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Keywords: Crohn's disease; Ulcerative colitis; Curcumin; Black pepper; Antioxidants

*Article*

# Effect of Curcumin plus Piperine on Redox Imbalance and Inflammation in Inflammatory Bowel Disease Patients: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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**Abstract:** Background: Inflammatory bowel diseases (IBD) present chronic inflammatory challenges marked by increased oxidative stress. This study assessed the collective impact of Curcumin plus piperine on oxidative stress and inflammation biomarkers in mild to moderate IBD patients. Methods: Patients diagnosed with CD or UC, aged ≥18 years with preserved kidney and liver functions, were randomized into three groups: placebo, Curcumin (1000 mg/day), and Curcumin plus piperine (1000 mg + 10 mg/day) for 12 weeks. Blood and fecal samples were collected to analyze oxidative stress and inflammatory markers, before and after the intervention period. Results: Curcumin plus piperine group had significantly higher serum SOD compared to the placebo group, after adjusting for sex, age, and type of IBD (4346.9 ± 879.0 vs 3614.5 ± 731.5; p=0.041). There were no significant differences in the other oxidative stress or inflammatory markers between groups. Conclusions: This study suggests that 12 weeks of Curcumin plus piperine supplementation effectively enhances the enzymatic antioxidant defense, particularly SOD, in IBD patients. These findings hold promise for managing redox imbalance in individuals with IBD, highlighting potential therapeutic benefits.

**Keywords:** crohn's disease; ulcerative colitis; curcumin; black pepper; antioxidants

## 1. Introduction

Inflammatory bowel diseases (IBD), represented by Crohn's disease (CD) and ulcerative colitis (UC), are chronic and recurrent gastrointestinal disorders, characterized by alternating periods of

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symptom activation and remission, including abdominal pain, diarrhea and rectal bleeding. These conditions significantly compromise patients' quality of life [1,2]

Epidemiological data indicate a rising global trend in new IBD cases, imposing a substantial burden on healthcare system [3]. Despite ongoing research, the etiology of IBD remains incompletely understood. The interplay between genetic and environmental factors, oxidative stress, and an exacerbated, uncontrolled immune response is implicated in both the onset and progression of the inflammatory process [4].

In inflammatory bowel diseases (IBD), a redox imbalance occurs, where the antioxidant defense is insufficient to counteract the excess of reactive oxygen and nitrogen species (RONS) generated. This contributes to impairment of the epithelial barrier and consequent increase in intestinal permeability, allowing the entry of luminal antigens that induce a dysregulated immune response characterized by elevated reactive species and pro-inflammatory cytokines. This results in oxidative damage to cellular components, further exacerbating the chronic inflammatory state [5,6].

Currently, drug therapy of IBD involves synthetic drugs and monoclonal antibodies designed to manage imminent inflammation. However, prolonged use of these therapies may lead adverse effects, limiting their effectiveness and causing many patients to become refractory to treatment, thereby increasing the risk of complications and surgical interventions [7,8].

To address these challenges, research has explored new therapeutic alternatives, with particular emphasis on the utilization of *Curcuma longa*, commonly known as turmeric, and their three main curcuminoids — curcumin, demethoxycurcumin, and bisdemethoxycurcumin—with curcumin being the primary bioactive component [9].

Curcumin, a hydrophobic polyphenol, exhibits potent antioxidant, anti-inflammatory, antitumor, antimicrobial, anti-glycating, anti-coagulant and healing properties, as evidenced by both experimental studies and clinical trials clinical trials in IBD [10] and other clinical situations such as Type 2 diabetes [11] and some cancers [12]. However, its therapeutic potential is hindered by low bioavailability, attributed to reduced solubility, rapid metabolization and systemic elimination [13,14].

Piperine, a bioactive compound from black pepper (*Piper nigrum*), emerges as a potentiation agent capable of inhibiting hepatic glucuronidation, thereby increasing curcumin bioavailability by up to 2000% [15]. The combination of curcumin and piperine has shown successful outcomes in diseases where oxidative stress is a significant etiological factor, such as metabolic syndrome [16,17]. Despite the positive effects of curcumin or turmeric in IBD, the combined impact with piperine, along with its antioxidant effects and influence on inflammatory markers like fecal calprotectin and cytokines, remains unexplored in clinical trials [18]. This highlights a crucial gap in understanding the comprehensive effects of the mixture, in patients with IBD.

Therefore, the objective of the present study was to clinically investigate the efficacy of using curcumin alone or in combination with piperine on markers of oxidative stress and inflammation in patients with IBD, in different matrixes (blood and feces).

## 2. Results

This section was divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

### *2.1. Baseline Characteristics*

The present study was carried out between July 2021 and March 2023. Initially, fifty-eight (58) patients were enrolled in the study. Among them, fifty-one completed the 12 weeks of supplementation, 14 in the placebo group, 20 in the Curcumin group and 17 in the Curcumin + piperine group. Participants were excluded due to loss of contact during follow-up of the clinical study, voluntary discontinuation of the supplement and incorrect intake of the recommended dosage (Figure 1).

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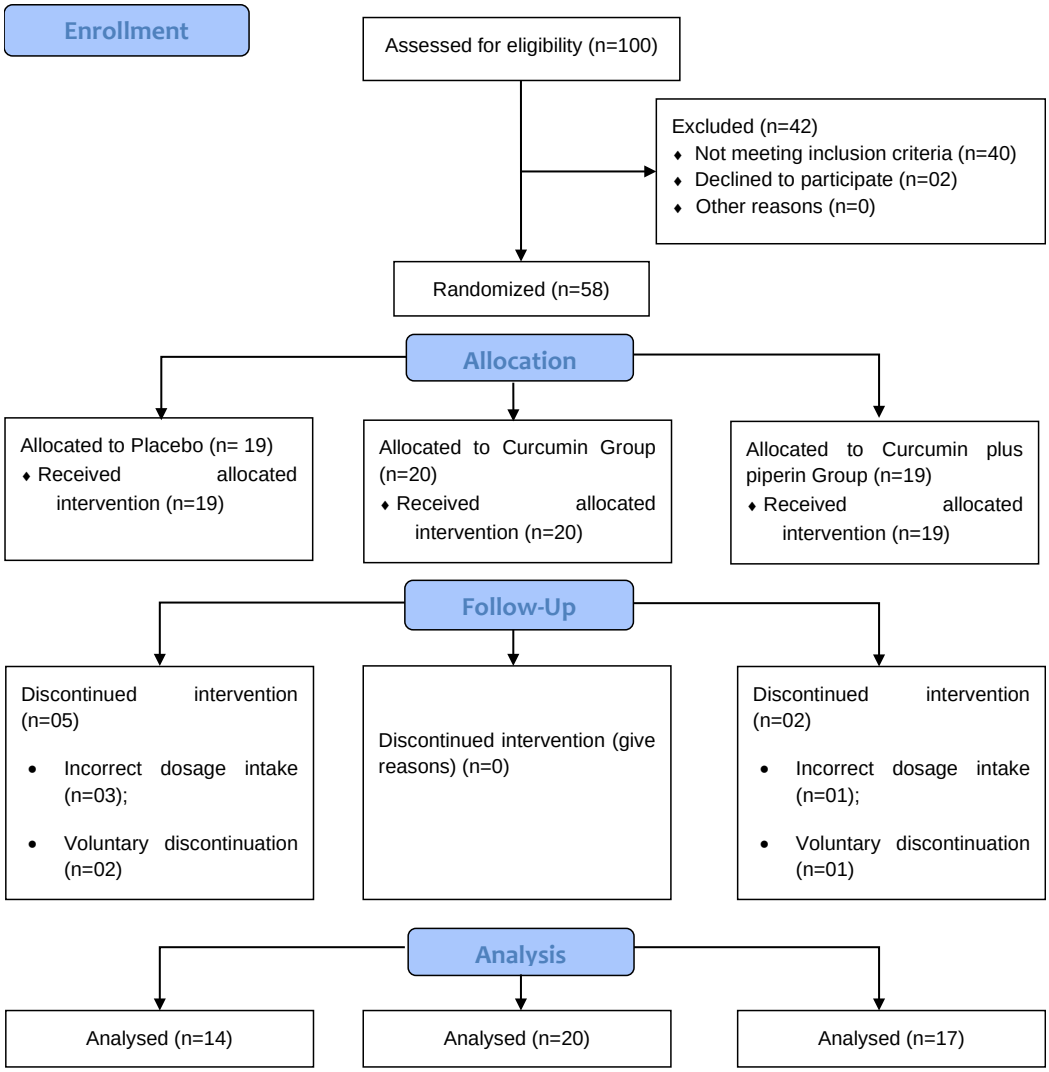


**Figure 1.** Flowchart of the clinical study.

The participants' mean age was 47.5 ± 15.5, with the majority being female (n=38; 65.5%) and in a stable relationship (n=44; 75.9%). None of the patients consumed alcoholic beverages, and physical activity was not a prevalent practice. Biologic therapy emerged as the primary pharmacological treatment among study participants (n=30; 51.7%), due to its effectiveness in better controlling the inflammatory condition. Notably, the observed data in Table 1 underscore a low prevalence of non-communicable chronic diseases (n=17; 29.7%) and extraintestinal manifestations (n=5; 8.6%).

However, despite the randomization conducted at the beginning of the clinical trial, a higher prevalence of education exceeding 4 years was significantly more frequent in the placebo group (p=0.031), along with a greater number of patients with UC allocated to the Curcumin group (p=0.026). Nevertheless, both situations ceased to be statistically significant by the end of the experiment (data not shown).

**Table 1.** Baseline Demographics and Clinical Characteristics of the IBD Patients.

| | | | Groups | | | |
|---|---|---|---|---|---|---|
| | | Total | Placebo n=19 | Curcumin n=20 | Curcumin + Piperine n=19 | *p-value* |
| Age | Mean (SD) | 47.5 ±15.5 | 50.9 ± 14.4 | 46.9 ± 18.9 | 44.7 ± 12.4 | 0.460[1] |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Sex | Female | 38 (65.5) | 13 (68.4) | 11 (55.0) | 14 (73.7) | 0.447[2] |
| | Male | 20 (34.5) | 6 (31.6) | 9 (45.0) | 5 (26.3) | |
| Schooling | < 4 years | 38 (65.5) | 8 (42.1) | 15 (75.0) | 15 (78.9) | 0.031[2] |
| | ≥ 4 years | 20 (34.5) | 11 (57.9)[a] | 5 (25.0)[b] | 4 (21.1)[b] | |
| Self-declared race | White | 14 (24.1) | 2 (10.5) | 7 (35.0) | 5 (26.3) | 0.196[2] |
| | Black/Brown | 44 (75.9) | 17 (89.5) | 13 (65.0) | 14 (73.7) | |
| Marital status | Single/Divorced | 19 (32.8) | 6 (31.6) | 8 (40.0) | 5 (26.3) | 0.668[2] |
| | Stable union | 33 (67.2) | 10 (52.6) | 10 (50.0) | 13 (68.4) | |
| Inflammatory Bowel Disease | Crohn's disease | 19 (32.8) | 9 (47.4)[b] | 2 (10.0)[a] | 8 (42.1)[ab] | 0.026[2] |
| | Ulcerative Colitis | 39 (67.2) | 10 (52.6) | 18 (90.0) | 11 (57.9) | |
| Diagnosis time | <10 years | 22 (37.9) | 11 (57.9) | 5 (25.0) | 6 (31.6) | 0.084[2] |
| | ≥ 10 years | 36 (62.1) | 8 (42.1) | 15 (75.0) | 13 (68.4) | |
| Chronic non-communicable diseases | No | 41 (70.7) | 14 (73.7) | 14 (70.0) | 13 (68.4) | 0.935[2] |
| | Yes | 17 (29.3) | 5 (26.3) | 6 (30.0) | 6 (31.6) | |
| History of COVID-19 | No | 47 (81.0) | 17 (89.5) | 15 (75.0) | 15 (78.9) | 0.495[2] |
| | Yes | 11 (19.0) | 2 (10.5) | 5 (25.0) | 4 (21.1) | |
| Extraintestinal manifestation | No | 53 (91.4) | 17 (89.5) | 19 (95.0) | 17 (89.5) | 0.776[2] |
| | Yes | 5 (8.6) | 2 (10.5) | 1 (5.0) | 2 (10.5) | |
| Smoking | No | 43 (74.1) | 13 (68.4) | 15 (75.0) | 15 (78.9) | 0.755[2] |
| | Yes | 15 (25.9) | 6 (31.6) | 5 (25.0) | 4 (21.1) | |
| Farmacologic treatment | Aminosalicylates | 26 (44.4) | 6 (31.6) | 10 (50.0) | 10 (52.6) | 0.301[2] |
| | Biological Therapy | 30 (51.7) | 11 (57.9) | 10 (50.0) | 9 (47.4) | |
| | No drug therapy | 2 (3.4) | 2 (10.5) | 0 (0.0) | 0 (0.0) | |
| Body mass index | Low weight | 9 (15.5) | 1 (5.3) | 4 (20.0) | 4 (21.1) | 0.588[2] |
| | Suitable weight | 24 (41.4) | 10 (52.6) | 7 (35.0) | 7 (36.8) | |
| | Overweight | 25 (43.1) | 8 (42.1) | 9 (45.0) | 8 (42.1) | |

Legend: 1 – ANOVA test; 2 – Chi square or Fisher's exact test; Categorical data expressed as n (%); a, b: different letters represent statistical significance.

Effects of Supplementation on Gastrointestinal Symptoms

In this study, as a secondary outcome, we assessed the impact of supplementation with Curcumin, with or without piperine, on various gastrointestinal complaints, common in patients with IBD. As depicted in supplementary 2, at baseline, there were no significant differences between the groups. However, at the study's conclusion, participants in the Curcumin + piperine group exhibited significantly higher complaints of heartburn compared to the placebo group. This distinction is evident in both the chi-square test (p=0.017) and the generalized estimating equation (group vs. time interaction), indicating 4.1 times higher (p=0,016; OR: 4.151; 95% IC, 1.309 – 13.169), likelihood of patients receiving this supplementation reporting heartburn compared to the placebo group (supplementary 1).

*2.2. Effects of Supplementation on Redox Imbalance and Inflammation*

Among the oxidative stress and inflammation markers (Tables 2 and 3) evaluated in this study, supplementation with Curcumin and piperine for 3 months proved effective in significantly increasing the levels of SOD compared to the placebo group after adjusting for sex, age, and type of IBD (4346.9 ± 879.0 vs 3614.5 ± 731.5; p=0.02; IC95%: 102.262 – 1528.186). Furthermore, it demonstrated a more pronounced impact when comparing endline to baseline (Δ) (538.8 ± 1040.1 vs -126.8 ± 762.7, placebo group; p = 0.027; IC95%: -351.986 – 1094.626). The other redox markers (CAT, MDA, and $H_2O_2$

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levels), disease activity (CalF), inflammation (MPO, TNF-α, IL-17A, and IL-22), and anti-inflammatory markers (IL-10) did not show significant changes after the experimental period.

**Table 2.** Serum Levels of Oxidative and Inflammatory Biomarkers in Patients with Inflammatory Bowel Disease According to Treatment Group: Baseline Data (T1) and Endline Data (T2).

| | | Group | | | *p-value* |
|---|---|---|---|---|---|
| | | Placebo | Curcumin | Curcumin + Piperine | ANCOVA/ Bonferroni* |
| MPO | T1 | 11.5 ± 2.5 | 10.8 ± 2.6 | 10.7 ± 2.4 | |
| | T2 | 11.8 ± 2.6 | 11.8 ± 1.6 | 12.9 ± 1.5 | 0.162 |
| | Δ | -0.0 ± 3.7 | 1.0 ± 2.2 | 1.8 ± 1.9 | 0.199 |
| TNF-α (pg/μL) [F] | T1 | 0.0 ± 0.6 | 0.1 ± 0.6 | 0.2 ± 0.5 | |
| | T2 | 0.0 ± 0.6 | -0.4 ± 0.5 | -0.2 ± 0.5 | 0.126 |
| IL-17A (pg/μL) [F] | T1 | 0.3 ± 0.2 | 0.1 ± 0.3 | 0.2 ± 0.4 | |
| | T2 | 0.3 (0.2) | 0.1 (0.5) | 0.3 (0.2) | 0.334[K] |
| IL 22 (pg/μL) | T1 | 3.4 ± 1.8 | 3.7 ± 2.0 | 3.9 ± 2.8 | |
| | T2 | 4.2 ± 1.9 | 4.7 ± 1.7 | 5.1 ± 2.9 | 0.498 |
| | Δ | 1.3 ± 1.8 | 1.0 ± 2.1 | 1.0 ± 3.0 | 0.853 |
| IL-10 (pg/μL) | T1 | 0.9 ± 0.7 | 1.0 ± 0.8 | 1.1 ± 0.8 | |
| | T2 | 1.5 ± 0.7 | 1.9 ± 1.0 | 1.9 ± 1.2 | 0.511 |
| | Δ | 0.6 ± 0.7 | 0.8 ± 1.3 | 0.7 ± 1.3 | 0.940 |
| SOD (U/μL) | T1 | 3599.8 ± 684.4 | 3728.1 ± 714.5 | 3761.3 ± 638.8 | |
| | T2 | 3614.5 ± 731.5 | 3870.2 ± 791.4 | 4346.9 ± 879.0 | 0.020[##] |
| | Δ | -126.8 ± 762.7 | 142.1 ± 906.9 | 538.8 ± 1040.1 | 0.027[##] |
| Catalase (U/min) | T1 | 7.6 ± 2.9 | 8.5 ± 2.8 | 9.0 ± 3.2 | |
| | T2 | 9.6 ± 4.2 | 9.2 ± 3.4 | 9.2 ± 3.4 | 0.781 |
| | Δ | 1.4 ± 6.1 | 0.6 ± 3.6 | 0.3 ± 2.6 | 0.576 |
| Hydrogen Peroxide (nmol/mL) | T1 | 218.3 ± 155.7 | 224.7 ± 154.9 | 259.5 ± 149.7 | |
| | T2 | 186.9 ± 118.7 | 215.5 ± 94.9 | 183.7 ± 98.1 | 0.307 |
| | Δ | -45.7 ± 220.0 | -9.1 ± 165.9 | -78.3 ± 168.3 | 0.476 |
| MDA (ng/μL) [F] | T1 | 1.0 ± 0.2 | 0.9 ± 0.2 | 0.9 ± 0.2 | |
| | T2 | 0.8 ± 0.4 | 0.7 ± 0.4 | 0.7 ± 0.3 | 0.602 |

Legend: Data expressed as mean ± Standard deviation or median (interquartile range); ◎ = logarized variable; ## = placebo group vs. Curcumin + piperine group; K = Kruskal-Wallis test; pg = picogram; μL = microliter; mL = milliliter; U = unit; Δ = (T2 – T1); ANCOVA adjusted for sex, age, and type of inflammatory bowel disease; *Bonferroni test has been realized when ANCOVA < 0.05.

**Table 3.** Fecal Calprotectin (FC) of patients with Inflammatory Bowel Disease according to the treatment group: baseline data (T1) and endline data (T2).

| | Group | | | | | | *p-value* |
|---|---|---|---|---|---|---|---|
| | Placebo | | Curcumin | | Curcumin + Piperine | | GEE p (OR; 95% CI) |
| | T1 n=19 | T2 n=14 | T1 n=20 | T2 n=20 | T1 n=19 | T2 n=17 | |
| <200 μg/g | 8 (42,1) | 5 (35,7) | 12 (60,0) | 7 (25,0) | 7 (36,8) | 12 (63,2) | 0,544 (1.392; 0,479 – 4.049)[#] |
| ≥200 μg/g | 11 (57,9) | 9 (64,3) | 8 (40,0) | 13 (75,0) | 9 (52,9) | 8 (47,1) | 0,710 (1.231; 0,413 – 3.670)[##] |

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Legend: Data expressed as n (%); GEE: Generalized estimating equations - group vs. time interaction, adjusted by sex, age and inflammatory bowel disease type; #placebo group vs. Curcumin + piperine group; ##placebo group vs. Curcumin group (Absence of treatment was used as a reference).

## 3. Discussion

Up to our knowledge, this is the first randomized, double-blind clinical trial assessing the impact of Curcumin supplementation, either alone or in combination with piperine, on oxidative, inflammatory, and disease activity markers in patients with inflammatory bowel disease (IBD). Our findings allow to affirm that the standard commercially available powder, rich in curcumin and combined with piperine, exhibits significant antioxidant action by substantially enhancing the endogenous defense mechanism, as evidenced by a noteworthy increase in serum SOD levels among supplemented patients over the three-month intervention period. These unprecedented results for this polyphenol underscore its beneficial effects on patients with IBD. This is further supported by a recent meta-analysis published by our research group [24].

The SOD family in mammals comprises three isoforms: SOD1 (Cu/ZnSOD, cytosolic), SOD2 (MnSOD, mitochondrial), and SOD3 (Cu/ZnSOD, extracellular). Their primary role is to scavenge superoxide radicals ($O_2^{-\bullet}$), reactive signaling molecules that can induce cell damage. SODs convert $O_2^{-\bullet}$ into $H_2O_2$, a less reactive form that can be further detoxified by catalase or glutathione peroxidase (GPx) [25,26]. Additionally, SOD activity reduces iron release, inhibiting hydroxy radical ($^{\bullet}OH$) formation, by Fenton reaction, and its downstream effects on lipids, proteins, and DNA [27].

Conversely, reducing circulating levels of the hydroxy radical impact the generation of reactive nitrogen species (RNS), as the superoxide radical can directly react with nitric oxide ($^{\bullet}NO$) to form peroxynitrite ($ONOO^-$), a potent oxidant and nitrating agent towards a wide range of macromolecules. Moreover, $ONOO^-$ is relatively stable under physiological conditions and can diffuse across several cell diameters, rendering it more toxic [26]. In this scenario, the increase of SOD levels not only diminishes peroxynitrite production, which also affects macromolecules, but also enables the utilization of nitric oxide as a vasodilator—an important factor in various biological contexts.

These results are corroborated by a randomized clinical trial in patients with metabolic syndrome, where the combination of curcumin plus piperine, supplemented at the same dosage used daily in this study (1000 mg + 10 mg), for 8 weeks, significantly increased SOD activity [28]. Another study conducted by Panahi et al. [29] also demonstrated the effectiveness of the Curcumin C3 Complex® formulation (1500 mg/day) in increasing SOD activity in patients with osteoarthritis, after 6 weeks [30], thus demonstrating the effectiveness of curcumin on this marker. of redox imbalance.

The observed increase in SOD levels in this study among patients receiving Curcumin plus piperine, with no alterations in $H_2O_2$ and MDA levels, suggests a beneficial effect of this supplementation. It is noteworthy that oxidative stress in IBD is considered both an etiological factor and a trigger for exacerbations, negatively impacting the quality of life of individuals with IBD. Studies indicate significant changes in SOD activity among IBD patients. In addition to lower levels of this enzyme compared to individuals without the disease, these low levels are associated with an increase in the inflammatory process [31]. Another change found in those individuals pertains to the expression of all three isoforms of SOD in the colonic mucosa. Kruidenier et al. [32] concluded in their study with 29 IBD patients that both CD and UC are accompanied by increased intestinal Mn-SOD and decreased Cu/Zn-SOD and EC-SOD levels, particularly in the inflamed mucosal epithelium.

In this context, it becomes clear that finding strategies, whether pharmacological or not, to improve the activity of this enzyme is crucial for the clinical therapy of the patients. The use of SOD or SOD mimetics has been tested as a pharmacological strategy in various *in vivo* and *in vitro* models [33], including induced colitis either through direct administration [34], or by stimulating the production of this enzyme in *Lactobacillus* [35]. However, these interventions are not yet considered a viable/safe option in humans, and hence, randomized clinical trials testing these formulations are not readily available. Considering this, antioxidant therapy with natural or synthetic substances has been the subject of numerous investigations.

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Recently, in a meta-analysis published by our group, evaluating clinical trials that investigated antioxidant or anti-inflammatory actions in their treatments for IBD, only SOD was significantly modulated, while markers such as MDA, like our findings, and total antioxidant capacity showed no significant relevance [18].

As mentioned earlier, despite Curcumin being a widely studied polyphenol in IBD, its antioxidant and anti-inflammatory roles have only been confirmed in animal models of ulcerative colitis and not in clinical trials, underscoring the novelty of our results.

Regarding the impact of supplementation on the inflammatory profile, in the present study, no significant changes were found in the plasma levels of the investigated markers (TNF-$\alpha$, IL-22, IL-17, IL-10, and MPO), nor in CalF, reflecting the inflammatory activity of the intestine. These results align with the study by Sadeghi et al. (2020), conducted in patients with UC, where the administration of 1500 mg/day of curcumin for 8 weeks did not cause changes in TNF-$\alpha$ levels [36].

While we acknowledge certain limitations in this study, such as the loss of five patients in the placebo group, the short duration of supplementation (3 months), and the inclusion of patients from a single follow-up center, it is crucial to emphasize that both treatment groups remain representative in relation to the calculated sample size. The brief supplementation period was strategically based on the standard interval between medical consultations in the department itself, and the single follow-up center serves as the state reference for patients with IBD. We employed common markers, enabling effective comparisons with other studies and strengthening the generalizability and applicability of the results.

Despite the higher incidence of heartburn complaints in the Curcumin plus piperine supplemented group, it is noteworthy that no patient reported the necessity to discontinue the supplementation. This suggests that the combination is well-tolerated and does not result in significant adverse effects. This self-reported symptom may be related to curcumin's ability to prominently inhibit the activity of cyclooxygenase-1 and 2, causing effects similar to some nonsteroidal anti-inflammatory drugs. The consequent blockade in prostaglandin synthesis, which acts as cytoprotective to the gastric mucosa, leads to increased acid production and reduced mucus in the stomach [37,38].

## 4. Materials and Methods

### *Study Type and Design*

This randomized, double-blind, placebo-controlled clinical study was conducted at the Coloproctology sector of Hospital Universitário Professor Alberto Antunes, Maceió-AL, Brazil, from July 2021 to March 2023.

Eligible participants were diagnosed with mild to moderate CD or UC, aged ≥18 years, and undergoing conventional drug therapy for IBD (aminosalicylates, immunosuppressants, biological therapy) or without a specific medication prescription for IBD, with preserved kidney and liver function. Exclusion criteria encompassed individuals with one or more of the following conditions: (1) fistulas, abscesses and strictures; (2) extensive resections of the small intestine (< 200 cm of remaining intestine); (3) patients admitted for acute treatment, in serious general condition; (4) cancer; (5) HIV positive; (6) Pregnant and breastfeeding women; and (7) hospitalization for IBD in the last three months. The exclusion of the clinical trial occurred if: (1) gestation; (2) change or withdrawal of conventional dosage/medication for IBD; (3) HIV-positive patients and renal function and compromised liver; (4) patients who voluntarily discontinued supplementation; and (5) hospitalization for IBD during clinical trial. All participants signed an Informed Consent Form.

This study was registered at ensaiosclinicos.gov.br as RBR-89q4ydz on July 20, 2023. It was approved by the Ethics Committee of the Federal University of Alagoas under the process no. 7829516.5.0000.5013, on February 28, 2019.

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*Intervention*

The Curcumin powder, along with piperine, was administered at doses of 1000 mg/day and 10 mg/day, respectively were prescribed to participants who maintained their clinical therapeutic regimen prescribed by a medical professional in the sector. These supplements were encapsulated in two capsules, meticulously designed to minimize color distortion. Patients were instructed to take these capsules after lunch consistently over a 12-week period. The co-administration of Curcumin (doses of 1000 mg/day) or Curcumin + piperine with lipid-containing meals was strategically employed to improve the bioavailability of these antioxidants, thereby facilitating optimal absorption. It is noteworthy that these capsules were gastro-resistant and free from lactose and gluten.

The capsules were prepared by an outsourced compounding pharmacy with Curcumin and piperine powders, depending on the treatment group (produced with gastro-resistant capsules, free from lactose and sucrose). Notably, the placebo capsules replicated all the physical characteristics (color, texture and appearance) of the treatment capsules, and were composed of starch + orange food coloring. The supplementation routine adhered to the standard procedures of the hospital's monitoring and medication delivery department, ensuring consistency in the delivery process to the patients.

*Ingredient Characterization*

The Curcumin and Piperine powder was obtained from the company Fragon® with curcumin making up a total of 98% of the curcuminoids. Chromatographic analysis was performed as previously described [19], with slight modifications. Curcumin and piperine extracts (0.25 mg/mL) and their combination (9.9:0.1 w/w), diluted in acetonitrile, were used to obtain the chromatograms. The mobile phase was composed from deionized water, acidified with phosphoric acid (0.1% v/v) (solvent A) and acetonitrile (solvent B). The chromatogram was obtained with phase A + B (30:70 v/v), in 9 min, with a flow of 1.0 mL/min and temperature of 35 °C and chromatograms were recorded at 345 nm (Supplementary 1).

*Blood Collection and Sample Preparation*

Participants' blood was collected in tubes containing EDTA and centrifuged at 4,000 x g at 4 ºC, for 10 minutes. Subsequently, the plasma was aspirated and stored in aliquots a -80 ºC in a biofreezer for analysis of inflammatory and oxidative stress markers (primary outcome).

*General Date and Anthropometric Measurements*

Socioeconomic data, clinical history, lifestyle and gastrointestinal symptoms (secondary outcome) were collected through a customized questionnaire. Anthropometric assessment was conducted by a trained professional both before and after the intervention period, encompassing measurements weight (kg) and height (m) to calculate the body mass index (BMI).

*Fecal Calprotectin Analysis (CalF)*

CalF levels were assessed using Bühlmann's Quantum Blue® test, according to the manufacturer's information. Elevated CalF values were considered when reached ≥ 200 µg/g of feces, indicative of active disease.

*Inflammation and Oxidative Stress Analyses*

Malondialdehyde (MDA) levels were measured using reversed-phase ion-pair high performance liquid chromatography (HPLC) with ultraviolet detection at 270 nm, expressed as ng/µL [20]. Superoxide dismutase (SOD) activity was quantified using the SOD Assay Kit per the manufacturer's instructions, measured at 450 nm absorbance in a spectrophotometer, and results expressed in U/µL. Hydrogen peroxide ($H_2O_2$) was measured according to the protocol established

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by Pick e Keisari (1980), and the results read at 610 nm and expressed as ng/μL [21]. Catalase (CAT) activity was determined following the colorimetric method adapted by Aebi (1974), monitored at 240 nm and was expressed in U/μL [22].

Myeloperoxidase (MPO) activity was measured by adapting the previously proposed method by Bradley et al. (1982), and results were expressed in U/μL. One unit of MPO was defined as the amount of enzyme required to decompose 1 μmol $H_2O_2$ [23].

Levels of tumor necrosis factor alfa (TNF-α), interleukin 17A (IL-17A), IL-22 and IL-10) were determined by enzyme-linked immunosorbent assay (ELISA), using the PeproTech® kit, according to the manufacturer's instructions. Absorbance reading was performed at 450 nm in an ELISA plate reader, and the results were expressed in pg/μL.

Furthermore, as a secondary outcome, the effect of curcumin supplementation, with or without piperine, was assessed concerning the primary gastrointestinal symptoms in patients with IBD.

*Sample Size*

The decision to explore the impact of curcumin supplementation on SOD levels was influenced by studies conducted within our research group, specifically in animal models. This choice was made because there is a lack of human studies, both randomized controlled trials (RCTs) and non-RCTs, that have assessed the effects of curcumin or curcumin + piperine on oxidative stress markers in individuals with inflammatory bowel disease (IBD). The relevant experimental clinical trial, on which the sample size calculation was based, is available in the thesis repository of the Universidade Federal de Alagoas at the following link: https://www.repositorio.ufal.br/handle/riufal/5281. The rationale behind using this study was driven by the absence of existing research in human subjects, making our animal studies a valuable reference. Considering this information and the patient population registered at the Coloproctology outpatient clinic at Hospital Universitário Professor Alberto Antunes (HUPAA), a minimum sample size of 19 patients in each group was calculated, aiming for 80% power to detect a significant difference between groups at a 5% significance level. Notably, HUPAA serves as the primary referral center for individuals with IBD in the state of Alagoas. The sample size calculation was based on the data of the 200 registered patients at the hospital's Coloproctology outpatient clinic.

*Randomization, Blinding and Allocation*

The participants' randomization process took place using the "runif" command of the "R" software (v. 4.1.3, The R Project Team, Vienna, Austria). A sequence of random numbers between 0 and 2 was requested to generate the list with a random sequence. This list was kept by a researcher without contact with the participants. They were included sequentially to guarantee the allocation concealment. By simple rounding of the generated random numbers, participants assigned with the number "0" were placed in the placebo group, those assigned with the number "1" in the Curcumin experimental group, and those assigned with the number "2" in the Curcumin + piperine experimental group. An independent evaluator, who had no contact at any point with the participants and researchers of the study, was responsible for conducting the statistical analyses.

*Statistical Analysis*

All analyses were conducted using the Statistical Package for Social Science (SPSS®), version 26.0 (SPSS, Chicago, IL, USA). Descriptive results were expressed as mean and standard deviation (SD) or median (interquartile range), and frequencies (n and %). Variance homogeneity was assessed using the Levene's test. Variables that were not homogeneous were logarithmized. Delta (Δ = endline -T2 - minus baseline – T1) was calculated only on homogeneous variables. The comparison of categorical outcomes between treatment groups was made using the chi-square test/Fisher's exact test. The comparison of categorical outcomes was made using Generalized estimating equations (GEE), adjusted by sex, age and IBD type. In continuous outcomes, it was utilized ANCOVA test,

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followed by Bonferroni test, adjusted for sex, age, and type of IBD. Results were considered significant with a p-value < 0.05.

## 5. Conclusions

In summary, the findings of this study are unprecedent and highly relevant to the scientific community and individuals with IBD. The results provide new insights into the use of a natural therapeutic strategy and underscore the effectiveness of its combination with piperine, in enhancing the antioxidant effects of Curcumin. This approach offers therapeutic alternatives without significant adverse effects, expanding the possibilities for individualized treatment and supporting the effective continuity of the treatment.

**Supplementary Materials:** The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: curcumin and piperine characterization; Table S2: Gastrointestinal complaints of patients with Inflammatory Bowel Disease according to the treatment group: baseline data (T1) and endline data (T2).

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**Institutional Review Board Statement:** This study was registered at ensaiosclinicos.gov.br as RBR-89q4ydz on July 20, 2023. It was approved by the Ethics Committee of the Federal University of Alagoas under process no. 7829516.5.0000.5013 on February 28, 2019.

**Informed Consent Statement:** All participants in this study signed the Informed Consent Form (ICF).

**Data Availability Statement:** This is an unpublished work, not under submission process in any other scientific journal. All data is privately accessible.

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