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TERAPIA ANTIOXIDANTE NA DOENÇA INFLAMATÓRIA
INTESTINAL: UMA REVISÃO SISTEMÁTICA COM METANÁLISE
DE ENSAIOS CLÍNICOS RANDOMIZADOS
(<https://doi.org/10.3390/ph16101374>)

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Faculdade de Nutrição da Universidade Federal de
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Graduação em Nutrição.

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RESUMO

O objetivo deste estudo foi avaliar a ação de substâncias usadas no tratamento de doenças inflamatórias intestinais (DII) sobre biomarcadores de estresse oxidativo e níveis de citocinas. Para esta revisão, foi realizada uma busca nas bases de dados PubMed, Science Direct e Scopus publicados até julho de 2023. Foram utilizadas as palavras-chave: “inflammatory bowel disease”, “ulcerative colitis”, “colitis”, “Crohn Disease”, “antioxidant”, “Antioxidant Effects”, “Anti Oxidants”, “Agents, Antiinflammatory”, “Anti Inflammatories”, “therapy”, “treatment”, “stress oxidative,” and “redox imbalance”. Foram elegíveis ensaios clínicos em humanos para a revisão sistemática e ensaios clínicos randomizados (ECRs) para metanálise que avaliassem marcadores de estresse oxidativo (inibição da geração de espécies reativas de oxigênio e nitrogênio - ERONS; ação sobre a defesa antioxidante enzimática ou não enzimática; inibição de dano oxidativo; e efeitos sobre citocinas pró/anti-inflamatórias. Foram encontrados 106 estudos e após a exclusão dos que não atenderam aos critérios de inclusão, 19 foram incluídos na revisão sistemática e 8 na meta-análise (6 para o desfecho capacidade antioxidante total - AOC; 6 para superóxido dismutase – SOD e 5 para peroxidação lipídica analisada através de malondialdeído - MDA). A SOD foi significativamente modulada ($RR = 0,3764$, IC 95% [0,0262 a 0,7267], $p = 0,035$), mas não a AOC ($RR: 0,3424$, IC 95% [0,0334 a 0,7183], $p = 0,0742$) ou MDA ($RR = -0,8534$, IC 95% [-1,9333 a 0,2265], $p = 0,1214$) pelos tratamentos testados. Esses resultados sugerem que a SOD pode ser um marcador importante no tratamento das DII. Sua elevação contribui para reduzir a formação de peroxinitrito, uma importante espécie reativa de nitrogênio que causa danos a diversas macromoléculas, incluindo membrana lipídica, DNA, o que reduziria lesões teciduais e mutações, respectivamente, contribuindo para um melhor prognóstico dos pacientes com DII. Dessa forma, é necessário que potenciais terapêuticas testadas para indivíduos com DII incluam os marcadores de estresse oxidativo, em especial a SOD, para que as abordagens terapêuticas para estes pacientes sejam melhor reconhecidas.

Palavras chaves: colite ulcerativa, doença de Crohn, estresse oxidativo, citocinas.

ABSTRACT

The objective of this study was to evaluate the action of substances used in the treatment of inflammatory bowel diseases (IBD) on biomarkers of oxidative stress and cytokine levels. For this review, a search was carried out in the PubMed, Science Direct and Scopus databases published until July 2023. The keywords were used: “inflammatory bowel disease”, “ulcerative colitis”, “colitis”, “Crohn Disease”, “antioxidant”, “Antioxidant Effects”, “Anti Oxidants”, “Agents, Antiinflammatory”, “Anti Inflammatories ”, “therapy”, “treatment”, “oxidative stress,” and “redox imbalance”. Clinical trials in humans were eligible for the systematic review and randomized clinical trials (RCTs) for meta-analysis that evaluated markers of oxidative stress (inhibition of the generation of reactive oxygen and nitrogen species - ERONS; action on enzymatic or non-enzymatic antioxidant defense; inhibition oxidative damage and effects on pro/anti-inflammatory cytokines. 106 studies were found and after excluding those that did not meet the inclusion criteria, 19 were included in the systematic review and 8 in the meta-analysis (6 for the outcome total antioxidant capacity - AOC; 6 for superoxide dismutase - SOD and 5 for peroxidation lipid analyzed using malondialdehyde - MDA). SOD was significantly modulated (RR = 0.3764, 95% CI [0.0262 to 0.7267], p = 0.035), but not AOC (RR: 0.3424, 95% CI [0.0334 to 0.7183], p = 0.0742) or MDA (RR = - 0.8534, 95% CI [-1.9333 to 0.2265], p = 0.1214) for the treatments tested. These results suggest that SOD may be an important marker in the treatment of IBD. Its elevation contributes to reducing the formation of peroxynitrite, an important reactive nitrogen species that causes damage to several macromolecules, including lipid membrane and DNA, which would reduce tissue damage and mutations, respectively, contributing to a better prognosis of patients with IBD. Therefore, it is necessary that potential therapies tested for individuals with IBD include markers of oxidative stress, especially SOD, so that therapeutic approaches for these patients are better recognized.

Keywords: ulcerative colitis, Crohn’s disease, oxidative stress, cytokines.

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1. APRESENTAÇÃO

As doenças inflamatórias intestinais (DII) são desordens crônicas que afetam o trato gastrointestinal (TGI), representadas principalmente pela doença de Crohn (DC) e colite ulcerativa (CUI). São caracterizadas por períodos de ativação e remissão dos sintomas, que incluem dor abdominal, diarreia e sangramento retal, causando impacto importante sobre o estado nutricional e qualidade de vida (Silva et al., 2010).

A DC pode acometer qualquer parte do TGI, desde a boca até o ânus, com lesões segmentadas que atingem todas as camadas histológicas. A CU, limita-se ao cólon e suas lesões são contínuas e progridem de forma ascendente, acometendo apenas a camada mucosa e submucosa (Feuerstein; Cheifetz, 2017; Gajendran et al., 2018).

Mundialmente, a prevalência de DII é de 396 casos por 100.000 habitantes, principalmente na Europa, América do Norte, Oriente Médio e Ásia. No Brasil, estudos regionais apontam para uma crescente incidência e prevalência (Molodecky et al., 2012). Tanto a DC, quanto a CU têm distribuição de idade e sexo semelhantes (Maranhão et al., 2015).

Não está totalmente elucidado a causa das DII, mas sabe-se que fatores genéticos, imunológicos, microbiológicos e ambientais como o tabagismo, o alto consumo de alimentos ultraprocessados e o estresse oxidativo estão envolvidos nas manifestações clínicas da doença (Tomasello et al., 2016).

O estresse oxidativo advém de uma desregulação entre a produção de espécies reativas de oxigênio e nitrogênio (ERONs) e capacidade do organismo combatê-las, através do sistema de defesa antioxidante, que atua por meios enzimáticos como a superóxido dismutase (SOD), a catalase (CAT) e a glutathione peroxidase (GPx), dentre outras, e não enzimáticos como a glutathione reduzida (GSH), vitamina E (alfa tocoferol) e vitamina C (ácido ascórbico) (Basílio; Santos; Branco, 2021). Estudos científicos apontam que há uma relação entre o estresse oxidativo e a progressão da patogênese das DII devido a inflamação crônica da mucosa intestinal que contribui com a diminuição dos antioxidantes no organismo (Tian et al., 2017; Bourgonje et al., 2019; Bourgonje et al., 2020).

O tratamento atual para a DII tem como principais representantes os medicamentos como o Ácido 5-aminosalicílico (5-ASA), corticosteróides e imunossupressores, porém esses fármacos apresentam ação limitada, podendo levar os pacientes a um efeito refratário, além disso diversos efeitos colaterais são relatados, podendo causar uma dependência

medicamentosa além de sintomas como náuseas, vômitos, azia e diarreia (Seyedian et al., 2019).

Deste modo se faz necessário investigar novas terapias que apresentem menos efeitos colaterais, sendo o estresse oxidativo e a inflamação crônica fatores que contribuem para o desenvolvimento e progressão da doença, nosso trabalho tem o objetivo de avaliar a ação de tratamentos da DII sobre biomarcadores de estresse oxidativo e citocinas.

2. REVISÃO DE LITERATURA

2.1 DOENÇAS INFLAMATÓRIAS INTESTINAIS

As DII são um conjunto de doenças que se caracterizam por uma inflamação crônica no TGI e têm como principais sintomas dor abdominal, diarreia e sangramento retal que podem vir acompanhados de perda de peso e febre. Os principais tipos são representados pela DC e CU (Silva et al., 2010).

A DC pode acometer qualquer porção do TGI, desde a boca ao ânus, sendo o íleo terminal a região mais afetada, com padrões de lesões transmuralis e descontínuas. Já a CU se limita ao cólon, causando uma inflamação difusa e inespecífica na mucosa e submucosa, com lesões contínuas e ascendentes (Maranhão et al., 2015).

Um estudo avaliou a prevalência e a incidência das DII no Brasil. Os dados demonstraram um aumento significativo na incidência de CUI e DC durante os anos do estudo, exceto em 2012 e 2013, em que a prevalência de DC se manteve estável ($P=0,99$). A incidência da DC foi de 0,66/100.000 habitantes em 2010 para 3,34/100.000 habitantes em 2019, enquanto a CUI foi de 1,34/100.000 habitantes para 10,43/100.000 habitantes em 2019. Durante o mesmo período, houve um notável aumento na prevalência, com os números passando de 5,29 casos por 100.000 habitantes em 2014 para 15,84 casos por 100.000 habitantes em 2019 para a DC e de 11,90 casos por 100.000 habitantes para 42,99 casos por 100.000 habitantes em 2019 para a CUI (Renuzza et al., 2022).

No Brasil, um estudo epidemiológico realizado entre os meses de janeiro de 2009 e novembro de 2019 observou que em 2018 ocorreu o maior número de internações por estas doenças, tendo a maior prevalência na região sudeste do Brasil. Já em relação a idade, a maior prevalência deu-se no grupo etário entre 30 a 39 anos, sendo observada uma maior prevalência em indivíduos do sexo feminino tanto para a DC, quanto para CU, enquanto

outros estudos apontam que apenas na DC a maior prevalência é em indivíduos do sexo feminino (Brito et al., 2020).

Não está totalmente evidenciado a causa das DII, mas sabe-se que fatores genéticos, imunitários, microbiológicos e ambientais como o tabagismo, o alto consumo de alimentos ultraprocessados e o estresse oxidativo estão envolvidos na manifestação da doença (Tomasello et al., 2016).

O diagnóstico é realizado por meio da colonoscopia, que é o padrão ouro, assim como sua classificação em DC e CU. Outro método que pode auxiliar no diagnóstico é o exame laboratorial com anticorpos, *antineutrophil cytoplasmic antibodies* (ANCA), que estão presentes em cerca de 60% dos pacientes com colite e em 40% dos pacientes com DC (Sairenji et al., 2017; Flynn; Eisenstein, 2019).

Os pacientes portadores de DII, tendem a apresentar marcadores inflamatórios elevados como a proteína C reativa (PCR), a velocidade de sedimentação de eritrócitos e a calprotectina fecal (CalF). A CalF constitui um marcador direto da inflamação da mucosa intestinal, e vem se mostrando uma excelente ferramenta rápida e não invasiva para avaliar a atividade inflamatória da doença e o risco de recidivas (Cabral; Abby, 2014).

O tratamento atual, tem como objetivo controlar a inflamação e induzir o paciente a fase de remissão, onde os principais tipos de medicamentos são os 5-ASA, corticosteróides e imunossupressores e imunorreguladores. A utilização desses medicamentos por longo prazo pode causar dependência, assim como os pacientes podem desenvolver um efeito refratário, ou seja, o medicamento passa a não apresentar o efeito desejado, além disso outros sintomas podem aparecer como diarreia, vômitos e náuseas. Com base nessas limitações dos medicamentos, justificando assim, a busca por outras formas de tratamentos, como as terapias antioxidantes (Seyedian et. al., 2019).

2.2 PAPEL DO ESTRESSE OXIDATIVO E DA INFLAMAÇÃO NA ETIOPATOGENESE DAS DOENÇAS INFLAMATÓRIAS INTESTINAIS

A produção de espécies reativas de oxigênio (EROs) e espécies reativas de nitrogênio (ERNs) é um processo comum no organismo humano, durante a oxidação aeróbica, essas espécies são produzidas no organismo com o intuito de realizar transferências de elétrons, mas também estão envolvidas em processos como fagocitose, sinalização intercelular, regulação do crescimento celular e etc, que geralmente ocorrem na mitocôndria, membrana celular e citoplasma. Os principais EROs são o radical superóxido (O_2^-), o radical hidroxila

(OH \cdot), radical hidroperoxila (HO $_2\cdot$) e o peróxido de hidrogênio (H $_2$ O $_2$), dentre os principais ERNs destacamos o peroxinitrito (ONOO \cdot), trióxido de nitrogênio (N $_2$ O $_3$) e radical óxido nítrico (NO \cdot) (Ferreira; Matsubara, 1997; Koury; Donangelo, 2003; Barbosa et al., 2010).

O organismo humano apresenta alguns meios de combater esses radicais livres, através do sistema antioxidante, esse sistema é dividido em enzimático e não enzimático. Entre os componentes do sistema enzimático, destacam-se as enzimas superóxido dismutase (SOD), a catalase (CAT) e a glutathione peroxidase (GPx). A SOD é uma enzima que apresenta três isoformas, a SOD citosólica, a SOD mitocondrial e a SOD extracelular, responsável por catalisar a reação que converte o O $_2\cdot^-$ em H $_2$ O $_2$, um composto menos reativo, mas que em condições como altas concentrações de ferro no organismo, o peróxido de hidrogênio passa a ser convertido no radical hidroxila que é altamente reativo (Ferreira; Matsubara, 1997; Barbosa et al., 2010).

A CAT, é uma enzima que pode ser encontrada no sangue, medula óssea, mucosas, rins e fígado, essa enzima irá atuar catalisando a reação que terá o H $_2$ O $_2$, convertendo essa molécula em água (Mayes, 1990; Barreiros et al., 2006). A GPx irá catalisar a mesma reação que a CAT, visando diminuir os danos que o H $_2$ O $_2$ pode vir a causar. Essa enzima existe em duas formas, podendo ser encontrada no citoplasma e na mitocôndria (Ferreira; Matsubara, 1997; Barbosa et al., 2010).

Em relação às formas não enzimáticas, geralmente estão associadas a elementos vindos da alimentação, alguns nutrientes irão apresentar atividade antioxidante, no qual podemos destacar as vitaminas C, A e E. Além dos minerais selênio, zinco e cobre (Ferreira; Matsubara, 1997).

Quando ocorre uma maior produção de ERNs e/ou uma diminuição das defesas antioxidantes do nosso organismo, surge então o EO. Esse EO pode ocasionar lesões à membrana celular, proteínas e DNA, impedindo que realize suas funções normais e favorecendo o aparecimento de diversas doenças, incluindo as DIIs (Leite et al., 2012).

Sabe-se que a patogênese das DII envolve um complexo desequilíbrio entre componentes genéticos, imunológicos e ambientais, resultando em uma resposta imunológica anormal contra a microbiota intestinal (Fiocchi, 1998). Esses fatores causam uma desregulada, contínua e exacerbada resposta inflamatória, envolvendo o EO, em indivíduos geneticamente predispostos.

O quadro de inflamação da mucosa, as células do epitélio intestinal, neutrófilos e macrófagos, produzem O $_2\cdot^-$ e NO \cdot , ativando assim as enzimas NOX e óxido nítrico sintase

induzível (iNOS), essa ativação é induzida por citocinas inflamatórias. As células do epitélio intestinal deste modo produzem mais EROs via ativação de NOX e iNOS. Essa sobrecarga de EROs pode danificar proteínas do citoesqueleto e levar a alterações nas junções de oclusão e permeabilidade epitelial no epitélio intestinal, ocasionando uma ruptura da barreira. Desta forma, o estresse oxidativo estimula a resposta inflamatória do epitélio intestinal e vice-versa, contribuindo para o aparecimento e progressão das DII (Bhattacharyya et al., 2014; Tian et al., 2017).

Algumas das principais enzimas produtoras de EROs no organismo são NADPH oxidase (NOX), xantina oxidase (XO), lipoxigenases (LOXs), mieloperoxidase (MPO), óxido nítrico sintase (NOS) e ciclooxygenases (COXs). As XOs, assim como as NOXs são enzimas produtoras de O_2^- , enquanto a primeira apresenta ação na mucosa intestinal, e em situações de desequilíbrio redox pode levar a lesões no trato gastrointestinal, contribuindo para o quadro inflamatório da DII, a segunda tem sido relacionada como fator de risco para as DIIs. Já a MPO contribui para a progressão de neoplasias em pacientes com CU (Tian et al., 2017; Basílio et al., 2021).

Dentre os diversos mediadores envolvidos nesse processo, as citocinas têm sido amplamente estudadas por seu papel fundamental na regulação da inflamação intestinal. Essas moléculas sinalizadoras desempenham um papel crucial na modulação da resposta imune, recrutamento de células inflamatórias e manutenção da inflamação crônica observada nas DII (Rogler; Andus, 1998).

As citocinas pró-inflamatórias, como a TNF- α , a IL-1 e a IL-6, têm sido implicadas na indução e amplificação da resposta inflamatória nas DII (Xavier; Podolsky, 2007). Essas citocinas promovem a ativação e recrutamento de células inflamatórias, a produção de moléculas pró-inflamatórias e a destruição do tecido intestinal (Xavier; Podolsky, 2007). Por outro lado, citocinas anti-inflamatórias, como a interleucina-10 (IL-10) e a interleucina-22 (IL-22), desempenham um papel na regulação negativa da inflamação e na manutenção da integridade da barreira intestinal (Sands, 2007; Khor; Gardet; Xavier, 2011).

É nesse cenário que as recentes terapias para DII têm se concentrado, visando o bloqueio de citocinas específicas como estratégias. Inibidores do TNF- α , como o infliximabe e adalimumabe, têm sido amplamente utilizados no tratamento da DC e CU, demonstrando eficácia na indução e manutenção da remissão clínica (Sands, 2007; Torres et al., 2009; Feagan et al., 2016; Torres et al., 2017). Porém, apresenta também suas limitações como já discutido.

Nesse sentido, o entendimento dos mecanismos de ação dos biomarcadores de desequilíbrio redox e das citocinas no contexto das DII, é essencial para o desenvolvimento de novas estratégias terapêuticas e identificação de alvos terapêuticos potenciais. A manipulação das citocinas, seja através do bloqueio de citocinas pró-inflamatórias ou da estimulação de citocinas anti-inflamatórias, representa uma abordagem promissora para o manejo das DII e melhoria da qualidade de vida dos pacientes.

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4. ARTIGO CIENTÍFICO

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel diseases (IBD), are currently recognized as a significant global public health concern. In 2019, the Global Burden of Disease (GBD) Study reported approximately 4.9 million IBD cases worldwide, with the highest prevalence rates found in China and the United States ^[1]. Despite the GBD study reporting an increase in the number of deaths and disability-adjusted life-years (DALY), which is an index of the overall disease burden, representing the loss of one year of full health, age-standardized indicators have shown a significant reduction when compared to the prevalence identified in the 1990s. The enhancement of patients' quality of life in IBD is primarily credited to advances in new biological therapies, specialized medical practices, and multidisciplinary treatment strategies ^[2].

Building upon this positive impact of multidisciplinary treatment approaches, there is a growing interest within the scientific community to identify alternative therapies that can help minimize the characteristic signs and symptoms of the disease. Among these therapies, the use of antioxidants, natural or synthetic, has gained attention due to their promising effects, particularly in animal models ^[3].

Oxidative stress, characterized by an imbalance between pro-oxidants and antioxidants favoring the former, plays a critical role not only in the development of IBD but also in the exacerbation of their signs and symptoms. This imbalance can result in damage to macromolecules and is graded on an intensity scale ranging from eustress (physiological stress) to distress (excessive and toxic oxidative burden)^[4]. The detrimental effects of oxidative stress in IBD is manifested through a range of symptoms including diarrhea, weight loss, ulceration, and even colorectal cancer (CRC) ^[5].

To assess these effects, several clinical trials have investigated substances with potential antioxidant and anti-inflammatory activity, as observed in experimental studies, in patients with IBD ^[6-10]. However, only a few studies have evaluated their impact on redox imbalance and cytokine profiles. In this context, the present systematic review with meta-analysis aims to determine the efficacy of antioxidant substances in modulating biomarkers of oxidative stress and pro- and anti-inflammatory cytokines in individuals with IBD. By summarizing the existing evidence, this study aims to offer valuable insights into the

potential advantages of antioxidant treatments in IBD management and contribute to the development of targeted interventions for this complex and debilitating condition.

2. Methods

2.1. Search Strategy and Selection of Studies

The search was conducted until July 2023 in the following databases: MEDLINE (via PubMed), Science Direct, and Scopus. The following keywords were used: “inflammatory bowel disease”, “ulcerative colitis”, “colitis”, “Crohn Disease”, “antioxidant”, “Antioxidant Effects”, “Anti Oxidants”, “Agents, Antiinflammatory”, “Anti Inflammatories”, “therapy”, “treatment”, “stress oxidative,” and “redox imbalance.” Boolean operators “OR” and “AND” were used adjusted according with database . All records retrieved had their titles and abstracts evaluated. Then, we evaluated titles for the removal of duplicate records. A similar search was used for the other two electronic databases. Some filters, referring to randomized trials and clinical trials and the number of humans available in each database, were used. To minimize result bias, the reference lists of relevant articles were manually searched to identify any missed publications. We included full articles that satisfied the inclusion and exclusion criteria.

2.2. Eligibility of Clinical Research

2.2.1. Clinical Studies

Human studies with participants of both sexes, diagnosed with UC or DC, examined the effects of oral consumption of antioxidants/drugs on oxidative stress and/or cytokine markers. There was no restriction on age, the severity of the disease (mild, moderate, or severe), or the location of the intestinal lesion (proximal or distal). Studies were excluded if they evaluated pregnant or lactating women and participants with other associated comorbidities, such as diabetes and hepatic, kidney, and autoimmune diseases.

2.2.2. Meta-Analysis

Randomized Clinical Trial (RCT) with participants of both sexes, aged 18 years or older, diagnosed with UC or DC, and oral consumption of antioxidants/drugs on oxidative stress and/or cytokines markers. There was no restriction on the severity of the disease (mild, moderate, or severe) or the intestinal lesion location (proximal or distal). Studies were excluded if they evaluated pregnant or lactating women and participants with other associated comorbidities, such as diabetes and hepatic, kidney, and autoimmune diseases.

This Systematic Review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) n° CDR42022335357.

2.3. Data Extraction

23.1. Clinical Studies

IBD clinical situation; number of randomized individuals (n)/age (years); intervention; dose and time of intervention; oxidative stress markers and cytokines effect.

2.3.2. Meta-Analysis

RCTs included in the meta-analysis were required to provide data on oxidative stress or cytokine biomarkers. The mean values of the biomarkers were then normalized by their standard deviation (SD) to standardize the data and reduce discrepancies resulting from different analytical methods. For studies that presented data using the standard error of the mean (SEM), the values were recalculated to the standard deviation (SD) for uniformity in the normalization process. Meta-analyses were conducted to assess the levels of superoxide dismutase (SOD), malondialdehyde (MDA), and antioxidant capacity.

2.3.3. Assessment of the Risk of Bias

The risk of bias of the randomized clinical trials (RCT) included was evaluated according to the Cochrane risk of bias tool. The risk of bias was independently assessed in six domains: random sequence generation, allocation concealment, blinding of participants and professionals, blinding of outcome assessors, incomplete outcomes (intention-to-treat or per-protocol analysis), and selective outcome reporting. All studies that did not present a registered clinical protocol were classified as high-risk of bias in the “selective outcome

report” domain. For non-randomized controlled studies, the ROBINS-I tool was used in seven domains: confounding, participant selection, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result.

2.3.4. Statistical Analysis

As all the metaanalyzed variables were categorized, the relative risk (RR) between groups for each variable was calculated for each study. Study weights were assigned according to the inverse variance method, and calculations were based on a random-effects model. An alpha value of 0.05 was adopted.

Statistical heterogeneity among the studies was tested using the Cochran Q test, and inconsistency was assessed using I² statistics. Whenever a result showed heterogeneity, it was explored by repeating the analysis with the removal of one study at a time to assess whether a particular study explained the heterogeneity. All analysis were conducted using the Jamovi® 2.3.26 program.

3. Results

3.1. Search Results

In this systematic review with meta-analysis, a total of 19 studies were identified according to the predefined inclusion criteria (Figure 1). Among them, 9 studies (47.3%) focused solely on patients with UC^[11-19], 6 studies (31.6%) included only patients with DC^[20-25], and 4 studies (21.0%) encompassed both diseases (CD and UC)^[26-29]. Most of the studies (n = 17; 89.5%) involved adult patients, while 2 studies (11.5%) were specifically conducted on children and adolescents^[24, 25]. The selected studies exhibited diverse designs, with 15 studies (78.9%) being double-blind, placebo-controlled randomized trials^[11,12,14-23,26-28].

A wide range of substances were tested in the included studies, including micronutrients such as antioxidant vitamin complexes^[21] or isolated vitamins^[13,22,29], zinc^[20], the amino acid glutamine^[23], functional foods such as flaxseed^[14], and omega-3 fatty acids^[11,17,20]. The polyphenols^[12,24] or polyphenol-rich foods^[16, 19], plant extracts^[15,26,27] and probiotics^[28] were also investigated. Co-enzyme Q10 and azathioprine—a traditional medication used in the treatment of IBD—were investigated by^[18,25] (Table 1).

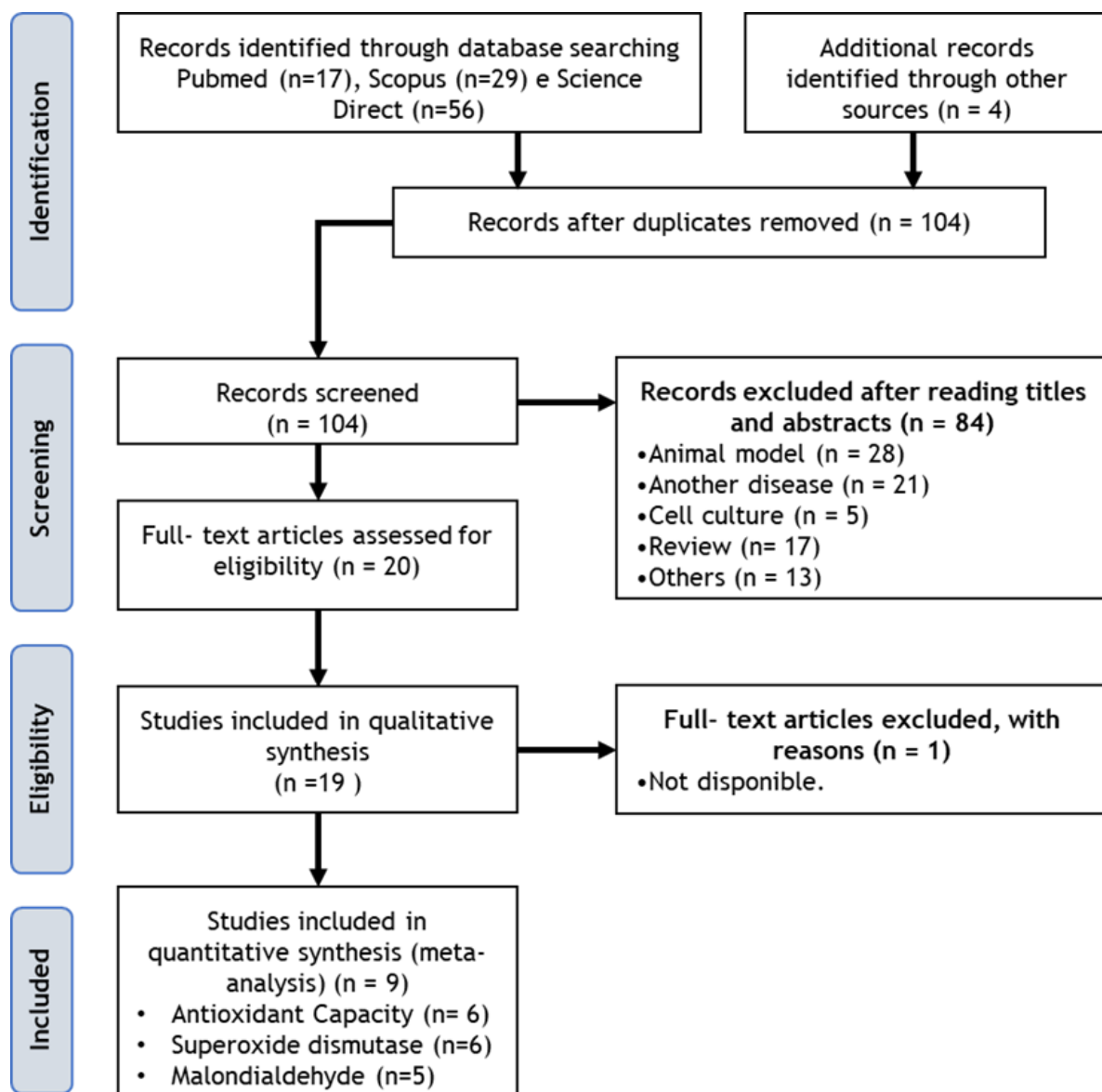


Figure 1. Flow diagram of study selection.

Table 1. Therapy for inflammatory bowel disease and its effects on biomarkers of oxidative stress and levels of pro- and anti-inflammatory cytokines

Authors, Year	IBD	Study	Intervention	Dose and Time of Intervention	Group Subjects (n) and Age [Mean \pm SD/SEM or Median (IQ)]	Oxidative Stress Markers					Cytokines	General Effects
						SOD	GPX	AOC	LP[M1]	Others		
Mulder et al., (1994) [20]	Inactive to moderately active CD	Randomized, double blind, placebo control	Zinc aspartate	300 mg For 4 weeks	Placebo: n = 22; age = 38 y (23–55) Intervention: n = 14; age = 42 y (22–47)	NS						No changes were found in the plasma and erythrocyte Metallothionein
Geerling et al., (2000) [21]	Remission CD	Randomized, double blind, placebo control	Intervention 1 (I1): Antioxidants intervention 2 (I2): AO complex + omega 3 (n-3)	For 12 weeks	Placebo: n = 8; age = 38 y (30–61) I1: n = 8; age = 43 y (33–52) I2: n = 9; age = 41 y (31–56)	I1 (\uparrow) I2 (\uparrow)	I2 (\downarrow)	NS				AO + n-[M2] 3 – decreased the proportion of arachidonic acid, and increased the proportion of eicosapentanoic acid and docosahexanoic acid in both plasma phospholipids and adipose tissue
Aghdassi et al., (2003) [22]	Remission CD	Randomized double blind, placebo control	Vit C + Vit E	Vit C: 1000 mg/d + Vit E: 800 UI/d For 4 weeks	Placebo: n = 29; age = 36.5 y \pm 1.7 61) Intervention: n = 28; age = 38.3 y \pm 2.9				\downarrow			Did not alter disease activity
Barbosa et al., (2003) [11]	Mild or moderate active UC	Randomized, cross-over, placebo control	Omega 3	4.5 g/d (90 mg of EPA + 60 mg of DHA) For 8 weeks	Placebo: n = 9; age = not informed Intervention: n = 9; age = 40 y \pm 11	NS		\uparrow	NS	Catalase: NS		Did not alter laboratory indicator or sigmoidoscopy or histology scores;

Ballini et al., (2019) [28]	DC or UC	Randomized, double blind, placebo control	Hyperbiotics Pro-15 Probiotics	12 weeks	Placebo: n = 20; age = 30–60 y Intervention: n = 20; age = 30–60 y				D-rom: ↓	↑ antioxidant defense
Akobeng et al., (2006) [23]	Active CD	Randomized, double blind, placebo control	Glutamine enriched polymeric diet	Placebo: Polymeric diet; Treatment: glutamine-enriched polymeric diet (42% of amino acid composition) For 4 weeks	Placebo: n = 8; age = 10.5 y ± 2.7 Intervention: n = 7; age = 12.2 y ± 2.8			NS		Did not alter plasma antioxidant concentrations
Kolacek et al., (2013) [24] [FM3]	Remission CD	Pilot	Pycnogenol	2 mg/d For 12 weeks	Healthy control: n = 15; age = 13.9 y ± 2.0 CD patients: n = 14; age = 16.3 y ± 1.5	NS	NS		NS	Serum AOC negatively correlated with disease activity and with CRP and fecal calprotectin
Samsamikor et al., (2016) [12]	Active mild to moderate UC	Randomized double blind, placebo control	Resveratrol	500 mg/d For 6 weeks	Placebo: n = 28; age = 38.8 ± 11.6 Intervention: n = 28; age = 37.4 y ± 16.5	↑	↑		↓	↓ severity of disease activity and ↑ the quality of life
Nematgorgani et al., (2017) [26]	Mild or moderate DC and UC	Randomized double blind, placebo control	<i>Urtica dioica</i> leaf extract	400 mg For 12 weeks	Placebo: n = 29; age = 38.3 y ± 13.3 Intervention: n = 30; age = 36.6 y ± 10.9	↑				↓ hs-CRP and platelet count; ↑ the quality of life; Did not alter levels of WBC and ESR
Papada et al., (2018) [27]	Remission DC and UC	Randomized double blind, placebo control	<i>Pistacia lentiscus</i>	2800 mg/d For 12 weeks	Placebo: n = 27; age = 45 y ± 17.4 Intervention: n = 33; age = 38.2 y ± 11.9		↑		Ox-LDL : ↓	↓ oxLDL/HDL, oxLDL/LDL and oxLDL/LDL

Karimi et al., (2019) [13]	Active mild to moderate UC	Randomized double blind	Vitamin D	Intervention 1: 1000 UI/d (I1) Intervention 2: 2000 UI (I2) For 12 weeks	I1: n = 22; age = 39.7 y ±15.6 I2: n = 24; age = 34 y ± 12.5	NS					IL-6 and IFN-γ: GF and FO (↓)	High dose group: ↑ the quality of life and ↓ severity of disease activity
Morshedzadeha et al., (2019) [14]	UC	Randomized double blind, placebo control	Intervention 1: Grounded flaxseed (GF) Intervention 2: Flaxseed oil (FO)	GF: 30,000 mg/d FO: 10,000 g/d For 2 weeks	Placebo: n = 25; age = 35.2 y ± 10.6 GF: n = 25; age = 29.9 y ± 9.1 FO: n = 25; age = 32.2 y ± 9.9							GF and FO: ↑ TGF-β and the quality of life; ↓ fecal calprotectin, Mayo score, ESR, waist circumference, diastolic and systolic blood pressure
Nikkhah-Bo daghi et al., (2019) [15]	Active mild to moderate UC	Randomized double blind, placebo control	<i>Nigella sativa</i>	2000 mg/d For 6 weeks	Placebo: n = 24; age = 39.2 y ± 11.8; Intervention: n = 24; age = 34.8 y ± 11.2		NS	↓		NFκB: NS	TNF-α: NS	↓ stool frequency score; Did not alter severity of disease activity and the quality of life
Nikkhah-Bo daghi et al., (2019) [16]	Active mild to moderate UC	Randomized double blind, placebo control	Zingiber	2000 mg/d For 12 weeks	Placebo: n = 24; age = 39.2 y ± 11.8 Intervention: n = 22; age = 41.4 y ± 11.4		NS	↓				↓ severity of disease activity; ↑ the quality of life
Abhari et al., (2020) [17]	Active mild to moderate UC	Randomized double blind, placebo control	Omega 3	4300 mg/d For 8 weeks	Placebo: n = 35; age = 69.7 y ±5.0. Intervention: n = 35; age = 69.7 y ±5.5	↑	↑		↓	· Catalase: ↑ · Ox-LDL: ↓	IL-6, IL-2, IL-1α and IL-1β: ↓	Did not alter BMI, waist circumference, diastolic and systolic blood pressure

von Martels et al., (2020) [29]	DC and UC	Prospective	Riboflavin	100 mg/d For 3 weeks	Group 1 (Fecal Calprotectin < 200 µg/g): n = 40; age = 44.2 y ± 11.6 Group 2 (Fecal Calprotectin > 200 µg/g): n = 30; age = 38.8 y ± 13.6					Free thiols: ↑ IL-6, TNF-α and IL-1β: NS IL-2: ↓	↓ severity disease activity, CRP and Enterobacteriaceae; No effects on diversity, taxonomy, or metabolic pathways of the fecal microbiome.
Farsi et al., 2021) [18]	Varying disease activity UC	Randomized double blind, placebo control	Coenzyme Q10	200 mg/d For 8 weeks	Placebo: n = 43; age = 40.2 y ± 11.5 Intervention: n = 43; age = 38.4 y ± 8.8					IL-10: ↑ IL-17: ↓	↓ severity disease activity; ↑ the quality of life and serum levels of cathelicidin LL-37; Did not alter β-defensin 2
Tahvilian et al., (2021) [19]	Active mild to moderate UC	Randomized double blind, placebo control	Saffron	100 mg/d For 8 weeks	Placebo: n = 35; age = 41.0 y ± 11.3 Intervention: n = 40; age = 40.5 y ± 12.7	↑	↑	↑	NS		
Tavassolifar et al., (2021) [25]	Active mild to moderate CD	Longitudinal	Azathioprine	50 mg/d For 12 weeks	Healthy control: n = 15; age = 33.6 y ± 1.2 CD patients: n = 15; age = 31.5 y ± 1.8	Normalized *				GP91PH OX, Nrf2, Catalase —normalized *	↓ severity disease activity

Khazdouz et al., (2023) [30]	Active mild to moderate UC	Randomized double blind, placebo control	Selenium	200 mcg/d 10 weeks	Placebo: n = 50; age = 37.9 ± 10.8 Intervention: n = 50; age = 34.5 ± 11.2		IL-17 ↓ IL-10 (NS)	↓ severity disease activity; ↑ the quality of life
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Legend: * = gene expression; n = total number; ↑ = increased; ↓ = reduced. AOC: Antioxidant capacity; DC: Crohn's disease; ESR—erythrocyte sedimentation rate; GP91PHOX: 91-kD glycoprotein component; GPx: Glutathione peroxidase; HDL: high density lipoprotein IFN-γ: Interferon gamma; IQ: interquartile range; IL: Interleukin; LDL: low density lipoprotein; LP: Lipid peroxidation; MDA: malondialdehyde; NFκB: nuclear factor kappa B; Nrf2: nuclear factor erythroid 2-related factor 2; NS: not significant; Ox-LDL: oxidized low-density lipoprotein; SEM: standard deviation of mean; SD: standard deviation; SOD: Superoxide dismutase; TGF-β: transforming growth factor beta; TNF- α: tumor necrosis factor alpha; UC: Ulcerative colitis; Vit: Vitamin; WBC: white blood cells y: Years; SEM: standard error of the mean.

The period of treatment varied from 2^[14] to 12 weeks^[24, 26-28, 16, 25], with the latter being the most common intervention time, among the evaluated studies.

Regarding the evaluated biomarkers of antioxidant defense, prominent ones included superoxide dismutase (SOD)^[20, 21, 11, 24, 12, 26, 17, 19, 25], glutathione peroxidase (GPX)^[21, 24, 17, 19], and catalase^[11, 17, 25], as well as antioxidant capacity^[21, 11, 12, 27, 13, 15, 16, 19]. Lipid membrane damage (lipid peroxidation - PL) was the most investigated macromolecular damage by the authors^[22, 11, 23, 24, 12, 15-17, 19], while only two studies assessed transcription factors such as factor nuclear kappa B (NFκB)^[15] and nuclear factor erythroid 2-related factor 2 (Nrf2)^[25].

Surprisingly, the anti-inflammatory action mediated by cytokines was not extensively investigated among the studies. Only five studies explored the impact of interventions on cytokines: IL-6^[14, 17, 29], tumor necrosis factor alpha (TNF- α)^[15, 29], IL-2^[17, 29], IL-1b^[17, 29] and IL-10^[29, 18]. Notably, omega-3 supplementation (4,300 mg/d for 8 weeks) reduced IL-1β levels; riboflavin (100 mg/d for 3 weeks) attenuated IL-2 levels (although it did not alter IL-6, IL-10, TNF-α, and IL-1β); and coenzyme-Q10 (200 mg/d for 8 weeks) not only reduced IL-17 levels but also increased the levels of IL-10, known for its anti-inflammatory properties.

Overall, the included studies shed light on the potential effects of various interventions on oxidative stress and inflammatory biomarkers in IBD patients. However, further research is required to fully understand the precise mechanisms and potential clinical implications of these interventions.

3.2. Risk of bias

The risk of bias analysis for the included studies is presented in Tables 2 and 3. Among the fourteen RCT studies included, nine were classified as having a low risk of bias. As for the three non-randomized controlled studies, two were classified as having a low risk of bias, while one was rated as moderate due to certain domains that might potentially influence the results analyzed in this meta-analysis.

Table 2. Bias risk of randomized included studies.

	DOM 1	DOM 2	DOM 3	DOM 4	DOM 5	DOM 6	Overall
Mulder, et al. (1994)[20]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Geerling et al. (2000)[21]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Barbosa et al. (2003)[11]	Unclear	Unclear	Unclear	Low	High	Unclear	Unclear

Aghdassi et al., (2003)[22]	Low	Unclear	Unclear	Unclear	High	High	Unclear
Akobeng et al., (2007)[23]	Low	Low	Low	Low	Low	High	Low
Samsamikor et al. (2016)[12]	Unclear	Unclear	Low	Low	Low	High	Low
Nematgorgani et al. (2017)[26]	Unclear	Low	Low	Low	Low	High	Low
Papada et al. (2018)[27]	Low	Low	Low	Low	Low	Low	Low
Ballini et al. (2019) [28]	Low	Low	Unclear	Unclear	Low	Low	Low
Nikkhah-Bodaghi et al. (2019)[15]	Low	Low	Low	Low	High	Unclear	Low
Nikkhah-Bodaghi et al. (2019)[16]	Low	Low	Low	Low	High	Unclear	Low
Karimi et al., (2019)[13]	Unclear	Low	Low	Unclear	Low	Unclear	Unclear
Morshedzadeh et al., (2019)[14]	Unclear	Unclear	High	Unclear	Low	Low	Unclear
Tahvilian et al., (2020)[19]	Low	Low	Low	Unclear	Low	Low	Low
Abhari et al. (2020) [17]	Unclear	Unclear	High	High	High	High	High
Farsi et al., (2021)[18]	Low	Low	Low	Low	Low	Low	Low
Khazdouz et al., (2023) [30]	Low	Low	Low	Low	Low	Low	Low

Legend: DOM 1: Sequence generation; DOM 2: Allocation concealment; DOM 3: Blinding of participants and professionals; DOM 4: Blinding of outcome assessors; DOM 5: Incomplete outcomes; DOM 6: Selective report.

Table 3. Bias risk of non-randomized included studies

	DOM 1	DOM 2	DOM 3	DOM 4	DOM 5	DOM 6	DOM 7	Overall
von Martels et al., (2020)[29]	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate
Kolářek et al. (2013)[24]	Low	Low	Low	Low	Low	Low	Low	Low
Tavassolifar et al., (2021)[25]	Moderate	Low	Low	Low	Low	Low	Low	Low

Legend: DOM 1: Confounding; DOM 2: Selection of participants into the study; DOM 3: Classification of interventions; DOM 4: Deviations from intended interventions; DOM 5: Missing data; DOM 6: Measurement of outcomes; DOM 7: Selection of the reported result.

3.3. Randomized Clinical Trial: Meta-analysis

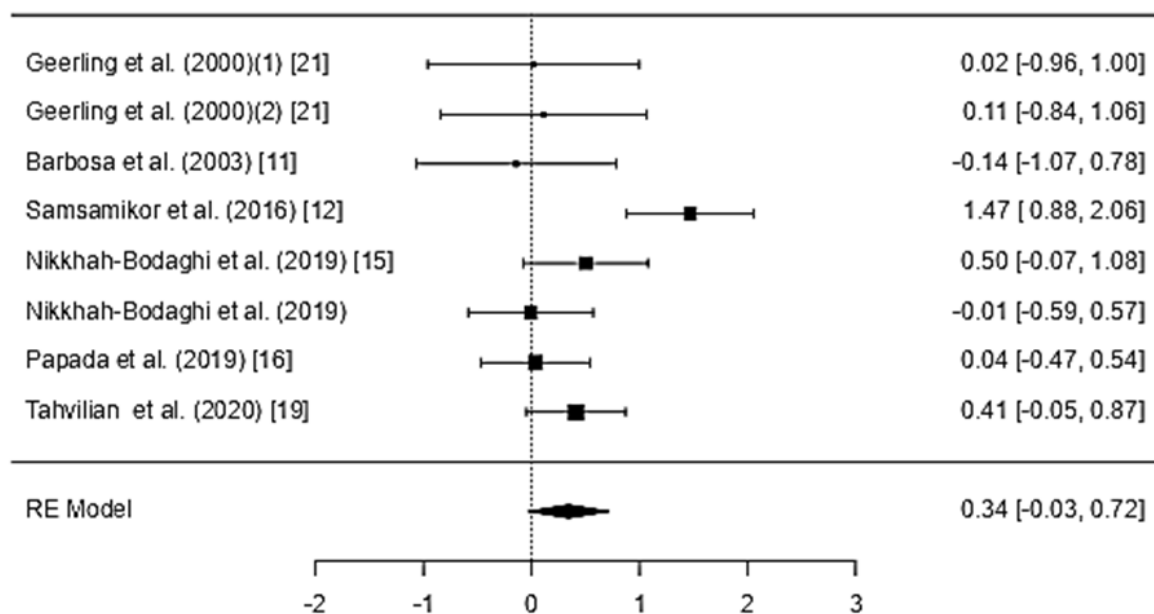
3.3.1. Antioxidant Capacity

Six studies were included in the analysis (the study of Geerling et al. analyzed two interventions group). The study by ^[24] was not included in the meta-analysis due to its inclusion of children and adolescents, which was a criterion for exclusion in this study. On the other hand, the RCT conducted by ^[13], while being an RCT, was not eligible for inclusion

because it did not compare the treatment to a placebo but instead compared two different doses of Vitamin D, making it unsuitable for the treatment versus non-treatment comparison required for this analysis. (Figure 2)

The observed standardized mean differences ranged from -0.1429 to 1.4691, with the majority of estimates being positive (75%). The estimated average standardized mean difference based on the random-effects model was = 0.3424 (95% CI: -0.0334 to 0.7183). Therefore, the average outcome did not differ significantly ($z = 1.7857$, $p = 0.0742$), indicating that there was no protective effect of the antioxidants included in this meta-analysis on the total antioxidant capacity.

According to the Q-test, the true outcomes appear to be heterogeneous ($Q(7) = 18.8116$, $p = 0.009$, $\tau^2 = 0.1749$, $I^2 = 62.7889\%$). A 95% prediction interval for the true outcomes is given by -0.5594 to 1.2443. Hence, although the average outcome is estimated to be positive, in some studies the true outcome may in fact be negative. An examination of the studentized residuals revealed that one study ^[12] had a value larger than ± 2.7344 and may be a potential outlier in the context of this model. According to the Cook's distances, one study ^[12] could be considered to be overly influential. Neither the rank correlation nor the regression test indicated any funnel plot asymmetry ($p = 0.9049$ and $p = 0.4033$, respectively).



Random-Effects Model (n = 8)						
	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	0.342	0.192	1.79	0.074	-0.033	0.718
Nota. Tau² Estimator: DerSimonian-Laird						
Heterogeneity Statistics						
Tau	Tau ²		I ²	H ²	df	Q
0.418	0.1749 (SE=0.1556)		62.79%	2.687	7.000	18.812

Figure 2. Forest plot for antioxidant capacity induced by inflammatory bowel disease therapy, according to a randomized clinical trial included in the meta-analysis Legend: df (Degrees of Freedom); H² (H-squared); Q: heterogeneity test; SE: standard error; Tau² (Tau squared).

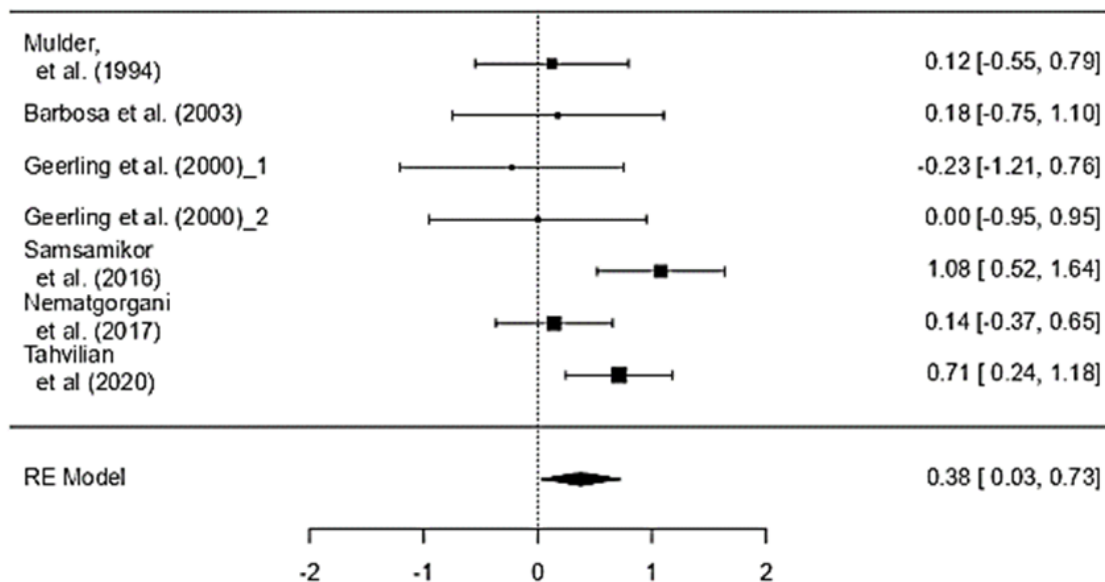
3.3.2. Superoxide Dismutase

Six studies were included in the SOD analysis (the study of ^[21] analyzed two interventions group). The study by ^[17], despite being an RCT, was not included in the meta-analysis due to the absence of standard deviation (SD) data for SOD in its results, which rendered the normalization of the data unfeasible. Similarly, the RCT conducted by ^[25] could not be included because its results were presented graphically without providing mean and SD values (Figure 3).

The standardized mean differences observed varied from -0.2277 to 1.0802, and notably, most of these estimates (71%) were positive. The calculated average standardized mean difference, using the random-effects model, was RR = 0.3764 (95% CI: 0.0262 to

0.7267). Consequently, the average outcome significantly differed from zero ($z = 2.1066$, $p = 0.035$), affirming the protective effect of the therapies included in this meta-analysis on SOD.

The Q-test for heterogeneity was not significant, but some heterogeneity may still be present in the true outcomes ($Q(6) = 11.3631$, $p = 0.0778$, $\tau^2 = 0.1002$, $I^2 = 47.1974\%$). The 95% prediction interval for the true outcomes ranges from -0.3360 to 1.0889. This means that although the estimated average outcome is positive, there is a possibility of negative outcomes in some studies. Examination of the studentized residuals showed that none of the studies had values exceeding ± 2.6901 , indicating the absence of outliers within this model. Cook's distances analysis revealed that none of the studies were excessively influential. Additionally, both the rank correlation and regression tests did not indicate any funnel plot asymmetry ($p = 0.3813$ and $p = 0.0961$, respectively).



Random-Effects Model (n = 7)						
	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	0.376	0.179	2.11	0.035	0.026	0.727
Nota. Tau ² Estimator: DerSimonian-Laird						
Heterogeneity Statistics						
Tau	Tau ²	I ²	H ²	df	Q	p
0.317	0.1002 (SE=0.1262)	47.2%	1.894	6.000	11.363	0.078

Figure 3. Forest plot for superoxide dismutase induced by inflammatory bowel disease therapy, according to a randomized clinical trial included in the meta-analysis. Legend: df (Degrees of Freedom); H² (H-squared); Q: heterogeneity test; SE: standard error; Tau² (Tau squared).

3.3.3. *Malondialdehyde (MDA)*

Five studies were included in the MDA analysis. Just like the meta-analysis for SOD, the study conducted by ^[17], although it was an RCT, was excluded from the meta-analysis. This was because it lacked SD data for MDA in its results, making it impossible to normalize the data for inclusion (Figure 4).

The standardized mean differences observed varied from -3.2454 to 0.6142, with a majority of these estimates (80%) being negative. The estimated average standard-ized mean difference, based on the random-effects model, was $RR = -0.8534$ (95% CI: -1.9333 to 0.2265). Consequently, the average outcome did not exhibit significant differences ($z = -1.5489$, $p = 0.1214$). This suggests that the use of antioxidant therapy did not significantly influence PL, as assessed through MDA levels.

Based on the Q-test, there is evidence of heterogeneity among the true outcomes ($Q(4) = 56.9046$, $p < 0.0001$, $\tau^2 = 1.3929$, $I^2 = 92.9707\%$). A 95% prediction interval for the true outcomes spans from -3.4062 to 1.6994. Consequently, although the average outcome is estimated to be negative, it is possible that in some studies, the true outcome may indeed be positive. An examination of the studentized residuals identified one potential outlier ^[12] with a value exceeding ± 2.5758 within the context of this model. According to the Cook's distances, none of the studies appeared to exert an overly influential effect.

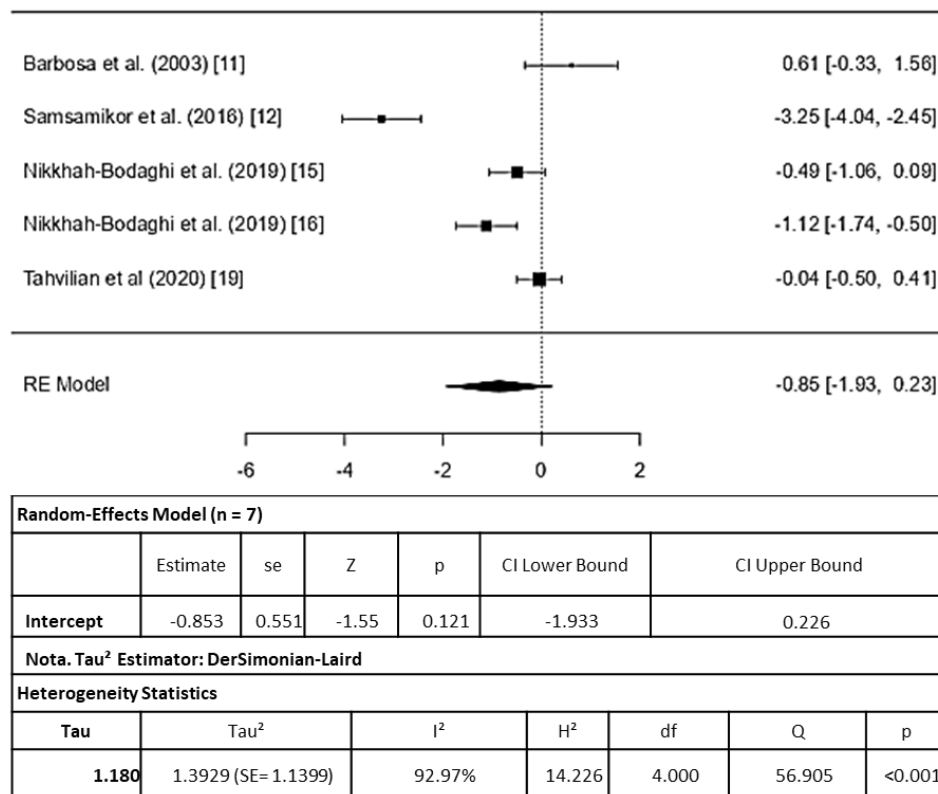


Figure 4. Forest plot for malondialdehyde induced by inflammatory bowel disease therapy, according to a randomized clinical trial included in the meta-analysis. Legend: df (Degrees of Freedom); H^2 (H-squared); Q: heterogeneity test; SE: standard error; Tau^2 (Tau squared).

4. Discussion

4.1. *Antioxidant Capacity*

The analysis of the oxidative stress biomarkers revealed that the serum antioxidant capacity received significant attention in the included articles of the systematic review. This marker, assessed through various techniques such as total antioxidant status (TAS) [21, 24, 13], total antioxidant potential/capacity (TAP/TAC) [11, 31, 12, 13, 15, 16, 19] and total serum oxidizability (TSO) [27], holds particular importance in the context of IBD. It is deemed a primary metric for assessing the extent and capacity of oxidative stress, not just in the context of aging but also in various age-related diseases. However, according to this meta-analysis this antioxidant marker did not undergo modulation and therefore was not influenced by the analyzed antioxidant therapy.

Serum antioxidant capacity, as an essential component of the antioxidant defense system, provides valuable insights into the overall redox balance among individuals with IBD. The human body employs a comprehensive array of mechanisms to combat redox imbalance, acting on reactive oxygen and nitrogen species and effectively repairing damage to macromolecules. This intricate defense system comprises both enzymatic and non-enzymatic endogenous components, with key enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), peroxyredoxin, and non-enzymatic compounds like reduced glutathione (GSH) [32-34].

In addition to the endogenous antioxidant defenses, the body benefits from exogenous antioxidants obtained through dietary sources. These compounds, including α -tocopherol (vitamin E), curcumin, β -carotene, ascorbic acid (vitamin C), flavonoids, selenium, and others, commonly found in fruits, vegetables, and grains [3, 35, 10]. However, when assessing the total antioxidant capacity, most methods estimate the cumulative effect of the enzymatic components of the antioxidant system, disregarding the complexity of endogenous and exogenous non-enzymatic systems.

Nevertheless, when assessing the total antioxidant capacity, it is crucial to consider the complexity of both endogenous and exogenous non-enzymatic systems. A comprehensive

evaluation becomes imperative to gain insights into the redox profile accurately. In this regard, a compelling series of tests conducted by Constantini and Verhulst (2009) highlighted the significance of associating antioxidant capacity with specific markers of oxidative damage to draw reliable conclusions about the redox status across different tissues [36].

4.2. Superoxide Dismutase

According to the data in Table 1, it is evident that 9 studies assessed the activity of SOD. Among them, 6 reported a significant effect of the intervention, involving various antioxidants, such as pycogenol [24], resveratrol [12], *Urtica dioica* [26], omega 3 [17], saffron [19] and the drug azathioprine [25]. Notably, the study by [24], had a focusing on children and adolescents, and [25], which analyzed genic expression, were excluded from the meta-analysis.

A noticeable increase in SOD levels/activity resulting from the use of these antioxidants in patients with IBD was observed. SOD is considered the first line of antioxidant defense and exists in three isoforms: cytosolic or copper-zinc SOD (CuZn-SOD), manganese SOD (Mn-SOD) located in mitochondria, and an extracellular form of CuZn-SOD (EC-SOD) [38]. Its role is to facilitate the conversion of the superoxide anion radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2 - a less reactive oxygen species (ROS), with a longer half-life, which can diffuse through the epithelial barrier and affect neighboring cells [39].

Furthermore, it is noteworthy that H_2O_2 , if not converted into water by the antioxidant enzymes catalase (CAT) and glutathione peroxidase (GPx), and when transition metals like Fe^{2+} are presented, it can swiftly convert into the extremely reactive hydroxyl radical (HO^{\bullet}), through the Fenton and Haber-Weiss reactions. HO^{\bullet} exhibits high reactivity and causes severe damage to macromolecules, including lipid peroxidation and the breakdown of peptide bonds in intercellular junctions, leading to alterations in membrane architecture and fluidity, respectively [40]. As such, it intensifies damage to the epithelial layer and intestinal permeability loss.

This complex interplay highlights the critical role of elevated SOD levels for individuals with IBD, as they often experience compromised cellular barrier integrity and subsequent increased intestinal permeability, allowing luminal antigens, such as pathogenic bacteria and their products, particularly lipopolysaccharide (LPS), to invade the previously sterile lamina propria and submucosa [41-43]. Consequently, the immune response is activated, mediated by cells of innate immunity (neutrophils, macrophages, natural killer cells) and acquired immunity (Th1, Th2, and Th17 lymphocytes), leading to the production of

pro-inflammatory cytokines and reactive species, with emphasis on $O_2^{\cdot-}$ synthesized by the NADPH-oxidase enzymatic complex, which is activated in the presence of neutrophils [44, 39, 45].

4.3. Malondialdehyde

According to this systematic review, eight studies analyzed lipid peroxidation through isoprostane levels^[22, 24], and MDA^[11, 23, 12, 15, 16, 19], enabling a meta-analysis of five RCTs that measured MDA levels. However, the study by^[23] was excluded, due to the inclusion of pediatric participants. The results of the meta-analysis demonstrated that there was no significant modulation of MDA levels by antioxidant therapy compared to the placebo group.

As previously discussed, IBD is characterized by a pronounced infiltration of immune cells into the intestinal tissue, leading to an excessive production of pro-inflammatory molecules and RONS. The primary objective of this immune response is to control microbial activity. Nevertheless, when this response becomes dysregulated, it leads to chronic activation of cellular mediators and transcription factors, such as NFkB, perpetuating the chronic oxidative and inflammatory response, resulting in severe cellular damage, including protein carbonylation, p53 mutation (p53M), DNA damage, and lipid peroxidation (LP)^[46]. LP, one of the most common forms of cellular damage, is particularly generated by nitrogen dioxide radical ($\cdot NO_2$), H_2O_2 , $\cdot OH$, peroxynitrite (ONOO $^-$), and hypochlorous acid (HOCl), which act on polyunsaturated fatty acids (PUFAs) and cholesterol, constituents of the colonic membrane. This process produces lipid-derived products, such as 4-hydroxynonenal (4-HNE), *trans*, *trans*-2,4-decadienal (tt-DDE), and epoxyketo-octadecenoic acid, as well as the widely studied malondialdehyde (MDA)^[3].

A recent review conducted by Lei et al., 2021 reported elevated MDA levels in plasma/serum/tissues of individuals with IBD or UC, confirming the close relationship of this marker with the oxidative/inflammatory damage characteristic of these conditions^[47]. However, the cause-and-effect relationship between LP and these events is not yet fully elucidated, requiring further research efforts from the scientific community^[48].

LP induces cell disruption and is associated with various symptoms of IBD, including diarrhea, ulceration, necrosis, blood loss, anemia, reduced nutrient and water absorption, resulting in weight loss and dehydration^[5]. Therefore, identifying substances that can reduce this process is crucial in determining the effectiveness of an antioxidant compound.

4.4. Oxidative Stress and inflammation mediated by Cytokines

Unfortunately, only four studies ^[14, 15, 29, 30] among those included in this systematic review analyzed cytokine levels, precluding the possibility of conducting a meta-analysis. The studies by [14,29] reported significant reductions in Interleukin-6 (IL-6) and Interferon gamma (IFN- γ) levels, following supplementation with grounded flaxseed and riboflavin, respectively. Additionally, ^[18] found that coenzyme Q10 supplementation significantly altered IL-10 and IL-17 levels, differing from ^[25] who did observe decreased of IL-17 levels, but not of IL-10 in subjects that received selenium for 10 weeks.

The connection between oxidative stress and alterations in pro and anti-inflammatory cytokines in IBD is well-established. RONS function as signaling molecules, recruiting and stimulating effector T lymphocyte differentiation and activating pathways of pro-inflammatory mediators and cytokines (e.g., tumor necrosis factor - TNF- α -, IL-1 β , IL-6, IL-8, IL-17, IL-23, IFN- γ), which have been extensively studied for their crucial role in regulating intestinal inflammation, modulating the immune response, recruiting inflammatory cells, and maintaining the chronic inflammation observed in IBD [49]. They also contribute to the expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and P-selectin ^[50, 44].

In the context of IBD, the nuclear factor kappa B (NF κ B) and active protein 1 (AP-1)/mitogen-activated protein kinase (MAPK) signaling pathways play crucial roles. Present in immune and intestinal epithelial cells, these transcription factors are essential for host homeostasis, immune tolerance, infection control, and tissue repair, by inducing the expression of pro-inflammatory genes, including IL-1 β , IL-6, IL-12, IL-23, nitric oxide synthase inducible (iNOS), cyclooxygenase-2 (COX-2), and TNF- α . However, their dysregulated or excessive activation can contribute to the observed chronic inflammatory response in IBD ^[51].

Both NF κ B and AP-1/MAPK are regulated by growth factors, cytokines, RONS, and pattern recognition receptors (PRRs), especially Toll-like receptor 4 (TLR4), which by stimulation, mainly through binding with lipopolysaccharides (LPS) from gram-negative bacteria, leading to the recruitment of innate and adaptive immune cells (macrophages, lymphocytes, neutrophils). Subsequently, additional quantities of pro-inflammatory cytokines, RONS, chemotactic molecules (e.g., Monocyte Chemoattractant Protein-1— MCP-1), adhesion molecules (Intercellular adhesion molecule 1—ICAM-1—and vascular cell adhesion molecule 1—VCAM-1), and other inflammatory mediators (e.g., eicosanoids,

platelet-activating factor, and matrix metalloproteinases) are generated, while anti-inflammatory genes such as IL-10 and transforming growth factor beta 1 (TGF- β 1) are downregulated [51,52].

Pro-inflammatory cytokines such as TNF- α , IL-1, and IL-6 are known to play a role in initiating and intensifying the inflammatory response in IBD [53]. TNF- α , produced by various immune cells, is involved in the activation and recruitment of inflammatory cells to the intestine, including neutrophils and T lymphocytes [54, 55]. This leads to a chronic inflammatory response in the gastrointestinal tract, resulting in intestinal tissue destruction, ulcers, fistulas, and strictures. Additionally, TNF- α disrupts the intestinal barrier by breaking the integrity of intercellular junctions, allowing antigens and bacteria from the gut lumen to enter the submucosal tissue [56]. This exacerbates the inflammatory response and contributes to its perpetuation. Furthermore, TNF- α stimulates the production of other pro-inflammatory cytokines such as IL-1 and IL-6, creating a positive inflammatory feedback loop that amplifies the immune response and inflammation [57]. Conversely, anti-inflammatory cytokines like IL-10 and IL-22 play a role in negatively regulating inflammation and maintaining intestinal barrier integrity [47].

The role of cytokines in IBD is so significant that various therapies aimed at their inhibition have been investigated. TNF- α inhibitors such as infliximab, adalimumab, and ustekinumab have been widely used in the treatment of CD and UC, demonstrating efficacy in inducing and maintaining clinical remission [48, 53]. Other therapeutic approaches targeting cytokines, such as interleukin-12/23 and interleukin-23 blockers, have also shown significant clinical benefits in IBD patients [54, 55].

On the other side, the prolonged use of these drugs resulted in side effects that limit their effectiveness and adherence, ranging from mild symptoms such as nausea and vomiting to severe conditions like insulin resistance and hepatic toxicity. In addition to their high cost, some IBD patients become refractory to treatment, increasing the risk of complications, such as fistulas, strictures, and abscesses, especially in CD, and requiring surgical interventions, thus affecting morbidity and mortality [56, 57].

This systematic review and meta-analysis have several limitations that warrant acknowledgment.

Firstly, the analyses encompassed studies involving patients with both CD and UC, including individuals in different disease phases, such as remission and active phases. The clinical heterogeneity among these studies, in terms of the types of patients included, may

introduce variability into the results. It is essential to recognize that the variation in the clinical characteristics of the study populations might have influenced the overall findings.

Secondly, the assessment of oxidative stress markers and cytokines involved diverse methodologies across the included studies. These methodological variations may have introduced inconsistencies and potential biases into the interpretation of the data. However, to mitigate this issue, we applied data normalization techniques by calculating the mean and SD for each parameter, allowing for a more reliable comparison across the studies. Despite these normalization efforts, the inherent variability associated with different measurement techniques and laboratory practices remains a limitation in this analysis.

Lastly, while every effort was made to provide a comprehensive overview of the impact of antioxidants on IBD, the inclusion of only RCTs may have introduced selection bias. Excluding other study designs, such as observational studies, might limit the generalizability of the findings.

The authors are encouraged to engage in a comprehensive discussion of the results, providing insights into their interpretation concerning previous studies and the underlying hypotheses. The implications of the findings should be explored within a broader context. Additionally, it is advisable to consider potential avenues for future research in the discussion.

5. Conclusions

Few RCTs currently include biomarkers of oxidative stress and cytokines in the analysis of the effectiveness of potential therapies, whether traditional or non-traditional, for the treatment of IBD. Among the markers of redox imbalance that show significant modulation are antioxidant capacity and SOD, but not MDA, a marker of lipid membrane damage.

The manipulation of ROS and cytokines represents a promising approach to manage IBD and improve the quality of life for patients. It is crucial for studies evaluating new therapeutic interventions to incorporate analyses of oxidative stress and cytokines into their assessments of therapeutic effectiveness. This integration will provide valuable insights into the potential benefits of novel treatments for IBD and contribute to the advancement of evidence-based medical interventions for this challenging condition.

Author Contributions: Conceptualization, data curation and methodology M.O.F.G. and F.A.M; J.I.R.J. and A.S.G. carried out the selection of papers; investigation, J.I.R.J., J.K.G.V.,

L.E.M.S.X and A.S.G; writing—original draft preparation J.K.G.V., A.S.P.M., J.C.F.S. and S.C.B; writing—review and editing, M.O.F.G. and F.A.M. All authors have read and agreed to the published version of the manuscript.

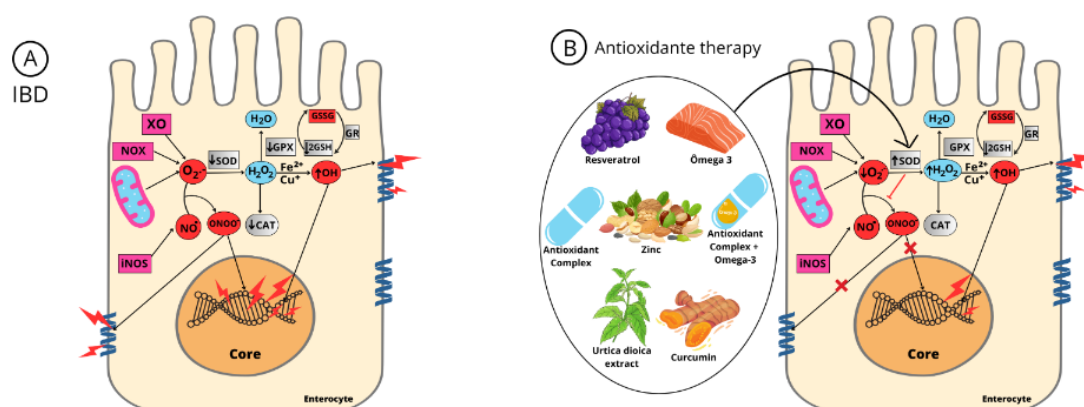
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Graphical Abstract

Legend: A - Redox Imbalance in patients with Inflammatory Bowel Disease: The generation of the superoxide anion radical ($O_2^{\cdot-}$) primarily arises from enzymes such as Nicotinamide adenine dinucleotide phosphate-oxidase (NOX) and xanthine oxidase (XO), in addition to the mitochondrial electron transport chain. On the other hand, nitric oxide (NO) is synthesized via Nitric Oxide Synthase inducible (iNOS). NO, in turn, can react with $O_2^{\cdot-}$ to generate peroxynitrite (ONOO $^-$) and other reactive nitrogen species (RNS) that act on the lipid membrane – causing lipid peroxidation (LP) –, and on deoxyribonucleic acid (DNA), – potentially leading to mutations. Simultaneously, $O_2^{\cdot-}$ undergoes dismutation by superoxide dismutase (SOD), generating hydrogen peroxide (H_2O_2), which can be neutralized by the enzymes glutathione peroxidase (GPx) and catalase (CAT) or react with ions such as Fe^{2+} or Cu^{+} , generating the highly reactive hydroxyl radical (OH $^{\cdot}$), an oxygen species capable of causing LP, DNA mutations, and disruption of proteins that are part of tight junctions. However, in individuals with IBD, antioxidant defenses such as SOD, GPx, CAT, glutathione reductase (GR) and glutathione reduced (GSH) are pathologically reduced, leading to an increased redox imbalance – characterized by elevated levels of reactive oxygen and nitrogen species (RONS) and oxidized glutathione (GSSG) –, as well as a reduction in both

enzymatic and non-enzymatic antioxidant defenses in these individuals; In Figure B, we can observe that treatment with the antioxidants included in this meta-analysis was effective in increasing SOD levels compared to the placebo, suggesting that there is less damage caused by RNS, demonstrating the protective effect of these therapies.

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7. ANEXOS

7.1 About Pharmaceutics

Pharmaceutics (ISSN 1999-4923) is an open access journal which provides an advanced forum for the science and technology of pharmaceutics and biopharmaceutics. Covered topics include pharmaceutical formulation, process development, drug delivery, pharmacokinetics, biopharmaceutics, pharmacogenetics, and interdisciplinary research involving, but not limited to, engineering, biomedical sciences, and cell biology. Our aim is to encourage scientists to publish their experimental results and theoretical assumptions in as much detail as possible. There is no restriction on the maximum length of the papers. The full experimental details must be provided so that the results can be reproduced. In addition, this journal presents the following unique features:

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- Biopharmaceutics
- Nanomedicine
- Drug targeting
- Drug design
- Pharmacokinetics, toxicokinetics

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- Pharmacokinetic analysis
- pharmacodynamics
 - Physiological and biochemical effects of drugs on the body
 - Drug–receptor interactions

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General Considerations

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 - **Front matter:** Title, Author list, Affiliations, Abstract, Keywords.
 - **Research manuscript sections:** Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).
 - **Back matter:** Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.
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