Arch Biol Sci. 2025;77(2):173-184 https://doi.org/10.2298/ABS250418014B

Preventive and curative effects of *Calendula officinalis* infusion in a dextran sulfate sodium (DSS)-induced colitis model in BALB/c mice

Youcef Bouferkas[1,2,*], Abir Haddi[2,3], Leila Amier[2], Kabir Khadija Argungu[2], Kawthar Belkaaloul[2], and Samia Addou[2]

[1]*Laboratory of Bioeconomics, Food Security and Health (LBSAS), Department of Food Sciences, Faculty of Natural and Life Sciences, University of Mostaganem Abdelhamid Ben Badis*, Mostaganem, Algeria

[2]*Laboratory of Physiology of Nutrition and Food Security (LPNSA), Department of Biology, Faculty of Natural and Life Sciences, University of Oran Ahmed Benbella*, Oran, Algeria

[3]*Higher School of Biological Sciences of Oran (ESSBO)*, Oran, Algeria

***Corresponding author**: youcef.bouferkas@univ-mosta.dz

Received: April 18, 2025; **Revised**: May 5, 2025; **Accepted**: May 16, 2025; **Published online**: May 20, 2025

Abstract: Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease (CD), is characterized by chronic intestinal inflammation. Current therapeutic strategies, including immunosuppressants and biologics, have limitations such as adverse effects and variable efficacy. *Calendula officinalis* (Co), a medicinal plant with recognized anti-inflammatory and antioxidant properties, may represent a promising alternative or complementary approach. This study aimed to evaluate the preventive and curative effects of Co infusion in a murine model of ulcerative colitis (UC) induced by dextran sodium sulfate (DSS). Fifty male BALB/c mice were randomly assigned to five groups: control, DSS+water (positive), Co+DSS (preventive), DSS+Co (curative), and DSS (positive). Colitis was induced by the administration of 4% DSS in drinking water for 7 days. Co infusion (2 g/150 mL) was administered orally for 14 days. Clinical parameters, including body weight and the Disease Activity Index (DAI), were recorded. Oxidative stress markers, including myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), were assessed. DSS administration induced significant weight loss, inflammation, and colonic oxidative stress. Co infusion significantly attenuated these effects and reduced inflammatory and oxidative markers. The findings suggest that Co may have potential as a preventive and curative agent in IBD management.

Keywords: ulcerative colitis; *Calendula officinalis*; oxidative stress; dextran sodium sulfate (DSS)

INTRODUCTION

Optimal gut health is a critical factor in maintaining overall health, with gut dysfunction as a risk factor for various diseases, including inflammatory bowel disease (IBD). IBD encompasses immune-mediated, chronic inflammatory disorders primarily affecting the gastrointestinal tract (GI) [1]. The two major forms of IBD, ulcerative colitis (UC) and Crohn's disease (CD), are characterized by inflammatory lesions and a spectrum of persistent clinical manifestations, including weight loss, diarrhea, hematochezia, and abdominal pain [2]. In IBD, accumulating evidence indicates that oxidative stress plays a pivotal role in both the initiation and progression of the pathology. It involved excessive production of reactive oxygen species (ROS) and a concomitant decline in antioxidant defenses within the inflamed mucosa, ultimately leading to chronic tissue damage [3].

The etiopathogenesis of IBD is not fully understood, although prevailing theories suggest a multifactorial origin involving environmental factors, such as increased intestinal permeability and alterations in gut microbiota, alongside genetic predisposition [4]. To elucidate the pathophysiology, therapeutic efficacy, and pharmacological mechanisms underlying IBD, the use of animal models, particularly rodents such

as mice and rats, has been extensively utilized in current research. These models have proven invaluable in advancing our understanding of the complex interactions between the immune system, gut microbiota, and mucosal epithelium [5].

The dextran sodium sulfate (DSS)-induced experimental model of colitis is the most widely utilized animal model for studying colitis, as it closely replicates the clinical and pathological characteristics of human UC. Key symptoms observed in this model include weight loss, diarrhea, and colonic shortening [6]. The DSS model is highly favored by the research community due to its rapid induction of colitis, ease of use, reproducibility, and controllability, making it an invaluable tool for studying disease mechanisms and evaluating potential therapeutic interventions [7]. The DSS-induced colitis model has been demonstrated to respond to drugs frequently utilized in the treatment of human UC, such as sulfasalazine, olsalazine, and mesalazine [8]. Oral administration of DSS via drinking water has been shown to induce both acute and chronic forms of colitis, depending on the duration and concentration of exposure [9]. This model is widely regarded as a valuable tool for investigating the inflammatory mechanisms involved in the pathogenesis of colitis, providing critical insights into disease progression and therapeutic targets [10].

In recent years, significant therapeutic advances have been made, leading to a more profound comprehension of the pathophysiological mechanisms underlying IBD. Among the newer treatments, anti-tumor necrosis factor-alpha (anti-TNF-α) agents, such as infliximab and adalimumab, have been shown to significantly improve health outcomes and reduce the need for surgical intervention [11]. Nevertheless, numerous patients encounter treatment failure, including primary and secondary non-response [12]. Given the activation of multiple inflammatory pathways in the IBD, the targeting of a single path may not be sufficient to control inflammation, as is currently attempted with targeted monotherapies [13]. The safety profiles of biological therapies have been extensively studied. These treatments have been associated with a range of side effects, including leukoencephalopathy, hypersensitivity reactions, myalgia, neoplasia, congestive heart failure, tuberculosis, and general malaise [14]. Furthermore, immunosuppressants have been linked to a variety of adverse effects, including hepatitis, pancreatitis, bone marrow toxicity, and leukopenia [15].

It is therefore crucial to develop new therapeutic modalities that can be administered repeatedly without diminishing efficacy and with a reduced risk of adverse effects [16]. Phytopharmaceuticals represent a promising therapeutic option for the treatment of IBD, offering potential advantages over conventional therapies [17]. This approach uses plants, which possess beneficial properties, including antiseptic, antibacterial, antifungal, anti-inflammatory, and antioxidant effects. Natural therapies for UC are more cost-effective and exhibit a reduced incidence of adverse effects when compared to standard pharmacological treatments [18].

One plant that has been extensively utilized within the domain of alternative medicine is *Calendula officinalis* Linn., which has been found to contain a diverse array of bioactive compounds. These include triterpenes [19], flavonoids, and phenolic acids [20], as well as quinones, coumarins [21], carotenoids [22], essential oils, fatty acids, minerals, saponins, and carbohydrates. In addition, sterols, tocopherols, and saponins have been identified in its composition [22]. It is also known for its cytotoxicity and tumor growth inhibition. Furthermore, it has demonstrated anti-inflammatory, antiviral, antiseptic, anticancer, anthelmintic, antidiabetic, wound healing, hepatoprotective, and antioxidant properties [23]. It is also deemed safe, given its therapeutic potential and proper dosage [24]. We evaluated the curative and preventive effects of orally administered Co infusion on serum inflammatory markers and colonic oxidative stress parameters in a DSS-induced colitis model in BALB/c mice.

MATERIALS AND METHODS

Ethics statement

All animal experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the Algerian Institutional Ethical Committee for Animal Research (agreement number 45/DGLPAG/DVA/SDA/14).

Animals

Seven-week-old male BALB/c mice, weighing 30-32 g, purchased from the Pasteur Institute of Algeria (Algiers, Algeria), were housed under specific pathogen-free (SPF) conditions at the Laboratory of Physiology of Nutrition and Food Safety (LPNSA), Faculty of Natural and Life Sciences, Oran 1 University Ahmed Ben Bella, Algeria. The mice were kept in a room with a 12-h light-dark cycle at constant temperature (20-25°C) and humidity (40-60%) with free access to water and food. The acclimatization period was one week.

Experimental design

The mice were randomly assigned to five experimental groups (n=10 per group). The control group (Ctl) received sterile water for 7 days without any treatment. The positive control group (DSS+water) was administered 4% DSS for 7 days, followed by sterile water for 14 days. The curative group (DSS+Co) received 4% DSS for 7 days, followed by Co infusion (Co) for 14 days. The preventive group, (Co+DSS) was treated with the infusion for 14 days before receiving 4% DSS for 7 days. The positive group (DSS) was treated with 4% DSS for 7 days without subsequent treatment. Therefore, the difference between the positive groups is the duration. In our protocol, the addition of the DSS group allowed us to validate the development of colitis for 7 days. The positive group was identical in protocol duration to the other experimental groups.

DSS and calendula preparation

The DSS solution was freshly prepared each day by dissolving 4 g of DSS powder in 100 mL of sterile water. The Co infusion was prepared by infusing 2 g of calendula flowers in 150 mL of sterile boiling water.

Clinical disease score

During the experimental period, the mice in each group were observed every morning; any changes in body weight, the occurrence of diarrhea, and signs of bleeding were recorded. Changes in body weight were calculated relative to day 1. The Disease Activity Index (DAI) was determined by adding the scores for weight loss, diarrhea, and bloody stools.

The following formula was used to determine

$$DAI=(\text{weight loss score}+\text{stool consistency}+\text{bleeding})/3.$$

Animal treatment, blood, and organ sampling

At the end of the experimental period, the mice were euthanized by cervical dislocation. Blood was collected from the retro-orbital sinus of the animals into ethylenediaminetetraacetic (EDTA) tubes to analyze C-reactive protein (CRP) levels and pro-inflammatory cytokine concentrations (IL-6, TNF-α). The colon segment was immediately transferred to ice-cold phosphate-buffered saline (pH 7.4) and used to estimate oxidative stress parameters.

C-reactive protein assay

CRP is an important biomarker of inflammation as it rises significantly in response to inflammation. This assay is a turbidimetric immunoassay for the quantitative measurement of serum CRP. CRP levels were measured strictly according to the manufacturer's instructions (C-Reactive Protein Kit, Mindray, CRP0102).

Measurement of pro-inflammatory cytokines

IL-6 and TNF-α levels were measured using a commercial mouse ELISA kit (IL-6 Cat: 555240; TNF-α Cat: 558534, BD OptEIA) according to the manufacturer's instructions.

Estimation of DSS-induced oxidative stress in colonic homogenates

Measurement of MPO activity

MPO activity was measured in colonic tissue using the method described by Lenoir et al. [25].

A section of colon tissue was homogenized on ice in 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide. After sonication and centrifugation (3,200 ×g, 10 min, 4°C), the supernatants underwent three freeze-thaw cycles and were centrifuged again. Subsequently, 50 µL of supernatant were mixed with phosphate buffer, guaiacol, and H_2O_2.

Absorbance at 470 nm was recorded every minute for 20 min, with each measurement performed in triplicate.

Estimation of GSH activity

GSH levels in colon tissues were measured using the method of Sedlak and Lindsay [26]. Tissue homogenates were treated with trichloroacetic acid, and centrifuged, and the supernatant was then reacted with Tris buffer and DTNB. After incubation, absorbance was recorded at 412 nm.

Estimation of lipid peroxidation

Colon homogenate samples were treated according to method described by Zielińska et al. [27]. The samples were treated with 3 mL of 1% phosphoric acid and 1 mL of 0.6% aqueous thiobarbituric acid. The mixture was incubated at 80°C for 45 min, then cooled on ice. Subsequently, 4 mL of n-butanol were added for extraction. The n-butanol phase was separated, and its absorbance was measured at 532 nm. The optical density was used to determine thiobarbituric acid reactive substances (TBARs), which represent the malondialdehyde content in the colonic homogenate.

Estimation of catalase (CAT) activity

Catalase activity in colon homogenates was estimated using the method described by Pervin et al. [28]. Fifty µL of homogenate were added to 1 mL of 10 mM H_2O_2 solution at room temperature. The optical density of the mixture was measured at 60-s intervals over a period of 180 s, and the decrease in absorbance was recorded.

Estimation of superoxide dismutase (SOD) activity

SOD activity was estimated using the nitro blue tetrazolium (NBT) dye reduction assay, as described [29]. Briefly, colon homogenates were centrifuged at 1,480 ×g for 15 min at 4°C, and the supernatant was used to determine SOD activity. The reduction rate of NBT-Triton in the presence of Na_2CO_3, hydroxylamine, and 0.3% Triton X-100 was measured at 560 nm and used to calculate SOD activity. The protein content was determined by the Bradford method [30].

Statistical analysis

Statistical analysis of the results was performed using GraphPad Prism® 9 software (version 9.5.1, GraphPad Software 2023, La Jolla, CA, USA). Values are presented as the mean±SE (standard error), and the significance level was set at $P<0.05$. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used for analysis.

RESULTS

Body weight gain

Calendula infusion significantly attenuated the DSS-induced reduction in body weight gain over the 21 days. The DSS group exhibited a marked suppression in weight gain ($P<0.001$), whereas both the DSS+Co and Co+DSS groups demonstrated substantial recovery, while the DSS+Co and Co+DSS groups showed substantial recovery compared to the DSS-treated groups ($P<0.001$). No weight change was observed in the calendula-treated groups (Fig. 1A). These results highlight the therapeutic efficacy of calendula in counteracting colitis-associated weight loss and promoting growth in mice.

Disease Activity Index (DAI) score

The DAI confirmed the protective and therapeutic effects of calendula infusion against DSS-induced colitis. The DSS group exhibited the highest DAI ($P<0.001$), indicating severe colitis, with similarly high scores observed in the DSS+water group. In contrast, the DSS+Co (curative) and Co+DSS (preventive) groups showed improvement, reflected in a significant decrease in DAI compared with the DSS-treated groups ($P<0.001$). The results obtained were comparable to those of the control group (Fig. 1B).

Colon length

DSS treatment (4%) induced a significant reduction in colon length, particularly in the DSS and DSS+water groups ($P<0.001$). In contrast, 14-day Co infusion administration significantly preserved colon length, restoring it to levels comparable to the control group.

It was also noted that no alteration in colon length was observed in the two groups treated with calendula. These findings highlight the curative and protective effects of Co against DSS-induced colonic damage (Fig. 1C and D).

Serum CRP concentrations

CRP levels were significantly elevated in the DSS, DSS+water, and DSS+Co groups compared to controls (P<0.001), indicating inflammation. However, the Co+DSS group exhibited CRP levels similar to those of the control, suggesting that calendula pretreatment effectively mitigates DSS-induced inflammation (Fig. 2A). Calendula has notable preventive anti-inflammatory effects, though its curative impact on CRP levels is less significant.

Measurement of serum IL-6 and TNF-α

DSS administration led to significant increases in the pro-inflammatory cytokines IL-6 and TNF-α (P<0.001). Calendula treatment, both preventive and curative, significantly reduced cytokine levels compared to the DSS+water and DSS groups (P<0.001), though levels in calendula-treated mice remained elevated relative to controls (P<0.001), indicating partial suppression of inflammation (Fig. 2B and 2C). Both preventive and curative treatments exhibited similar effects on cytokine reduction (P<0.05).

Measurement of MPO activity

MPO activity, an indirect marker of inflammation, was significantly elevated in the DSS and DSS+water

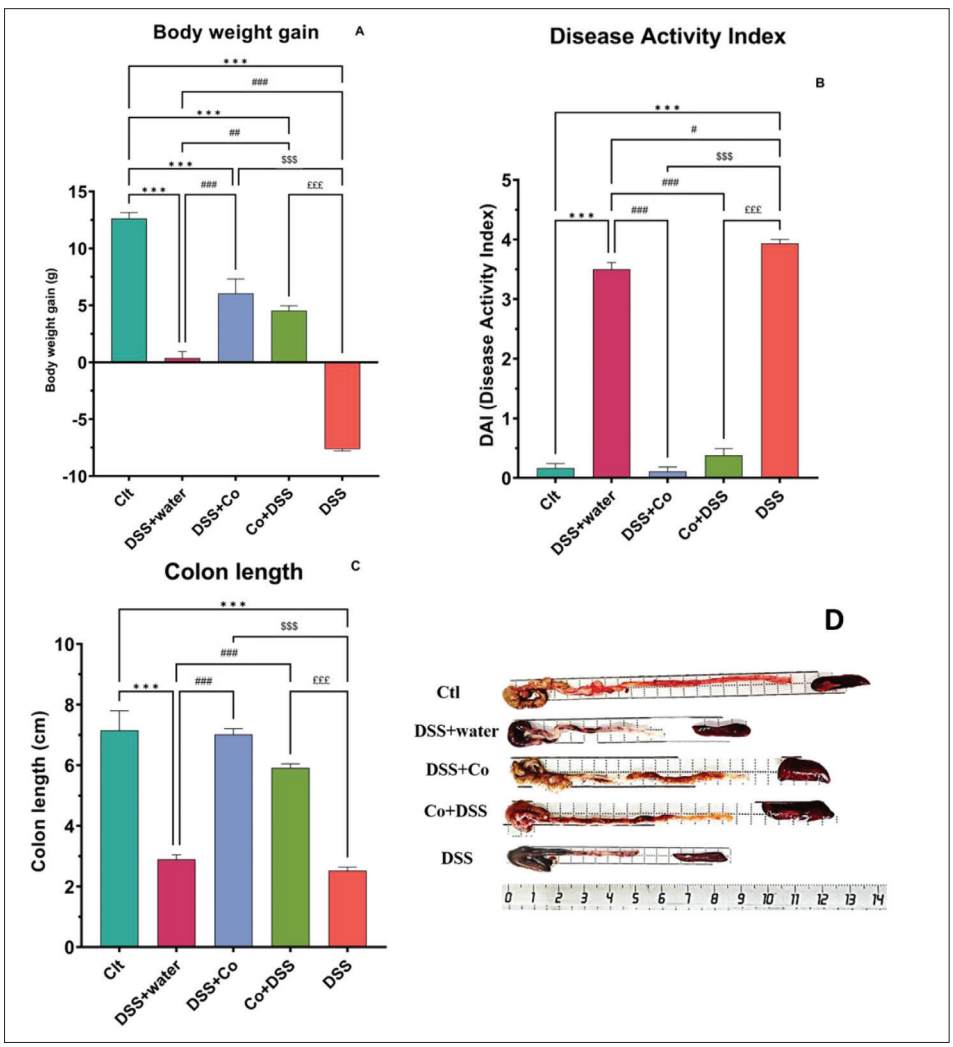

Fig. 1. Effect of Co administration on body weight gain **(A)**, Disease Activity Index score **(B)**, and colon length **(C)** of mice in different groups over 21 days (n=50). **D** – Representative photograph of the macroscopic appearance of colon tissues in each group. The values are presented as the means±SEM. One-way ANOVA was used for statistical analysis, followed by Tukey's post hoc test; *, #, $, £ significantly different from control, DSS+water, DSS+Co, Co+DSS, and DSS groups at P<0.05, respectively.

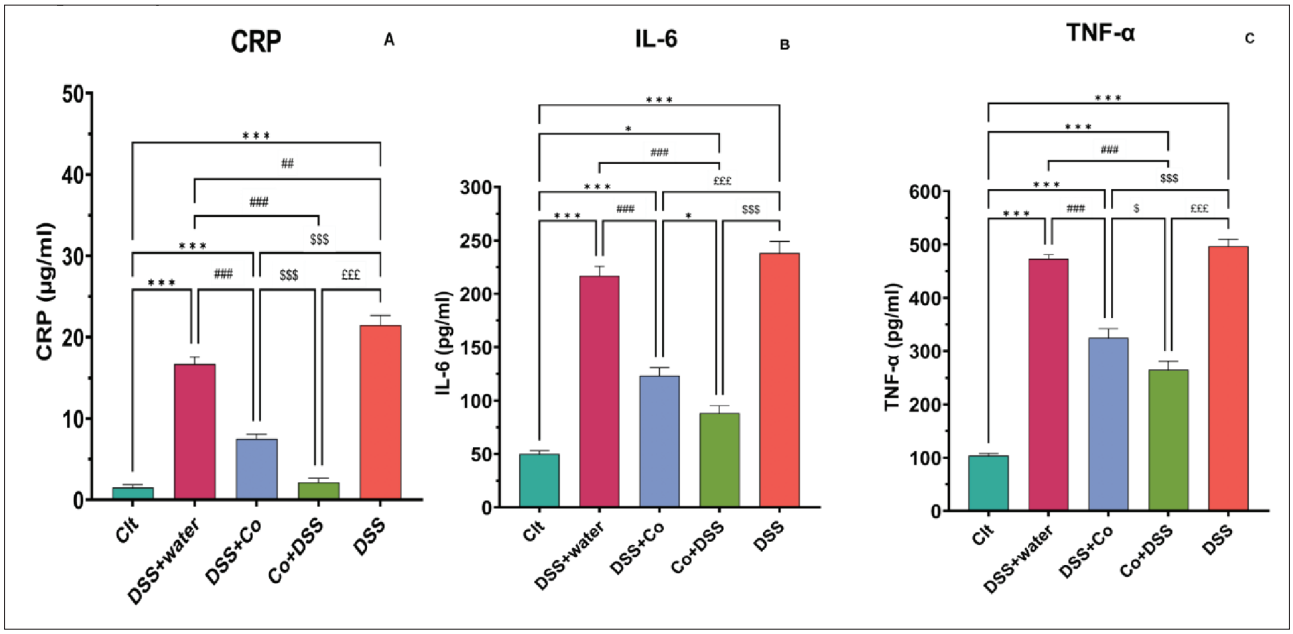

Fig. 2. Effect of Co administration on serum CRP **(A)**, IL-6 **(B)**, and TNF-α **(C)** concentrations in mice in different groups over 21 days (n=50). The values are presented as the means± SEM. One-way ANOVA was used for statistical analysis, followed by Tukey's post hoc test; *, $^{\#}$, $^{\$}$, $^{£}$ significantly different from control, DSS+water, DSS+Co, Co+DSS and DSS groups at P<0.05, respectively.

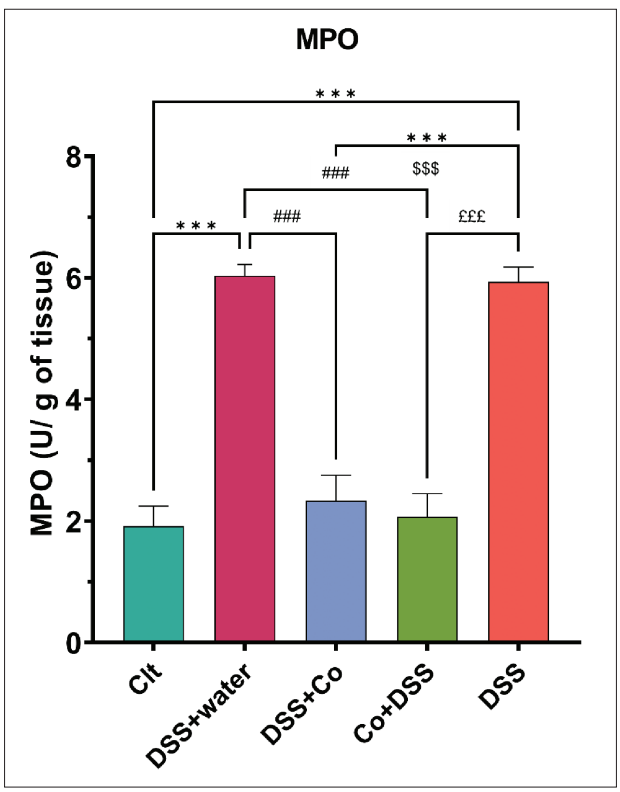

Fig. 3. Effect of Co on MPO activity in the colonic tissues of DSS-treated mice (n=50). The values are presented as the means±SEM. One-way ANOVA was used for statistical analysis, followed by Tukey's post hoc test; *, $^{\#}$, $^{\$}$, $^{£}$ significantly different from control, DSS+water, DSS+Co, Co+DSS, and DSS groups at P<0.05.

groups compared to the control (P<0.001). Calendula treatment significantly reduced MPO activity (P<0.001), with no significant difference observed between the calendula-treated groups (DSS+Co and Co+DSS) and the control group (Fig. 3). These results suggest that calendula effectively inhibits MPO activity, mitigating both inflammation and oxidative stress, regardless of whether administered preventively or curatively.

Evaluation of the colonic mucosa's oxidative state (GSH, MDA, CAT, and SOD)

DSS-induced colitis in mice (DSS+water and DSS groups) led to a significant reduction in GSH, CAT, and SOD levels (P<0.001), indicating oxidative stress. Calendula treatment, both preventive and curative, significantly restored these levels compared to DSS-treated groups (Fig. 4A, 4B, 4C), with no difference observed between the two treatment groups. Additionally, the activity of MDA was increased in DSS-induced colitis mice, but calendula supplementation reversed these changes, restoring MDA activity to control levels (Fig. 4D). These results highlight the effective antioxidant properties of calendula, regardless of the treatment timing.

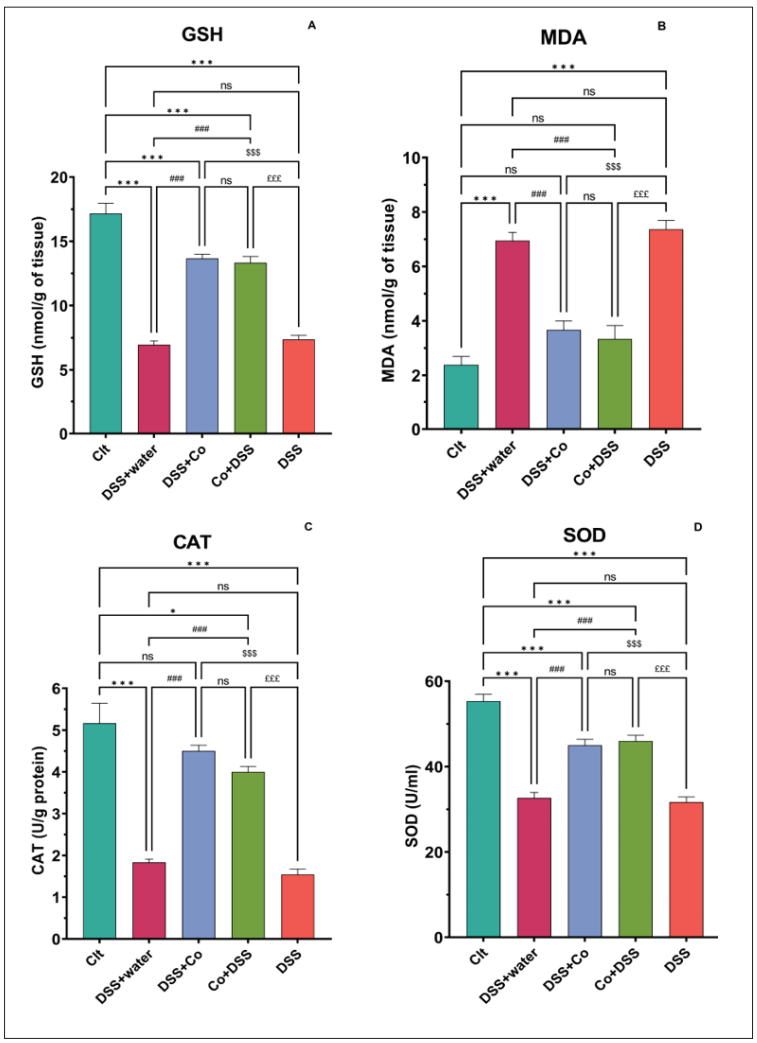

Fig. 4. Effect of Co on total GSH **(A)**, MDA **(B)**, catalase **(C)** CAT, and **(D)** SOD activities in the colonic tissues of DSS-treated mice (n=50). The values are presented as the means± SEM. One-way ANOVA was used for statistical analysis, followed by Tukey's post hoc test; *, $^{\#}$, $^{\$}$, $^{£}$ significantly different from control, DSS+water, DSS+Co, Co+DSS, and DSS groups at P<0.05, respectively.

DISCUSSION

This study aimed to evaluate the preventive and curative effects of *Calendula officinalis* administration on the inflammatory and oxidative status of mice subjected to DSS-induced colitis. DSS is a well-established model for inducing colitis in rodents, mimicking several aspects of human IBD. In integrative medicine, *C. officinalis* is known for its anti-inflammatory, antioxidant, and potential therapeutic effects in IBD. This study analyzed the various parameters measured during the experimental period to determine the effectiveness of the administration of calendula flower infusion in mitigating DSS-induced inflammation.

Calendula flowers are used in traditional folk medicine to prepare aqueous extracts and ointments for treating, among other things, inflammations and wounds [31]. *C. officinalis* is an important medicinal plant with diverse phytochemicals and biological activities, such as antioxidant, anti-inflammatory, antibacterial, gastroprotective, and hepatoprotective [32].

The effect of calendula infusion on inflammation induced by dextran sodium sulfate (DSS) in animal models remains underexplored. However, the few existing studies on related compounds and extracts provide insights into potential mechanisms and outcomes that could be relevant for calendula's application in treating DSS-induced colitis.

DSS administration causes significant weight loss, diarrhea, hematochezia, and a reduced Disease Activity Index (DAI) in mice. These scores reflect the extent of inflammation and ulceration. Our findings are similar to those obtained by Kim et al. [33,]. However, when calendula infusion is administered before and after the induction of colitis, body weight and DAI are significantly improved at the end of the experiment.

In this study, mice treated with Co showed an improvement in the length of the colon compared to mice treated with DSS. The overall decrease in colon length in DSS-fed mice was an indicator of DSS-induced inflammatory damage [34]. These results suggest a potential protective effect of calendula against DSS-induced inflammatory damage [35]. To date, no studies have investigated the effect of calendula on body weight or disease activity. In general, the induction of colitis in animals leads to increased diarrhea, resulting in water loss and impaired nutrient absorption, ultimately causing weight loss.

The preservation of colon length suggests a reduction in inflammation and maintenance of tissue integrity, supporting the hypothesis that *C. officinalis*

possesses anti-inflammatory properties in colitis models. Mice treated with *C. officinalis* exhibited higher CRP levels compared to those treated with DSS, indicating a reduction in inflammation. These results are consistent with those of Preethi et al. [36], who reported that increased CRP levels were observed following lipopolysaccharide (LPS) administration in mice. LPS is a structural molecule that helps maintain the integrity of the bacterial membrane and is a critical component of the outer membrane of Gram-negative bacteria. LPS is a strong PAMP recognized by the immune system. Calendula infusion significantly inhibited CRP production.

This decrease in CRP levels is consistent with the reduction observed in other inflammatory markers such as IL-6 and TNF-α. In both groups treated with Co, there was a significant decrease in the pro-inflammatory cytokines IL-6 and TNF-α compared to the positive groups. These results support the anti-inflammatory properties of *C. officinalis* and suggest its potential efficacy in mitigating inflammation in DSS-induced colitis. A similar study indicated that *C. officinalis* extract's anti-inflammatory effects might result from inhibiting pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, IFN-γ) and CRP in LPS-injected mice [36].

Calendula officinalis demonstrates significant potential in modulating inflammatory responses, potentially surpassing the effects of DSS by inhibiting key pro-inflammatory pathways. Herbal formulas containing *C. officinalis*, particularly in tea form, demonstrate significant anti-inflammatory effects by inhibiting pro-inflammatory mediators such as IL-6, TNF-α, and prostaglandin E2 (PGE2) [37].

The findings regarding the anti-inflammatory effects of calendula flower extracts align with previous research, particularly the study by Alexandre et al. [38], which underscores the potential of calendula as a natural anti-inflammatory agent. The efficacy of calendula flowers can be attributed to their bioactive compounds, which play a crucial role in modulating inflammatory mediators. These compounds are responsible for the observed reduction in inflammation, making calendula flowers a viable alternative to synthetic medications.

Measurement of the activity of the various redox status parameters in the colon of mice treated with calendula showed a highly significant improvement in the activities of the various oxidative stress enzymes. Oral administration of DSS showed a significant increase in the concentrations of MPO and MDA and decreased GSH, CAT, and SOD levels in colonic tissue when compared to the control group. Furthermore, the administration of a Co infusion was observed to enhance the antioxidant status of mice treated with DSS. This was evidenced by an increase in the concentration of GSH, CAT, and SOD, and a reduction in the concentration of MPO and MDA.

Myeloperoxidase (MPO) is an important enzyme in neutrophils, lymphocytes, monocytes, and macrophages, crucial for inflammation and immune responses. It serves as a specific biomarker for colonic mucosa inflammation, particularly in conditions like inflammatory bowel disease (IBD) [39].

Elevated MPO activity can also be indicative of neutrophil infiltrates and inflammatory processes. Furthermore, it has been established that during the acute inflammatory response, activated neutrophils migrate from the bloodstream to the inflamed mucosa and submucosa of the large intestine, a process critical for the development of intestinal inflammation [40]. This process gives rise to an overproduction of reactive oxygen and nitrogen species, and lipid mediators, resulting in intestinal damage. It can therefore be concluded that a reduction in the activity of this enzyme is indicative of anti-inflammatory processes [41].

IBD has been demonstrated to be initiated and advanced by oxidative stress. ROS attack macromolecules in cells, thereby disrupting epithelial cell integrity and impeding mucosal healing. This process is especially impaired in cases where endogenous defense mechanisms are compromised. Elevated levels of lipid peroxidation (MDA) and diminished levels of reduced glutathione, catalase, and superoxide dismutase in colonic tissue are indicative of oxidative stress [42].

MDA is a biomarker for oxidative stress and lipid peroxidation, which reflects the extent of tissue damage in experimentally acetic acid-induced ulcerative colitis in adult male albino rats [43]. Increased LPO in colonic tissue generates higher ROS levels, depleting cellular antioxidants and causing colon damage, inflammation, and ulceration [35]. On the other hand, reduced glutathione (GSH), an endogenous non-enzymatic

compound that functions as an antioxidant, plays a pivotal role in eliminating reactive oxygen intermediates and free radicals generated during metabolic processes [3]. GSH functions as a substrate for the antioxidant enzyme glutathione peroxidase (GPx), facilitating the detoxification of reactive species. During this process, GSH is oxidized to glutathione disulfide (GSSG), which can be regenerated back to its reduced form by glutathione reductase. However, excessive production of free radicals can impair this recycling mechanism, leading to the intracellular accumulation of GSSG [44].

The present study supports the conclusions of Banakar et al. [35], who reported that pretreatment with *C. officinalis* enhanced GSH levels that were depleted by acetic acid-induced ulcerative colitis in rats and contributed to their restoration toward baseline values. CAT has been identified as a promising therapeutic candidate for the management of oxidative stress and inflammation in IBD. It has been extensively studied as a therapeutic antioxidant. This antioxidant enzyme scavenges free radicals and converts them to hydrogen peroxide molecules. These hydrogen peroxide molecules are then further hydrolyzed into a non-toxic molecule of water and oxygen [45].

SOD represents the primary enzymatic antioxidant defense system within cells. SOD performance is usually linked with UC severity in IBD patients, where increased SOD activity during IBD contributes to oxidative injury [46]. An extract of *C. officinalis* Linn. was evaluated for its antioxidant potential by oral administration of an alcoholic extract to inhibit superoxide dismutase generation in macrophages in female Swiss albino mice by 12.6% and 38.7% at doses of 100 and 250 mg/kg body wt., respectively. Oral administration of *C. officinalis* to mice for 1 month significantly increased catalase activity. The extract produced a significant increase in glutathione levels in the blood and liver [47]. Glutathione reductase was found to be increased, whereas glutathione peroxidase was decreased after administration of the calendula extract. These results indicated *C. officinalis* has significant antioxidant activity *in vitro* and *in vivo* [48].

Calendula officinalis extract possesses flavonoids, flavoxanthin, saponins, triterpenoid esters, and auroxanthin, and by blocking the arachidonic acid pathway and inhibiting the mechanisms of MPO and MDA, it induces its anti-inflammatory and antioxidant properties [49]. Due to these effects, *C. officinalis* extract could prevent colitis by increasing the transcriptional activity of nuclear factor erythroid-derived 2-like 2 (NRF2), which prompts the formation of SOD and CAT, protecting tissues from oxidative stress.

The evaluation of the antioxidant and anti-inflammatory potentials of an herbal formulation containing calendula flower tea has revealed significant therapeutic benefits. *In vitro* assays of Prabhu Venkatesh et al. [37] found that calendula tea exhibits both antioxidant (DPPH assay) and anti-inflammatory (albumin denaturation and anti-protease assay) activities, making it a promising candidate for medicinal applications. The aqueous extract of calendula flowers contains phytochemicals such as flavonoids and phenols, which contribute to its antioxidant activity [48]. Additionally, the anti-inflammatory properties were attributed to compounds like triterpenoids and flavonoids, which have been shown to inhibit inflammatory processes [32].

An alcoholic extract of *C. officinalis* Linn. was evaluated for its *in vitro* antioxidant potential. The extract demonstrated the ability to scavenge superoxide radicals generated by riboflavin photoreduction, as well as hydroxyl radicals produced via the Fenton reaction. Additionally, it effectively inhibited lipid peroxidation (LPO) *in vitro*. The concentrations required to achieve 50% inhibition (IC_{50}) were 500 μg/mL for superoxide radicals, 480 μg/mL for hydroxyl radicals, and 2,000 μg/mL for LPO. The extract also showed significant scavenging activity against ABTS and DPPH radicals, with IC_{50} values of 6.5 μg/mL and 100 μg/mL, respectively [47]. |At a concentration of 7.5 mg/mL, LPO decreased gradually from 68% to 40% with increasing irradiation up to 20 kGy. At a lower concentration of 3.75 mg/mL, LPO dropped sharply from 56% to 28% at 1 kGy, followed by a more gradual decline with increasing doses [50].

Compared to conventional pharmacological treatments for IBD, such as corticosteroids and immunosuppressants, *Calendula officinalis* offers a natural and potentially safer alternative with fewer side effects, and it also targets various pathways. While conventional therapies are effective in managing acute inflammation, their long-term use is often limited by adverse effects. *C. officinalis*, with its anti-inflammatory and

antioxidant properties, presents a promising adjunct or alternative treatment, particularly for patients seeking more natural therapeutic options.

More research is needed to understand how *C. officinalis* works at the molecular level and its effectiveness in humans. Clinical trials are crucial to determine the best dosing and assess the long-term safety and effectiveness of *C. officinalis* for managing IBD, as current research on its anti-inflammatory effects is limited.

CONCLUSIONS

This study shows that *Calendula officinalis* can prevent and treat ulcerative colitis by reducing CRP and pro-inflammatory cytokines, indicating its therapeutic potential. Both curative and preventive treatments improved inflammatory markers and reduced oxidative stress. Calendula's ability to modulate inflammatory and antioxidant pathways, coupled with its favorable safety profile, makes it a promising candidate for developing novel treatments for IBD.

Funding: The authors received no specific funding for this work.

Author contributions: YB designed and performed the experiments, data analysis and also wrote the manuscript. YB, AH and KKA performed animal treatments and tissue collection, AH, KB and SA provided scientific advice, YB, AH and KB designed experiments and revised the manuscript. All authors reviewed and approved the final version.

Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The raw data underlying this article is available as an online supplementary research dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Bouferkas%20et%20al_Dataset.pdf

REFERENCES

1. Geier MS, Butler RN, Howarth GS. Inflammatory bowel disease: Current insights into pathogenesis and new therapeutic options; probiotics, prebiotics and synbiotics. Int J Food Microbiol. 2007;115(1):1-11. http://doi.org/10.1016/j.ijfoodmicro.2006.10.006
2. Wang Z, Chang Y, Sun H, Li Y, Tang T. Advances in molecular mechanisms of inflammatory bowel disease-associated colorectal cancer (Review). Oncol Lett. 2024;27(6):257. http://doi.org/10.3892/ol.2024.14390
3. Muro P, Zhang L, Li S, Zhao Z, Jin T, Mao F, Mao Z. The emerging role of oxidative stress in inflammatory bowel disease. Front Endocrinol (Lausanne). 2024;15:1390351. https://doi.org/10.3389/fendo.2024.1390351
4. Liu D, Saikam V, Skrada KA, Merlin D, Iyer SS. Inflammatory bowel disease biomarkers. Med Res Rev. 2022;42(5):1856-87. http://doi.org/10.1002/med.21893
5. Baydi Z, Limami Y, Khalki L, Zaid N, Naya A, Mtairag EM, Oudghiri M, Zaid Y. An update of research animal models of inflammatory bowel disease. Sci World J. 2021;2021:7479540. http://doi.org/10.1155/2021/7479540
6. Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. Nat Protoc. 2007;2(3):541-6. http://doi.org/10.1038/nprot.2007.41
7. Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF. Chemically induced mouse models of acute and chronic intestinal inflammation. Nat Protoc. 2017;12(7):1295-1309. http://doi.org/10.1038/nprot.2017.044
8. Axelsson LG, Landström E, Goldschmidt TJ, Grönberg A, Bylund-Fellenius AC. Dextran sulfate sodium (DSS)-induced experimental colitis in immunodeficient mice: Effects in CD4(+) cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm Res*. 1996;45(4):181-91. http://doi.org/10.1007/BF02285159
9. Kim TW, Seo JN, Suh YH, Park HJ, Kim JH, Kim JY, Oh KI. Involvement of lymphocytes in dextran sulfate sodium-induced experimental colitis. World J Gastroenterol. 2006;12(2):302-5. http://doi.org/10.3748/wjg.v12.i2.302
10. Huang TC, Tsai SS, Liu LF, Liu YL, Liu HJ, Chuang KP. Effect of *Arctium lappa* L. in the dextran sulfate sodium colitis mouse model. World J Gastroenterol. 2010;16(33):4193-9. http://doi.org/10.3748/wjg.v16.i33.4193
11. Theodoraki E, Orfanoudaki E, Petroulaki E, Foteinogiannopoulou K, Koutroubakis IE. Active smoking is associated with the development of adverse events of biological therapy in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2023;35(1):15-20. http://doi.org/10.1097/MEG.0000000000002445
12. Marsal J, Barreiro-de Acosta M, Blumenstein I, Cappello M, Bazin T, Sebastian S. Management of non-response and loss of response to anti-tumor necrosis factor therapy in inflammatory bowel disease. Front Med (Lausanne). 2022;9:897936. http://doi.org/10.3389/fmed.2022.897936
13. Higashiyama M, Hokari R. New and emerging treatments for inflammatory bowel disease. Digestion. 2023;104(1):74-81. http://doi.org/10.1159/000527422
14. Kondamudi PK, Malayandi R, Eaga C, Aggarwal D. Drugs as causative agents and therapeutic agents in inflammatory bowel disease. Acta Pharm Sin B. 2013;3(5):289-96. http://doi.org/10.1016/j.apsb.2013.06.004
15. Bamba S, Tsujikawa T, Sasaki M, Fujiyama Y, Andoh A. Immunomodulators and immunosuppressants for Japanese patients with ulcerative colitis. ISRN Gastroenterol. 2011;2011:194324. http://doi.org/10.5402/2011/194324
16. Triantafillidis JK, Triantafyllidi A, Vagianos C, Papalois A. Favorable results from the use of herbal and plant products in inflammatory bowel disease: Evidence from experimental animal studies. Ann Gastroenterol. 2016;29(3):268-81. http://doi.org/10.20524/aog.2016.0059

17. Triantafyllidi A, Xanthos T, Papalois A, Triantafillidis JK. Herbal and plant therapy in patients with inflammatory bowel disease. Ann Gastroenterol. 2015;28(2):210-220.
18. Gupta M, Mishra V, Gulati M, Kapoor B, Kaur A, Gupta R, Tambuwala MM. Natural compounds as safe therapeutic options for ulcerative colitis. Inflammopharmacology. 2022;30(2):397-434. http://doi.org/10.1007/s10787-022-00931-1
19. Arora D, Rani A, Sharma A. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. Pharmacogn Rev. 2013;7(14):179-87. http://doi.org/10.4103/0973-7847.120520
20. Olennikov DN, Kashchenko NI. Componential profile and amylase inhibiting activity of phenolic compounds from *Calendula officinalis* L. leaves. Sci World J. 2014;2014:654193. http://doi.org/10.1155/2014/654193
21. Re TA, Mooney D, Antignac E, Dufour E, Bark I, Srinivasan V, Nohynek G. Application of the threshold of toxicological concern approach for the safety evaluation of *Calendula officinalis* petals and extracts used in cosmetic and personal care products. Food Chem Toxicol. 2009;47(6):1246-54. http://doi.org/10.1016/j.fct.2009.02.016
22. Olennikov DN, Kashchenko NI, Chirikova NK, Akobirshoeva A, Zilfikarov IN, Vennos C. Isorhamnetin and quercetin derivatives as anti-acetylcholinesterase principles of marigold (*Calendula officinalis*) flowers and preparations. Int J Mol Sci. 2017;18(8):1685. http://doi.org/10.3390/ijms18081685
23. Garrido-Suárez BB, Garrido G, Menéndez AB, Merino N, Valdés O, de la Paz N, Romero A, Delgado L, Fernández MD, Piñeros O, Delgado-Hernández R. Topical *Calendula officinalis* L. inhibits inflammatory pain through antioxidant, anti-inflammatory, and peripheral opioid mechanisms. J Integr Med. 2022;21:34-46. http://doi.org/10.1016/j.joim.2022.10.002
24. Basch E, Bent S, Foppa I, Haskmi S, Kroll D, Mele M, Szapary P, Ulbricht C, Vora M, Yong S; Natural Standard Research Collaboration. Marigold (*Calendula officinalis* L.): An evidence-based systematic review by the Natural Standard Research Collaboration. J Herb Pharmacother. 2006;6(3-4):135-59. http://doi.org/10.1080/j157v06n03_08
25. Lenoir L, Rossary A, Joubert-Zakeyh J, Vergnaud-Gauduchon J, Farges MC, Fraisse D, Texier O, Lamaison JL, Vasson MP, Felgines C. Lemon verbena infusion consumption attenuates oxidative stress in dextran sulfate sodium-induced colitis in the rat. Dig Dis Sci. 2011;56(12):3534-45. http://doi.org/10.1007/s10620-011-1784-x
26. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205. https://doi.org/10.1016/0003-2697(68)90092-4
27. Zielińska M, Lewandowska U, Podsędek A, Cygankiewicz AI, Jacenik D, Sałaga M, et Kordek R, Krajewska WM, Fichna J. Orally available extract from *Brassica oleracea* var. *capitata rubra* attenuates experimental colitis in mouse models of inflammatory bowel diseases. J Funct Foods. 2015;17:587-99.
28. Pervin M, Hasnat MA, Lim JH, Lee YM, Kim EO, Um BH, Lim BO. Preventive and therapeutic effects of blueberry (*Vaccinium corymbosum*) extract against DSS-induced ulcerative colitis by regulation of antioxidant and inflammatory mediators. J Nutr Biochem. 2016;28:103-13. http://doi.org/10.1016/j.jnutbio.2015.10.006
29. Wang F, Fu Y, Cai W, Sinclair AJ, Li D. Anti-inflammatory activity and mechanisms of a lipid extract from hard-shelled mussel (*Mytilus coruscus*) in mice with dextran sulphate sodium-induced colitis. J Funct Foods. 2016;23:389-99.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. http://doi.org/10.1016/0003-2697(76)90527-3
31. Bragueto Escher G, Cardoso Borges LDC, Sousa Santos J, Mendanha Cruz T, Boscacci Marques M, Vieira do Carmo MA, Azevedo L, M Furtado M, Sant'Ana AS, Wen M, Zhang L, Granato D. From the field to the pot: Phytochemical and functional analyses of *Calendula officinalis* L. flower for incorporation in an organic yogurt. Antioxidants. 2019;8(11):559. http://doi.org/10.3390/antiox8110559
32. Sapkota B, Kunwar P. A Review on Traditional Uses, Phytochemistry and Pharmacological Activities ofCalendula officinalis Linn. Nat Prod Commun. 2024;19(6):1-22. http://doi.org/10.1177/1934578X241259021
33. Kim JJ, Shajib MS, Manocha MM, Khan WI. Investigating intestinal inflammation in DSS-induced model of IBD. J Vis Exp. 2012;(60):3678. http://doi.org/10.3791/3678
34. Zhao LP, Wu J, Quan W, Zhou Y, Hong H, Niu GY, Li T, Huang SB, Qiao CM, Zhao WJ, Cui C, Shen YQ. DSS-induced colitis activates the kynurenine pathway in serum and brain by affecting IDO-1 and gut microbiota. Front Immunol. 2023;13:1089200. http://doi.org/10.3389/fimmu.2022.1089200
35. Banakar SM, Veerapur VP, Thippeswamy BS, Jagadeesh NV, Gavimath CC, Alshehri ZS. Protective effect of Calendula officinalis (L.) flower extract in acetic acid–induced ulcerative colitis in rats. J Herbs Spices Med Plants. 2016;22(3):225-37. http://doi.org/10.1080/10496475.2016.1190952
36. Preethi KC, Kuttan G, Kuttan R. Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. and its possible mechanism of action. Indian J Exp Biol. 2009;47(2):113-20.
37. Prabhu Venkatesh DSG, Ramani PSR, Ramalingam K. In vitro evaluation of antioxidant and anti-inflammatory potentials of herbal formulation containing marigold flower (*Calendula officinalis* L.) tea. Cureus. 2023;15(8):e43308. http://doi.org/10.7759/cureus.43308
38. Alexandre JTM, Sousa LHT, Lisboa MRP, Furlaneto FAC, Val DRD. Anti-inflammatory and antiresorptive effects of *Calendula officinalis* on inflammatory bone loss in rats. Clin Oral Investig. 2018;22:2175-85. http://doi.org/10.1007/s00784-017-2308-7
39. Lin W, Chen H, Chen X, Guo C. The roles of neutrophil-derived myeloperoxidase (MPO) in diseases: The new progress. Antioxidants. 2024;13(1):132. http://doi.org/10.3390/antiox13010132
40. Choudhary S, Keshavarzian A, Yong S, Wade M, Bocckino S, Day BJ, Banan A. Novel antioxidants zolimid and AEOL11201 ameliorate colitis in rats. Dig Dis Sci. 2001;46(10):2222-30. http://doi.org/10.1023/a:1011975218006

41. Keshavarzian A, Sedghi S, Kanofsky J, List T, Robinson C, Ibrahim C, Winship D. Excessive production of reactive oxygen metabolites by inflamed colon: Analysis by chemiluminescence probe. Gastroenterology. 1992;103(1):177-85. http://doi.org/10.1016/0016-5085(92)91111-G
42. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem.2017;86:715-48. http://doi.org/10.1146/annurev-biochem-061516-045037
43. Hashem H, Sadek AM. Antioxidant ameliorating effect of setria (reduced glutathione) on experimentally induced colitis in adult male albino rats. Egypt J Histol. 2021;44(1):188-205.
44. Averill-Bates DA. The antioxidant glutathione. Vitam Horm. 2023;121:109-41. http://doi.org/10.1016/bs.vh.2022.09.002
45. Parihar AK, Srivastava S, Patel S, Singh MR, Singh D. Novel catalase loaded nanocores for the treatment of inflammatory bowel diseases. Artif Cells Nanomed Biotechnol. 2017;45(5):981-9. http://doi.org/10.1080/21691401.2016.1198363
46. Ashique S, Kumar S, Hussain A, Mishra N, Garg A, Gowda BHJ, Farid A, Gupta G, Dua K, Taghizadeh-Hesary F. A narrative review on the role of magnesium in immune regulation, inflammation, infectious diseases, and cancer. J Health Popul Nutr. 2023;42(1):117. http://doi.org/10.1186/s41043-023-00461-8
47. Preethi KC, Kuttan G, Kuttan R. Antioxidant potential of an extract of *Calendula officinalis* flowers in vitro and in vivo. Pharm Biol. 2006;44(9):691-7.
48. Tanideh N, Jamshidzadeh A, Sepehrimanesh M, Hosseinzadeh M, Koohi-Hosseinabadi O, Najibi A, Raam M, Daneshi S, Asadi-Yousefabad SL. Healing acceleration of acetic acid-induced colitis by marigold (*Calendula officinalis*) in male rats. Saudi J Gastroenterol. 2016;22(1):50-6. http://doi.org/10.4103/1319-3767.173759
49. Meenatchisundaram S, Parameswar G, Subbraj T, Suganya T, Michael A. Note on pharmacological activities of *Calendula officinalis* L. Ethnobot Leaflets. 2009;2009(1):5.
50. Singh MK, Sahu P, Nagori K, Dewangan D, Kumar T, Alexander A, Badwaik H, Tripathi DK. Organoleptic properties in-vitro and in-vivo pharmacological activities of Calendula officinalis Linn: An over review. J Chem Pharm Res. 2011; 3(4):655-63.

ONLINE SUPPLEMENTARY RESEARCH DATASET

The raw data underlying this article is available as an online supplementary research dataset: https://www.serbiosoc.org.rs/NewUploads/Uploads/Bouferkas%20et%20al_Dataset.pdf