilize cellular membranes (Salehi *et al*., 2019; Kiprono *et al*., 2021).

The marked elevation of TNF-α and IL-6 observed in infected untreated rats confirms the central role of these cytokines in mediating intestinal inflammation during *Salmonella* infection. TNF-α is known to increase epithelial permeability and induce apoptosis, while IL-6 contributes to chronic inflammation and tissue injury (Fàbrega & Vila, 2019; Farhana & Khan, 2021). The significant reduction of these cytokines following treatment indicates that the combined plant extracts exert potent anti-inflammatory and immunomodulatory effects.

Mechanistically, the anti-inflammatory activity of *A. sativum* is largely attributed to sulfur-containing compounds such as allicin (Arreola *et al*., 2015), which inhibit NF-κB signaling pathways and suppress cytokine production. Similarly, diterpenoids and phenolic compounds in *P. comosus* have been shown to inhibit inflammatory mediators and reduce leukocyte infiltration (Arreola *et al*., 2021; Mwangi *et al*., 2022). The observed dose-dependent reduction in TNF-α expression from 8.0 to 1.5-fold and in IL-6 expression from 9.5 to 1.6-fold suggests a synergistic interaction between these bioactive compounds, enhancing their overall therapeutic efficacy.

The concordance between immunohistochemical and qPCR findings further strengthens the validity of the results, indicating that the extracts modulate inflammatory responses at both transcriptional and protein levels. This dual-level regulation is particularly important in controlling infection-induced tissue damage and promoting mucosal healing.

While ciprofloxacin effectively reduced inflammation and restored tissue structure, the comparable efficacy of the aqueous plant extracts highlights their potential as complementary therapeutic agents. Unlike conventional antibiotics, which primarily target bacterial survival, plant-derived compounds offer additional benefits by modulating host immune responses and promoting tissue repair. This multifaceted mechanism may be especially valuable in the context of increasing antimicrobial resistance.

Overall, the findings of this study provide strong evidence that combined aqueous extracts of *P. comosus* and *A. sativum* not only mitigate inflammation but also facilitate structural recovery of the gastrointestinal mucosa. These effects underscore their potential as adjunct therapies in the management of typhoid-associated intestinal damage. Future studies should focus on elucidating specific molecular pathways involved, as well as evaluating long-term safety and clinical applicability.

Conclusion

Combined aqueous extracts of *Plectranthus comosus* and *Allium sativum* effectively ameliorated gastrointestinal histopathological damage and suppressed pro-inflammatory cytokine expression in *Salmonella typhi*-infected rats. The dose-dependent restoration of intestinal structure and reduction of TNF-α and IL-6 levels, particularly at 200 mg/kg, demonstrates strong therapeutic potential comparable to standard antibiotic treatment. These findings support further exploration of these plant extracts as complementary agents in the management of typhoid-associated intestinal inflammation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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