

Exploration of Possible Effects of Cannabis sativa Seed Oil Applied in Different Doses in Ulcerative Colitis Model

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Abstract

BACKGROUND

Ulcerative colitis is a chronic inflammatory disease characterized by extensive mucosal damage and insufficient treatment options. *Cannabis sativa* seed oil, distinguished by its rich bioactive lipid profile, offers significant potential in modulating inflammation and oxidative stress. Given that its dose-dependent efficacy remains largely unexplored, this study investigates the therapeutic effects of varying doses of *Cannabis sativa* seed oil to establish a novel intervention strategy for ulcerative colitis.

MATERIALS AND METHODS

24 Wistar albino rats were distributed into four groups: control, TNBS-induced colitis, and two treatment groups receiving *Cannabis sativa* seed oil via intragastric gavage at doses of 1 ml/kg (CS1) and 3 ml/kg (CS3). Following a 72-hour protocol, colon tissues were harvested to evaluate oxidative stress markers (SOD, GPx, MDA, MPO, CAT) and pro-inflammatory cytokines (TNF- α , IL-1 β).

RESULTS

Cannabis sativa seed oil administration significantly attenuated apoptotic and inflammatory signaling while restoring antioxidant enzyme homeostasis. Both biochemical and histopathological analyses confirmed that the treatment conferred robust protection against colitogenic damage ($p < 0.05$).

CONCLUSION

Our findings demonstrate that *Cannabis sativa* seed oil effectively ameliorates ulcerative colitis. Notably, the 3 ml/kg dose exhibited superior efficacy, yielding results comparable to the healthy control group, which underscores its potential as a novel pharmacological agent for inflammatory bowel diseases.

1. INTRODUCTION

Inflammatory bowel disease (IBD), primarily classified as Crohn's disease (CD) and ulcerative colitis (UC), is a chronic gastrointestinal condition (1). Globally, approximately 11.2 million individuals are affected, with the highest prevalence in Europe and North America (2). The disease typically manifests between ages 10–35 and occurs more frequently in females (1, 2). Notably, 20% to 30% of ulcerative colitis patients show an inadequate response to medical therapy, often necessitating surgical intervention or colostomy (3). Long-term pharmacological management is essential in chronic diseases, yet complications and poor adherence often compromise patient outcomes. Reported non-adherence rates for IBD treatments vary significantly, ranging from 9.6–65% for aminosalicylic acid to 3–64.7% for thiopurines (4, 5). Such non-adherence severely impairs symptom control and increases the risk of

surgical interventions (6) Consequently, identifying natural phytochemicals to enhance treatment response and quality of life has become a key research focus, offering promising strategies to target oxidative stress and proinflammatory cytokines (7).

Ulcerative colitis (UC) is a multifactorial immunological disorder characterized by recurrent gastrointestinal inflammation and mucosal injury. Pathogenesis involves an uncontrolled immune response where disrupted intestinal barrier integrity permits luminal antigens to infiltrate the submucosa. This barrier defect triggers massive immune cell infiltration and activates the nuclear factor-kappa B (NF- κ B) pathway, resulting in the excessive production of proinflammatory cytokines such as TNF- α and IL-1 β (8). This cytokine dysregulation accelerates tissue destruction and intensifies oxidative stress via reactive oxygen species (ROS) production. Oxidative stress promotes lipid peroxidation (increased MDA) and depletes endogenous antioxidants like SOD and GPx, escalating mucosal damage to a transmural scale (9). Growing interest in bioactive natural components stems from the adverse effects of conventional IBD therapies. *Cannabis sativa* seed oil emerges as a therapeutic agent due to its optimal 3:1 Omega-6/Omega-3 ratio and rich polyphenolic content. Its anti-inflammatory capacity primarily involves components like ALA and GLA, which suppress proinflammatory pathways via PPAR- γ activation (10). Furthermore, tocopherols within the oil directly neutralize ROS, protecting the mucosal barrier from oxidative assault and reducing immune cell infiltration through the modulation of MPO activity (11). This mechanistic synergy positions CS seed oil as a potent natural adjuvant strategy that repairs the intestinal barrier and mitigates the cytokine storm.

Used since 3000 BC in traditional Chinese medicine, *Cannabis sativa* has long treated fatigue, rheumatism, and inflammatory disorders (12). *Cannabis sativa* seed oil (CSSO) is a unique plant-derived source of vitamin D (13). Beyond nutrition, its essential fatty acids contribute to anti-inflammatory, anticancer, and neuroprotective effects (14). CSSO lacks psychoactive properties as it bypasses cannabinoid 1 receptors. Instead, it exerts potent antioxidant and immune-modulating effects via vitamin E and cannabinoid 2 receptors (14).

Inflammation is a fundamental biological defense mechanism that protects tissues from microorganisms and toxins but may also result in tissue damage (15). This process is mediated by blood vessels, leukocytes, and plasma proteins. Inflammation can also be triggered by tissue necrosis, toxins, and chemical agents. Recent studies have significantly associated ulcerative colitis with systemic indicators, specifically the platelet-to-lymphocyte and neutrophil-to-lymphocyte ratios (16, 17). Ulcerative colitis pathophysiology involves diffuse mucosal inflammation extending from the rectum to proximal segments of the colon (18, 19).

This study aimed to evaluate the therapeutic potential of *Cannabis sativa* seed oil as a natural adjuvant for ulcerative colitis, addressing the limitations of current pharmacological interventions. Our objective was to investigate whether its rich antioxidant and anti-inflammatory profile could effectively mitigate mucosal damage and oxidative stress in an experimental colitis model.

2. MATERIALS AND METHODS

2.1. Study Design and Setting

This study was conducted in accordance with the principles outlined in the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health and was approved by the Ethics Committee for Animal Experiments of Aydın Adnan Menderes University. The clinical trial number is 64583101/2023/06, Approval date 19/01/2023.

2.2. Experimental Animals and Conditions

A total of 24 male Wistar albino rats, weighing between 250–400 g and aged 10–12 weeks, were obtained from the Animal Production Center of Aydın Adnan Menderes University Faculty of Medicine. The animals were housed under semi-controlled laboratory conditions with a temperature of $22 \pm 1^\circ\text{C}$, relative humidity of 40–50%, and a 12/12-hour light/dark cycle. Standard laboratory chow and tap water were provided *ad libitum* until 8–12 hours before the experimental procedures.

2.3. Characterization and Standardization of *Cannabis sativa* Seed Oil

The *Cannabis sativa* seed oil used in this study was a standardized, commercially available product (Mecitefendi, Turkey) manufactured under ISO 9001 and ISO 22000 quality management systems (The fat content was analyzed using chromatographic methods and submitted to the official authorities). To ensure the reproducibility and scientific validity of the results, the chemical profile of the specific batch utilized was documented, and a single, uniform batch was used for all experimental groups to maintain consistency. The oil was extracted through a controlled cold-pressing process (below 40°C) and is characterized by a high concentration of polyunsaturated fatty acids ($\sim 80\text{--}90\%$), a significant tocopherol content (800–1100 mg/kg), and a specific 3:1 Omega-6 to Omega-3 ratio (20). This rigorous standardization ensures that the observed therapeutic effects are consistently attributable to the established phytochemical profile of high-quality hemp seed oil.

2.4. Experimental Design

The rats were randomly allocated into four experimental groups ($n = 6$ per group): a healthy Control group, a Colitis group (TNBS-induced, untreated), and two treatment groups receiving *Cannabis sativa* seed oil at doses of 1 mL/kg (CS1) and 3 mL/kg (CS3). In the treatment arms, the oil was administered daily via intragastric gavage for five consecutive doses, with the initial dose starting six hours post-induction. Seventy-two hours after the induction of colitis, all rats were sacrificed under anesthesia, and colon tissues were harvested for subsequent biochemical and histopathological analyses.

2.5. Induction of Experimental Colitis

Experimental colitis was induced by intracolonic administration of TNBS. The rats were fasted for 24 hours prior to induction, with free access to water. Under anesthesia with ketamine (75 mg/kg) and

xylazine (8 mg/kg) administered intraperitoneally, a polyethylene cannula (6 Fr diameter) was gently inserted 8 cm into the anus. A 1 mL solution containing 25 mg of 2,4,6-trinitrobenzene sulfonic acid (TNBS) dissolved in 37% ethanol was slowly injected. The rats were maintained in the Trendelenburg position for 30 seconds to ensure uniform distribution of the solution, then returned to their cages for recovery.

2.6. Histopathological Evaluation and Scoring

Colonic tissue samples were fixed in 10% neutral buffered formalin, dehydrated through a graded series of ethanol, and embedded in paraffin blocks. Sections of 5- μ m thickness were obtained via microtome and stained with Hematoxylin and Eosin (H&E). The stained sections were examined under a light microscope to evaluate mucosal architecture and inflammatory infiltration. Histological damage was quantified using a validated semi-quantitative scoring system based on established literature. Parameters including epithelial loss, crypt architectural distortion, inflammatory cell infiltration, submucosal edema, and the presence of hemorrhage or necrosis were graded on a scale (typically 0–3 or 0–4). The total histological score, representing the sum of these individual parameters, was calculated to provide a comprehensive assessment of colonic mucosal injury.

2.7. Tissue Sampling and Processing

At the end of the 72-hour experimental period, anesthesia was induced with xylazine (8 mg/kg) and ketamine (75 mg/kg) intraperitoneally. The abdominal cavity was opened under sterile conditions for macroscopic inspection of the colon to evaluate perforation or adhesion. Tissue samples obtained for biochemical analysis were stored at -80°C until further processing. Portions of the colon designated for histopathological evaluation were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μ m thickness, and stained with hematoxylin–eosin (H&E) for microscopic examination.

2.8. Biochemical Analysis

Colon tissue samples were homogenized in 50 mM phosphate buffer (w/v = 1/10) at $0-4^{\circ}\text{C}$. The homogenates were centrifuged at $500 \times g$ for 15 minutes at 4°C , and the supernatants were collected for analysis. The activities and concentrations of myeloperoxidase (MPO), catalase (CAT), interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were determined using commercially available ELISA kits (BioVision and ScienceCell, USA) according to the manufacturers' instructions.

2.9. Statistical Analysis

Statistical analyses were performed using GraphPad Prism (version 7.04, GraphPad Software Inc., San Diego, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. For normally distributed data, statistical differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test for post-hoc analysis. Data are expressed as Mean $\pm$ Standard Error of the Mean (SEM), and a p-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1. Histopathological Findings

Histopathological findings are summarized in Table 1. No tissue damage was observed in the control group. In the colitis group, severe mucosal disruption, hemorrhage, necrosis, submucosal edema, crypt distortion, epithelial damage, reduced Goblet cell count, and wall thickening were evident. In the CS1 group, moderate necrosis, hemorrhage, and inflammation were observed. In contrast, the CS3 group exhibited no necrosis or crypt distortion, and the Goblet cell count remained unaltered. Only mild edema and inflammation were detected in this group.

Microscopic examination of Hematoxylin-Eosin (H&E, x10) stained sections revealed no structural alterations in the control group (Fig. 1A). In contrast, the TNBS group exhibited severe histopathological changes in all evaluated parameters (Fig. 1C). The CS1 group showed improvements in submucosal edema, hemorrhage, wall thickening, epithelial damage, crypt distortion, necrosis, inflammation, and Goblet cell count relative to the TNBS group (Fig. 1B). Similarly, the CS3 group demonstrated marked reductions in edema, hemorrhage, inflammation, necrosis, crypt distortion, Goblet cell loss, and wall thickening compared to the TNBS group (Fig. 1D).

3.2. Biochemical Findings

Comparisons among the study groups revealed significant differences in several biochemical parameters (Table 2, Fig. 2). TNBS administration successfully induced oxidative damage, evidenced by the marked elevation of MDA (355.8 ± 23.36) and the significant increase in MPO levels (618.0 ± 33.76), reflecting extensive lipid peroxidation and neutrophil infiltration, respectively. Furthermore, the TNBS group exhibited severe inflammation, demonstrated by the substantial rise in pro-inflammatory cytokines TNF-alpha (1549 ± 70.24) and IL-1 beta (1406 ± 67.31). This pathological state was further underscored by the dysregulation of the endogenous antioxidant system, shown by the diminished levels of CAT (277.7 ± 19.76) and GPx (619.3 ± 47.72) and the increased SOD activity (40.17 ± 1.682).

Conversely, treatment with *Cannabis sativa* seed oil significantly mitigated these pathological changes in a dose-dependent manner. Specifically, the highest dose (CS3) significantly reduced the levels of MDA (220.5 ± 23.48) and MPO (400.5 ± 36.48) compared to the TNBS group ($p < 0.05$). Similarly, CS treatment markedly suppressed the inflammatory response; specifically, TNF- α (1234 ± 54.72 in CS3) and IL-1 β (1081 ± 49.69 in CS3) levels demonstrated a significant restoration toward baseline values. The intervention also restored the antioxidant capacity, as evidenced by the significant elevation of CAT (380.7 ± 15.07 in CS3) and GPx (856.3 ± 23.19 in CS1) compared to the TNBS group. These findings collectively affirm the potent anti-inflammatory and antioxidant properties of *Cannabis sativa* in the experimental colitis model.

Tablo 1 Shows the tissue histopathology scores ($p < 0.05$).

	Control	Colitis	CS1	CS3
Submucosal edema	0.22	2.87	2.25	1.88
Hemorrhage	0	2.87	1.50	1.33
Inflammation	0.33	2.21	1.50	1.44
Necrosis	0	3	1.62	1.33
Decrease in Goblet cell count	0	2.75	1.62	1.33
Epithelial damage	0	2.87	1.62	1.33
Crypt distortion	0	2.87	1.50	1.22
Wall thickening	0	2.87	1.75	1.33

The histopathological findings, evaluated by an expert pathologist blinded to the experimental groups to ensure objectivity, are summarized in Table 1. The untreated Colitis group exhibited extensive mucosal injury, characterized by significantly elevated scores for submucosal edema, hemorrhage, necrosis, and epithelial damage compared to the Control group ($p < 0.05$). Significant crypt distortion and a reduction in goblet cell count further confirmed the severity of the TNBS-induced model. Conversely, *Cannabis sativa* (CS) seed oil treatment attenuated these pathological changes in a dose-dependent manner. While the CS1 group showed moderate recovery, the CS3 group demonstrated superior therapeutic efficacy, significantly reducing inflammation and necrosis scores. These results suggest that CS treatment, particularly at higher doses, effectively preserves structural mucosal integrity in experimental colitis.

Table 2 *Collective Biochemistry Results Between Groups (mean $\pm$ SEM). $p < 0.05$:*, $p < 0.01$:**, $p < 0.005$:*** Comparisons were made according to the TNBS group in different biochemical parameters.*

	CONTROL	TNBS	CS1	CS3
MDA (nmol/g tissue)	137.7 ± 4.120****	355.8 ± 23.36	245.3 ± 16.58**	220.5 ± 23.48***
MPO (mU/mL)	338.7 ± 26.83***	618.0 ± 33.76	478.3 ± 27.75*	400.5 ± 36.48***
GPx (mU/mL)	1138 ± 53.53****	619.3 ± 47.72	856.3 ± 23.19**	792.3 ± 55.20*
CAT (mU/mL)	412.5 ± 28.52**	277.7 ± 19.76	348.2 ± 29.48	380.7 ± 15.07*
TNF alfa (pg/g tissue)	1225 ± 63.60**	1549 ± 70.24	1366 ± 60.65	1231 ± 54.72**
SOD %	22.83 ± 2.915***	40.17 ± 1.682	31.83 ± 1.778*	26.83 ± 2.242**
IL1 beta (pg/mL)	939.5 ± 31.91****	1046 ± 67.31	1174 ± 63.90*	1081 ± 49.69**

The biochemical parameters summarized in Table 2 demonstrate that intrarectal TNBS administration induced significant oxidative damage and an inflammatory response. In the TNBS group, a marked elevation in lipid peroxidation (MDA) and neutrophil infiltration (MPO) was observed alongside a significant depletion of antioxidant enzymes (GPx and CAT) compared to the Control group ($p < 0.05$). Furthermore, proinflammatory cytokine levels (TNF- α and IL-1 β) were increased in the colitis model. Treatment with *Cannabis sativa* seed oil (CS) effectively modulated these parameters. Specifically, the CS3 group (high dose) showed superior efficacy in reducing MDA and MPO levels ($p < 0.005$ and $p < 0.001$, respectively) and significantly suppressing TNF- α and IL-1 β expression compared to the untreated TNBS group. While GPx levels were partially restored in both treatment groups, the CS1 group exhibited a more pronounced recovery in GPx activity compared to CS3. These results suggest that CS treatment strengthens the antioxidant defense system and attenuates the inflammatory cascade in experimental colitis.

4. DISCUSSION

Inflammatory bowel disease (IBD) is a chronic condition characterized by persistent inflammation of the gastrointestinal tract, often leading to mucosal injury, edema, and ulcer formation (21, 22). In the present study, macroscopic and histopathological examinations revealed ulceration and edema in rats with TNBS-induced colitis, whereas biochemical analyses indicated an imbalance in oxidative stress and

antioxidant systems, alongside elevated inflammatory markers. Results show that TNBS administration successfully induced colitis and oxidative-inflammatory responses in the experimental model.

Both Crohn's disease and ulcerative colitis are primary forms of IBD, and their clinical management typically involves corticosteroids, aminosalicylates, immunosuppressive agents, and TNF-alpha inhibitors (23–25). Although effective, these conventional therapies are often limited by significant adverse effects. For instance, TNF-alpha inhibitors have been associated with increased risks of lymphoma and heart failure (26), aminosalicylates may cause gastrointestinal distress (27, 28), and long-term corticosteroid use is linked to osteoporosis and edema (29–31). Given these clinical challenges, the current study hypothesized that *Cannabis sativa* seed oil could mitigate mucosal damage and inflammatory responses. Unlike synthetic agents, *Cannabis sativa* seed oil is characterized by a favorable safety profile in experimental models, as it is composed of essential fatty acids and bioactive compounds that lack the systemic toxicity associated with conventional immunosuppressants, thereby offering a promising complementary approach for IBD management.

Experimental colitis models are indispensable tools for elucidating the pathophysiology of IBD. While various induction methods, such as oxazolone, dinitrochlorobenzene, acetic acid, and dextran sulfate sodium (DSS), are commonly employed (32, 33), the intrarectal TNBS model was selected for the present study. TNBS is particularly valued for its capacity to induce a transmural inflammatory response and Th1-mediated immune profile that closely mimics the clinical and histopathological features of human Crohn's disease. This model provides a reliable and reproducible framework for evaluating the therapeutic efficacy of natural compounds like *Cannabis sativa* seed oil, allowing for a standardized assessment of its protective effects on mucosal integrity.

Oxidative stress markers were significantly altered in the TNBS group. MDA, a marker of lipid peroxidation, was significantly elevated, reflecting oxidative damage (34). Alves et al. (2024) also supported that MDA was significantly elevated, reflecting oxidative damage, in their colitis study (11). A study by Mo et al. (2022) demonstrated that the oral administration of mulberry anthocyanins successfully suppressed oxidative stress in the colon in a DSS-induced ulcerative colitis model. Consequently, a significant reduction in MDA levels, a marker of lipid peroxidation, was reported (35). MPO levels, widely recognized as indicators of neutrophil infiltration and inflammatory activity, were also increased in untreated rats (36). These findings are consistent with previous studies in which plant extracts attenuated MPO elevation in experimental colitis models (37). Wang et al. (2014) demonstrated that the sulfated polysaccharide isolated from the plant *Ulva lactuca* significantly suppressed MPO activity in an experimental colitis model, proving the ability of natural compounds, similar to what *Cannabis sativa* also shows, to attenuate the inflammatory response (38). Conversely, in the CS-treated groups, both MDA and MPO levels were significantly reduced in a dose-dependent manner, indicating the antioxidant and anti-inflammatory properties of *Cannabis sativa*.

The dysregulation of endogenous antioxidant systems, including SOD, CAT, and GPx, was observed in TNBS-induced colitis. SOD, which catalyzes the dismutation of superoxide radicals, plays a key role in

preventing lipid peroxidation and mitigating inflammatory damage (39, 40). Qian et al. (2024) concluded in their 2024 study that they ameliorated colitis symptoms by enhancing antioxidant enzyme function. They demonstrated a protective effect against oxidative damage by restoring the levels of SOD, CAT, and GPx (41). A systematic review by Wu et al. (2021) investigated the protective effects of various natural compounds on inflammatory bowel diseases. The study reported that one of the fundamental mechanisms of action of these compounds is to alleviate oxidative stress by directly and indirectly upregulating the activity of mucosal SOD, CAT, and GPx enzymes. The *Cannabis sativa* seed oil study similarly showed a protective effect against oxidative damage by restoring SOD, CAT, and GPx levels (42). Results show that, TNBS administration resulted in decreased SOD levels, whereas treatment with *Cannabis sativa* significantly restored SOD activity, suggesting a protective effect against oxidative injury. Similarly, CAT and GPx levels were elevated in CS-treated groups, further supporting the antioxidant and anti-inflammatory role of the intervention (43–45).

Proinflammatory cytokines such as TNF-alpha and IL-1 beta are critical mediators in the pathogenesis of IBD and serve as key biomarkers of inflammation (46–48). Alhasbary et al. (2025), in a review examining the potential of natural compounds in the treatment of ulcerative colitis, emphasized that flavonoids, in particular, modulate the immune response by suppressing the production and release of proinflammatory cytokines such as TNF-alpha and IL-1 beta. This effect is critical for breaking the inflammatory cycle caused by the cytokines. Liang et al. found that natural compounds successfully reduced TNF-alpha and IL-1 beta by targeting inflammatory signaling pathways, which supports our study with *Cannabis sativa* (8, 49). Our results demonstrated that TNBS-induced colitis significantly increased serum and tissue levels of TNF-alpha and IL-1 beta, while *Cannabis sativa* treatment markedly reduced these cytokine levels, indicating a strong anti-inflammatory effect. These findings are in agreement with previous studies; for instance, Zhu et al. (2019) reported that gallic acid significantly decreased TNF-alpha and IL-1 beta levels in TNBS-induced colitis, paralleling our observations in the CS-treated groups.

The observed therapeutic effects can be attributed to the unique lipid profile of *Cannabis sativa* seed oil. The selection of *Cannabis sativa* seed oil is predicated on its unique nutritional profile, specifically the optimal 3:1 ratio of Omega-6 to Omega-3 polyunsaturated fatty acids, which is essential for modulating systemic inflammation (50). Unlike conventional vegetable oils, it contains significant levels of Gamma-Linolenic Acid, a precursor to anti-inflammatory eicosanoids. Furthermore, its rich composition of bioactive polyphenols and tocopherols provides a synergistic antioxidant effect, making it a superior candidate for mitigating the oxidative stress and mucosal damage associated with ulcerative colitis compared to other lipid sources.

Finally, while other studies investigating DSS-induced colitis have highlighted potential issues with reproducibility and standardization (51), the intrarectal TNBS method employed in our study provided consistent histopathological and biochemical results. Histopathological evaluation confirmed that CS treatment mitigated mucosal damage, edema, necrosis, and inflammatory infiltration in a dose-dependent manner, further supporting the therapeutic potential of *Cannabis sativa* in IBD.

5. CONCLUSIONS

This study demonstrates that *Cannabis sativa* seed oil significantly improves mucosal and histopathological damage in TNBS-induced colitis. The therapeutic effect is primarily driven by the reduction of oxidative stress and the suppression of pro-inflammatory cytokines. Our findings highlight that CS seed oil exerts these protective effects in a dose-dependent manner. Consequently, this natural intervention represents a promising adjunctive therapy for managing inflammatory bowel disease (IBD).

However, certain limitations warrant further investigation. Future research should focus on evaluating the long-term safety and efficacy of CS seed oil in human populations. Detailed studies are needed to optimize dosages and treatment durations. Additionally, exploring potential synergistic effects between CS seed oil and conventional IBD treatments could provide new clinical perspectives. Such investigations will be crucial in determining the viability of CS seed oil as a supportive strategy for IBD patients.

Declarations

Ethics Committee Approval

The study protocol was approved by the Aydın Adnan Menderes University Animal Experiments Ethics Committee (Aydın, Türkiye; Approval number: 64583101/2023/06, Date: 19/01/2023). All experimental procedures were conducted at the Aydın Adnan Menderes University Animal Facility in strict accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH). Research and publication ethics were rigorously followed throughout the investigation.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Financial Disclosure

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Author Contributions

Idea/Conceptualization: FŞ, İK; Study Design: FŞ, İK; Data Collection/Processing: FŞ, İK; Analysis/Interpretation: FŞ. ; Literature Review: İK; Manuscript Drafting/Writing: İK; Critical Revision: FŞ.

References

1. Mahalli AA, Alharthi HM. Assessment of health-related quality of life of patients with inflammatory bowel diseases in Eastern Province, Saudi Arabia. *J Infect Public Health*. 2017;10(1):93–101. <https://doi.org/10.1016/j.jiph.2016.02.008>.
2. Malinowski B, Wiciński M, Sokolowska MM, Hill NA, Szambelan M. The rundown of dietary supplements and their effects on inflammatory bowel disease—A review. *Nutrients*. 2020;12(5):1423. <https://doi.org/10.3390/nu12051423>.
3. Fiorino G, Allocca M, Chaparro M, Coenen S, Fidalgo C, Younge L, et al. Quality of Care's standards in inflammatory bowel disease: a systematic review. *J Crohn's Colitis*. 2019;13(1):127–37. <https://doi.org/10.1093/ecco-jcc/jjy140>.
4. Ghadir MR, Bagheri M, Vahedi H, Daryani NE, Malekzadeh R, Hormati A, et al. Nonadherence to medication in inflammatory bowel disease: rate and reasons. *Middle East J Dig Dis*. 2016;8(2):116. 10.15171/mejdd.2016.16.
5. Martelli L, Lopez A, Strobel S, Danese S, Roblin X, Baumann C, et al. Adherence to infliximab therapy in inflammatory bowel disease patients in a real-life setting. *J Digest Dis*. 2017;18(10):566–73. <https://doi.org/10.1111/1751-2980.12539>.
6. Chan W, Chen A, Tiao D, Selinger C, Leong R. Medication adherence in inflammatory bowel disease. *Intest Res*. 2017;15(4):434. <https://doi.org/10.5217/ir.2017.15.4.434>.
7. Leong R, Tiao DK, Chan W, Chen A, Selinger CP. (2017). Medication adherence in inflammatory bowel disease. <https://doi.org/10.5217/ir.2017.15.4.434> 7
8. Lu X, Sun Y, Zhang Z, Sun Z, Wang S, Xu E. Regulation of pyroptosis by natural products in ulcerative colitis: mechanisms and therapeutic potential. *Front Pharmacol*. 2025;16:1573684. <https://doi.org/10.3389/fphar.2025.1573684>.
9. Qian J, Liang T, Xu Y, Liu ZP, Jing LL, Luo HB. Effect of the Novel Free Radical Scavenger 4'-Hydroxyl-2-Substituted Phenylnitronyl Nitroxide on Oxidative Stress, Mitochondrial Dysfunction and Apoptosis Induced by Cerebral Ischemia–Reperfusion in Rats. *Neuroscience*. 2024;540:1–11. <https://doi.org/10.1016/j.neuroscience.2023.12.016>.
10. Bukowska B. Current and Potential Use of Biologically Active Compounds Derived from *Cannabis sativa* L. in the Treatment of Selected Diseases. *Int J Mol Sci*. 2024;25(23):12738. <https://doi.org/10.3390/ijms252312738>.
11. Araruna MEC, Alves Júnior EB, de Lima Serafim CA, Pessoa MMB, de Souza Pessôa ML, Alves VP, et al. (-)-Fenchone Ameliorates TNBS-Induced Colitis in Rats via Antioxidant, Immunomodulatory, and Cytoprotective Mechanisms. *Pharmaceuticals*. 2024;18(1):18. <https://doi.org/10.3390/ph18010018>.
12. Abel EL. Marijuana the first twelve thousand years Plenum Press. New York, New York, USA. 1980.
13. Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S. Gastric Cytoprotection of the Non-Steroidal Anti-Inflammatory Sesquiterpene, β -Caryophyllene. *Planta Med*. 1996;62(05):469–70. 10.1055/s-2006-957942.
14. Leizer C, Ribnicky D, Poulev A, Dushenkov S, Raskin I. The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition. *J Nutraceuticals Funct Med Foods*. 2000;2(4):35–53.

https://doi.org/10.1300/J133v02n04_04.

15. Morgan GE Jr, Mikail MS. Clinical anesthesiology. In: Clinical anesthesiology [Internet]. 1996. Oct (17). pp. 881–881. Available from: <https://pesquisa.bvsalud.org/portal/resource/pt/biblio-931314>. id:biblio-931314.
16. Endo K, Satoh T, Yoshino Y, Kondo S, Kawakami Y, Katayama T, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as noninvasive predictors of the therapeutic outcomes of systemic corticosteroid therapy in ulcerative colitis. *Inflamm Intestinal Dis*. 2021;6(4):218–24. <https://doi.org/10.1159/000520523>.
17. Akpinar MY, Ozin YO, Kaplan M, Ates I, Kalkan IH, Kilic ZMY, et al. Platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio predict mucosal disease severity in ulcerative colitis. *J Med Biochem*. 2018;37(2):155. 10.1515/jomb-2017-0050.
18. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol*. 2010;105(3):501–23. quiz 524.
19. Fidan F, Alkan BM, Tosun A. Pandemic of the age: Vitamin D deficiency and insufficiency. *Turkish J Osteoporos*. 2014;20(2):71–4. 10.4274/tod.94830.
20. Fathordoobady F, Singh A, Kitts DD, Pratap Singh A. Hemp (*Cannabis Sativa* L.) Extract: Anti-Microbial Properties, Methods of Extraction, and Potential Oral Delivery. *Food Reviews Int*. 2019;35(7):664–84. <https://doi.org/10.1080/87559129.2019.1600539>.
21. Lih-Brody L, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Digest Dis Sci*. 1996;41(10):2078–86. <https://doi.org/10.1007/BF02093613>.
22. Cromer WE, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J gastroenterology: WJG*. 2011;17(5):578. 10.3748/wjg.v17.i5.578.
23. Fiasse R, Denis MA, Dewit O. Chronic inflammatory bowel disease: Crohn's disease and ulcerative colitis. *J Pharm Belg*. 2010;(1):1–9.
24. Ananthakrishnan AN, Aspirin. Nonsteroidal Anti-inflammatory Drug Use, and Risk for Crohn Disease and Ulcerative Colitis: A Cohort Study. *Ann Intern Med*. 2012;156(5):350. <https://doi.org/10.7326/0003-4819-156-5-201203060-00007>.
25. Cornish JA, Tan E, Simillis C, Clark SK, Teare J, Tekkis PP. The risk of oral contraceptives in the etiology of inflammatory bowel disease: a meta-analysis. *Official J Am Coll Gastroenterology| ACG*. 2008;103(9):2394–400.
26. Panaccione R, Fedorak RN, Aumais G, Bernard EJ, Bernstein CN, Bitton A, et al. Review and Clinical Perspectives for the Use of Infliximab in Ulcerative Colitis. *Can J Gastroenterol*. 2008;22(3):261–72. <https://doi.org/10.1155/2008/493405>.

27. Gisbert JP, González-Lama Y, Maté J. 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis*. 2007;13(5):629–38. <https://doi.org/10.1002/ibd.20099>.
28. Hanauer SB. Aminosalicylates in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2004;20(s4):60–5. <https://doi.org/10.1111/j.1365-2036.2004.02048.x>.
29. Herfarth HH, Long MD, Rubinas TC, Sandridge M, Miller MB. Evaluation of a Non-invasive Method to Detect Cytomegalovirus (CMV)-DNA in Stool Samples of Patients with Inflammatory Bowel Disease (IBD): A Pilot Study. *Dig Dis Sci*. 2010;55(4):1053–8.
30. Tung J, Loftus EV, Freese DK, El-Youssef M, Zinsmeister AR, Melton JL, et al. A population-based study of the frequency of corticosteroid resistance and dependence in pediatric patients with Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis*. 2006;12(12):1093–100. <https://doi.org/10.1097/01.mib.0000235835.32176.85>.
31. Seo J, Kim S, Hong SW, Hwang SW, Park SH, Yang D, et al. Continuing or stopping 5-aminosalicylates in patients with inflammatory bowel disease on anti- TNF therapy: A nationwide population-based study. *Aliment Pharmacol Ther*. 2024;60(3):389–400. <https://doi.org/10.1111/apt.18102>.
32. Randhawa PK, Singh K, Singh N, Jaggi AS. A Review on Chemical-Induced Inflammatory Bowel Disease Models in Rodents. *Korean J Physiol Pharmacol*. 2014;18(4):279. <http://dx.do.org/10.4196/kjpp.2014.18.4.279>.
33. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. *Gastroenterology*. 1995;109(4):1344–67. [https://doi.org/10.1016/0016-5085\(95\)90599-5](https://doi.org/10.1016/0016-5085(95)90599-5).
34. Güvenç M, Cellat M, Özkan H, Tekeli İO, Uyar A, Gökçek İ, et al. Protective Effects of Tyrosol Against DSS-Induced Ulcerative Colitis in Rats. *Inflammation*. 2019;42(5):1680–91.
35. Mo J, Ni J, Zhang M, Xu Y, Li Y, Karim N, et al. Mulberry anthocyanins ameliorate DSS-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota. *Antioxidants*. 2022;11(9):1674. <https://doi.org/10.3390/antiox11091674>.
36. Da Silva S, Keita ÅV, Mohlin S, Pålman S, Theodorou V, Pålman I, et al. A novel topical PPAR γ agonist induces PPAR γ activity in ulcerative colitis mucosa and prevents and reverses inflammation in induced colitis models. *Inflamm Bowel Dis*. 2018;24(4):792–805. <https://doi.org/10.1093/ibd/izx079>.
37. Çevik Ö, Şener A, Kumral ZÖ, Çetinel Ş, Altıntaş A, Oba R, et al. Protective and therapeutic effects of *Polygonum cognatum* Meissn aqueous extract in experimental colitis. *Marmara Pharm J*. 2014;18(3):126–34. 10.12991/mpj.2014187239.
38. Zhu Y, Wang D, Zhou S, Zhou T. Hypoglycemic Effects of *Gynura divaricata* (L.) DC Polysaccharide and Action Mechanisms via Modulation of Gut Microbiota in Diabetic Mice. *J Agric Food Chem*. 2024;72(17):9893–905.
39. Sanyal J, Bandyopadhyay SK, Banerjee TK, Mukherjee SC, Chakraborty DP, Ray BC, et al. Plasma levels of lipid peroxides in patients with Parkinson's disease. *Eur Rev Med Pharmacol Sci*. 2009;13(2):129–32.

40. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. *Lab Invest.* 1982;47(5):412–26.
41. Qian Z, Zhang M, Lu T, Yu J, Yin S, Wang H, et al. Propolis alleviates ulcerative colitis injury by inhibiting the protein kinase C - transient receptor potential cation channel subfamily V member 1 - calcitonin gene-related peptide/substance P (PKC-TRPV1-CGRP/SP) signaling axis. *PLoS ONE.* 2024;19(1):e0294169. <https://doi.org/10.1371/journal.pone.0294169>.
42. Hu L, Liu J, Yin J. Eriodictyol attenuates TNBS -induced ulcerative colitis through repressing TLR4 / NF-κB signaling pathway in rats. *Kaohsiung J Med Scie.* 2021 Sept;37(9):812–8. <https://doi.org/10.1002/kjm2.12400>.
43. Ek RO, Serter M, Ergin K, Cecen S, Unsal C, Yildiz Y, et al. Protective effects of citicoline on TNBS-induced experimental colitis in rats. *Int J Clin Exp Med.* 2014;7(4):989.
44. Iborra M, Moret I, Rausell F, Bastida G, Aguas M, Cerrillo E, et al. Role of oxidative stress and antioxidant enzymes in Crohn's disease. *Biochem Soc Trans.* 2011;39(4):1102–6. <https://doi.org/10.1042/BST0391102>.
45. Fattman CL, Schaefer LM, Oury TD. Extracellular superoxide dismutase in biology and medicine. *Free Radic Biol Med.* 2003;35(3):236–56. [https://doi.org/10.1016/S0891-5849\(03\)00275-2](https://doi.org/10.1016/S0891-5849(03)00275-2).
46. Nishitani Y, Yamamoto K, Yoshida M, Azuma T, Kanazawa K, Hashimoto T, et al. Intestinal anti-inflammatory activity of luteolin: Role of the aglycone in NF-κB inactivation in macrophages co-cultured with intestinal epithelial cells. *BioFactors.* 2013 Sept;39(5):522–33. <https://doi.org/10.1002/biof.1091>.
47. Zhou YH, Yu JP, Liu YF, Teng XJ, Ming M, Lv P, et al. Effects of Ginkgo biloba Extract on Inflammatory Mediators (SOD, MDA, TNF- α, NF- κ Bp65, IL-6) in TNBS-Induced Colitis in Rats. *Mediat Inflamm.* 2006;2006(1):092642. <https://doi.org/10.1155/MI/2006/92642>.
48. Zhu L, Gu P, Shen H. Gallic acid improved inflammation via NF-κB pathway in TNBS-induced ulcerative colitis. *Int Immunopharmacol.* 2019;67:129–37. <https://doi.org/10.1016/j.intimp.2018.11.049>.
49. Saurabh NK, Khan MM, Kirabo A. A future avenue of treatment ulcerative colitis targeting macrophage polarization: a phytochemical application. *Crohn's Colitis 360.* 2024;6(4):otae070. <https://doi.org/10.1093/crocol/otae070>.
50. Callaway JC. Hempseed as a nutritional resource: An overview. *Euphytica.* 2004;140(1):65–72.
51. Dönder Y, Arikan TB, Baykan M, Akyüz M, Öz AB. Effects of quercitrin on bacterial translocation in a rat model of experimental colitis. *Asian J Surg.* 2018;41(6):543–50. <https://doi.org/10.1016/j.asjsur.2017.12.002>.

Figures

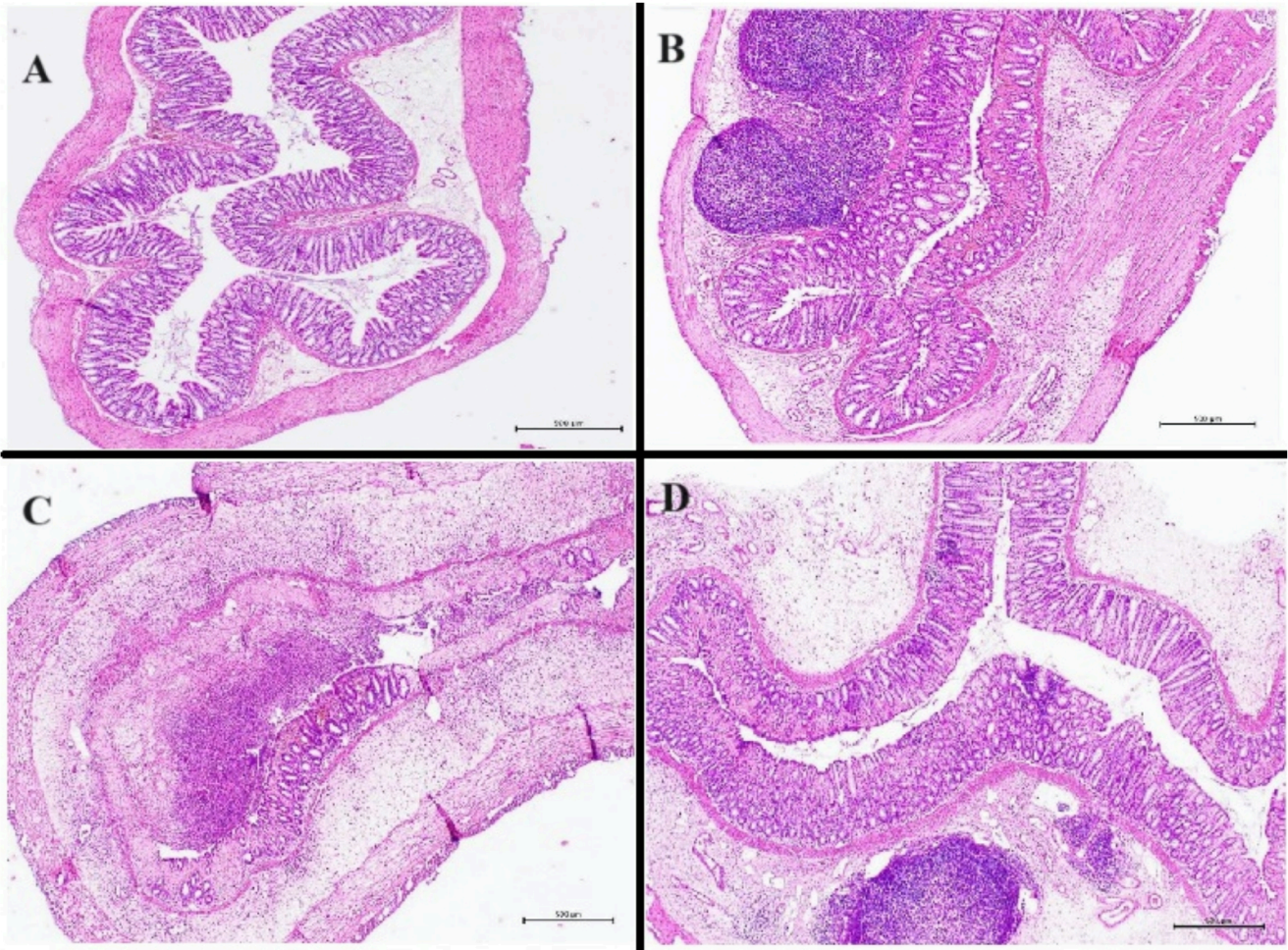


Figure 1

Depicts the following: Control group, normal colon appearance (A); CS1 group, area with mild edema and mild inflammation (B); colitis group, necrotic tissue, submucosal edema, crypt distortion, decreased goblet cells, wall thickening, epithelial damage (C); CS3 group, preserved colon tissue with mild edema (D).

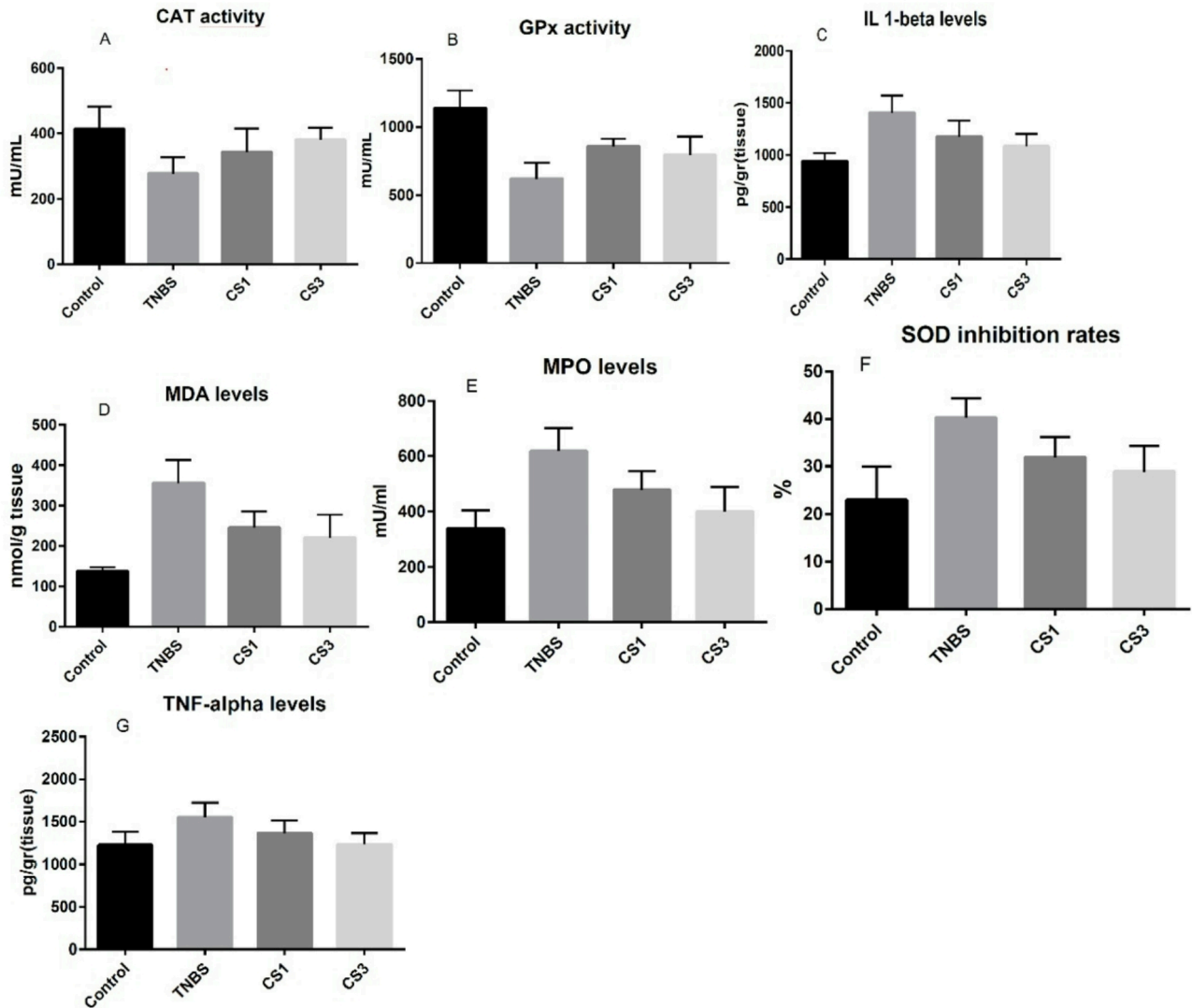


Figure 2

Effect of Cannabis sativa seed oil on biochemical markers in TNBS-induced ulcerative colitis. (A) Catalase (CAT) activity, (B) Glutathione peroxidase (GPx) activity, (C) Interleukin-1 beta (IL-1 β) levels, (D) Malondialdehyde (MDA) concentrations, (E) Myeloperoxidase (MPO) activity, (F) Superoxide dismutase (SOD) activity, and (G) Tumor necrosis factor-alpha (TNF- α) levels. Data are expressed as Mean $\pm$ SEM (n=6 per group). Statistical significance was evaluated by one-way ANOVA followed by Tukey's multiple comparisons test. *p < 0.05 denotes statistical significance.

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