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RESEARCH ARTICLE

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Anti-inflammatory and anti-hyperalgesic effects induced by an aqueous aged black garlic extract in rodent models of ulcerative colitis and colitis-associated visceral pain

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Abstract

Inflammatory bowel disease (IBD) is a morbid condition characterized by relapsing–remitting inflammation of the colon, accompanied by persistent gut dysmotility and abdominal pain. Different reports demonstrated biological activities of aged black garlic (ABG), including anti-inflammatory and antioxidant effects. We aimed to investigate beneficial effects exerted by ABGE on colon inflammation by using ex vivo and in vivo experimental models. We investigated the anti-inflammatory effects of an ABG water extract (ABGE) on rat colon specimens exposed to *E. coli* lipopolysaccharide (LPS), a known ex vivo experimental model of ulcerative colitis. We determined gene expression of various biomarkers involved in inflammation, including interleukin (IL)-1β, IL-6, nuclear factor-kB (NF-kB), tumor necrosis factor (TNF)-α. Moreover, we studied the acute effects of ABGE on visceral pain associated with colitis induced by 2,4-dinitrobenzene sulfonic acid (DNBS) injection in rats. ABGE suppressed LPS-induced gene expression of IL-1β, IL-6, NF-kB, and TNF-α. In addition, the acute administration of ABGE (0.03–1 g kg^{-1}) dose-dependently relieved post-inflammatory visceral pain, with the higher dose (1 g kg^{-1}) able to significantly reduce both the behavioral nociceptive response and the entity of abdominal contraction (assessed by electromyography) in response to colorectal distension after the acute administration in DNBS-treated rats. Present findings showed that ABGE could represent a potential strategy for treatment of colitis-associated inflammatory process and visceral pain. The beneficial effects induced by the extract could be related to the pattern of polyphenolic composition, with particular regard to gallic acid and catechin.

KEYWORDS
aged black garlic, colon, inflammation, oxidative stress, pain

Maria Loreta Libero and Elena Lucarini have contributed equally to this study.

1 | INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic and multifactorial inflammatory condition of the gastrointestinal tract, characterized by unbalanced and increased immune response to external stimuli (Achitei et al., 2013). As a consequence of this condition, enteric mucosa produces various pro-inflammatory biomarkers, such as cytokines and prostaglandins, as well as reactive oxygen species (ROS), which reinforce the inflammatory status, thus contributing to tissue functional impairment (Kang et al., 2011a; Kruidenier et al., 2003). An increasing number of studies reported that up to 60% of patients with diagnosis of IBD frequently show abdominal pain. Abdominal pain perception and pathogenesis involve several mechanisms. In patients affected by IBD, chronic abdominal pain is sustained by a sensitization of primary neurons induced by inflammation, immune response derangement, and dysbiosis in the gut microbiota, which involves the maintenance of a low-grade inflammation within the gut (Bakshi et al., 2021; Coates et al., 2021; Colombel et al., 2019; Lucarini et al., 2022; Srinath et al., 2012). Together these mechanisms contribute to the multifactorial nature of post-inflammatory visceral pain, combining inflammatory as well as neuropathic aspects, that make it difficult to treat (Takahashi et al., 2021).

Current therapeutic strategies for IBD display several disadvantages such as numerous side effects, ineffectiveness in counteracting pain establishment and persistence, as well as weak responsiveness (Choi et al., 2017; Zeitz et al., 2016). In this context, the identification of new pharmacological and non-pharmacological approaches is necessary. Noteworthy, herbal extracts have long been reported to contrast IBD-related oxidative stress and inflammatory response (Chung et al., 2007; Lenoir et al., 2012; Recinella et al., 2022). Garlic was shown to possess protective effects against ulcerative colitis (Harisa et al., 2009). Accordingly, we recently showed that a hydroalcoholic and a water extract of garlic (*Allium sativum* L.) exert beneficial effects in colon, suggesting their possible role in preventing and managing ulcerative colitis (Recinella et al., 2022).

Aged black garlic (ABG) is obtained by processing fresh garlic under controlled high temperature (70–90°C) and humidity (70%–90%) involving intrinsic chemical reactions with spontaneous fermentation. ABG has been suggested to exert multiple biological properties, such as antioxidant, anti-inflammatory, anticancer, cardiovascular protection, hepatoprotective, neuroprotective, anti-obesity, anti-allergy, immunomodulation, and hepatoprotection (Javed & Ahmed, 2022; Jeong et al., 2016; Mathew & Biju, 2008; Najman et al., 2021; Shin et al., 2014; Vinayagam et al., 2023). Interestingly, bioactive compounds, including polyphenols, organosulfur, and S-allyl cysteine, are critically involved in beneficial effects induced by ABG (Kodera et al., 2002; Sasmaz et al., 2022). After processing, ABG content in phenolic compounds is 5–8 folds higher compared to fresh garlic, resulting in higher antioxidant activity than fresh garlic (Kim et al., 2013). Moreover, a wide body of evidence suggested protective effects of polyphenols against various oxidative stress- and inflammation-related diseases, including cardiovascular diseases, IBD and cancer (Biasi et al., 2011; Das & Das, 2010; Kang, Kugathasan, et al., 2011a; Kang, Shin, et al., 2011b; Singh et al., 2008). Accordingly, our previous study showed that an ABG water extract (ABGE) exhibited anti-inflammatory and antioxidant properties on mouse heart specimens exposed to LPS, which have been suggested to be related, at least in part, to the ABGE content in polyphenolic compounds, with particular regards to gallic acid and catechin. In this context, the polyphenolic content in the extract was previously quantified using high-performance liquid chromatography coupled with a photo diode array detector (HPLC-DAD) analytical method. In particular, a total of 12 compounds were identified at a wavelength of 254 nm, with gallic acid (#1) and catechin (#4) being the prominent phytochemicals (Figure 1) (Recinella et al., 2023).

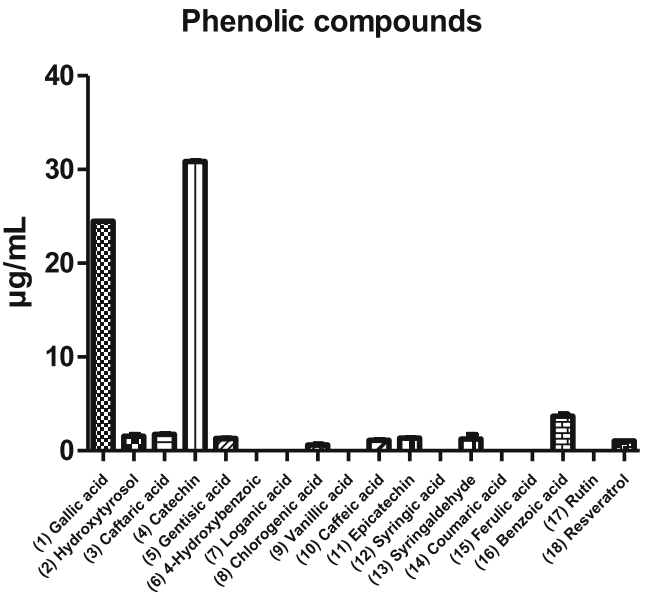

FIGURE 1 Identified phenolic compounds in aged black garlic water extract (ABGE), by high-performance liquid chromatography coupled with a photo diode array detector (HPLC-DAD) analytical method. Gallic acid (#1) and catechin (#4) were the prominent phytochemicals (Recinella et al., 2023).

Based on these findings, we aimed to investigate potential protective effects exerted by ABGE on colon damage caused by inflammation by using both ex vivo and in vivo experimental models. We evaluated the potential anti-inflammatory effects induced by ABGE, on a validated ex vivo experimental model of ulcerative colitis constituted by rat colon specimens exposed to *Escherichia coli* lipopolysaccharide (LPS) (Recinella et al., 2020; Recinella et al., 2022). In this context, we assessed gene expression of various biomarkers involved in inflammation, including interleukin (IL)-1β, IL-6, nuclear factor-kB (NF-kB), and tumor necrosis factor (TNF)-α. In addition, we used the experimental model of colitis induced by the intrarectal injection of 2,4-dinitrobenzenesulfonic acid (DNBS) (Lucarini et al., 2020), to evaluate the potential beneficial effects of ABGE on colitis-associated persistent visceral pain in rats.

2 | MATERIALS AND METHODS

2.1 | Preparation of ABGE

ABG cloves were supplied as dried material by il Grappolo S.r.l. (Soliera, Modena, Italy). 10 g of garlic cloves were crushed with a

utility garlic crusher by hand, and the juice and debris of the garlic were collected in a centrifuge tube by pouring 10 ml of water onto the crusher. After having been shaken 10 times, the tube was placed in a Trans-sonic T460 ultrasonic bath supplied by Elma (Singen, Germany) for 15 min at room temperature and then centrifuged twice at 4000 rpm for 10 min each time (Fujisawa, Suma, Origuchi, Kumagai, et al., 2008a; Fujisawa, Suma, Origuchi, Seki, & Ariga, 2008b). The supernatant was filtered, until chemical analyses were performed.

3 | TOXICOLOGICAL AND PHARMACOLOGICAL STUDIES

3.1 | Cell lines and treatments

Colorectal cancer cell line SW-480 (ATCC, Manassas, VA) were cultured in RPMI1640 (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO), 1% Pen/Strep (EuroClone, Milan, Italy) and 1% L-glutamine (EuroClone, Milan, Italy). Human fibroblast HFF-1 cell line (ATCC, Manassas, VA) was cultured in DMEM high glucose (4.5 g/L; Sigma, St. Louis, MO), supplemented with 15% FBS, 1% Pen/Strep and 1% L-glutamine. Both cell lines were maintained in a humidified incubator at 37°C, 5% CO_2.

3.2 | Cell viability assay

Cell viability was evaluated by MTT assay [3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] (Sigma, St. Louis, MO), as previously described (Veschi et al., 2018). Briefly, SW-480 and HFF-1 cell lines were seeded in 96-well plates (5×10^3 cells/well) and were treated the following day with ABGE at various concentrations (1–1000 μg/ml), or with vehicle (control). After 72 h of treatment, the MTT solution was added to each well and incubated at 37°C for at least 3 h, until purple formazan crystals were formed. In order to dissolve the precipitate, the culture medium was replaced with dimethyl sulfoxide (DMSO, Euroclone, Milan, Italy). Absorbance of each well was quantified at 540 and 690 nm, using a Synergy H1 microplate reader (BioTek Instruments Inc., Winooski, VT). IC50 values were calculated using the CompuSyn software.

3.3 | Animals

Adult Sprague–Dawley male rats (200–250 g, Envigo, Varese, Italy) were housed in Plexiglass cages (40 cm × 25 cm × 15 cm), in colony rooms (22 ± 1°C; 60% humidity), on a 12 h/12 h light/dark cycle (light phase: 07:00–19:00 h), with free access to tap water and food, 24 h/day throughout the study, with no fasting periods. Rats were fed with a standard laboratory diet.

Colon specimens were obtained from rats treated with the vehicle from our previous experiments, approved by Local Ethical Committee (University "G. d'Annunzio" of Chieti-Pescara) and Italian Health Ministry (Italian Health Ministry Authorization N. 880, delivered on 24 August 2015). All animal manipulations were performed according to the Directive 2010/63/EU of the European parliament and of the European Union council (September 22, 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the described experiments was obtained from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines (McGrath & Lilley, 2015). All efforts were made to minimize animal suffering and to reduce the number of animals used.

3.3.1 | Ex vivo studies

Rats were sacrificed by CO_2 inhalation (100% CO_2 at a flow rate of 20% of the chamber volume per min), and colon specimens were immediately collected and maintained in a humidified incubator with 5% CO_2 at 37°C for 4 h, in RPMI buffer (Sigma, St. Louis, MO) with added bacterial LPS (Sigma–Aldrich, St. Louis, MO) (10 μg/ml) (incubation period) (Recinella et al., 2019; Recinella et al., 2020). During the incubation period, colon specimens were treated with scalar concentrations of ABGE (1 μg/ml, 50 μg/ml, 500 μg/ml).

After collection, total RNA was extracted from the colon specimens using TRI Reagent (Sigma–Aldrich, St. Louis, MO), according to the manufacturer's protocol. Contaminating DNA was removed using 2 units of RNase-free DNase 1 (DNA-free kit, Ambion, Austin, TX). The RNA concentration was quantified at 260 nm by spectrophotometer reading (BioPhotometer, Eppendorf, Hamburg, Germany) and its purity was assessed by the ratio at 260 and 280 nm readings. The quality of the extracted RNA samples was also determined by electrophoresis through agarose gels and staining with ethidium bromide, under UV light. One microgram of total RNA extracted from each sample in a 20 μl reaction volume was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., Monza, Italy). Reactions were incubated in a 2720 Thermal Cycler (Thermo Fisher Scientific Inc., Monza, Italy) initially at 25°C for 10 min, then at 37°C for 120 min, and finally at 85°C for 5 s. Gene expression of IL-1β, IL-6, NF-kB, and TNF-α was determined by quantitative real-time PCR using TaqMan probe-based chemistry, as previously described (Recinella et al., 2021). PCR primers and TaqMan probes were purchased from Thermo Fisher Scientific Inc. (Assays-on-Demand Gene Expression Products, Rn00580432_m1 for IL-1β gene, Rn01410330_m1 for IL-6 gene, Rn01399572_m1 for NF-kB gene, Rn99999017_m1 for TNF-α gene). β-actin (Thermo Fisher Scientific Inc., Monza, Italy, Part No. 4352340E) was used as the housekeeping gene. The real-time PCR was carried out in triplicate for each cDNA sample in relation to each of the investigated genes. Data were elaborated with the Sequence Detection System (SDS) software version 2.3 (Thermo Fisher Scientific Inc., Monza, Italy). Gene expression was relatively quantified by the comparative $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

3.4 | In vivo studies

3.4.1 | Induction of colitis and drug administration

Colitis has been induced in rats in conformity with the method described by Lucarini et al. (2020). During a brief period of anesthesia with isoflurane (2%; VIRBAC S.r.l., Milan, Italy), inhaled into animas by means of a Compact Gas Anesthesia System (UGO Basile, Varese, Italy), 30 mg of 2,4-dinitrobenzenesulfonic acid (DNBS; Sigma-Aldrich, Milan, Italy) dissolved in 0.25 ml of 50% ethanol was intrarectally injected using a polyethylene PE-60 catheter inserted 8 cm proximal to the anus. Further, 0.25 ml of saline solution (0.9% NaCl; Sigma-Aldrich, Milan, Italy) was injected in control rats.

On day 14 after DNBS injection, subgroups of animals affected by colitis-associated visceral pain were orally administered with ABGE (0.03–1 g kg^{-1}; 10 ml kg^{-1}) or vehicle (10 ml kg^{-1}). Control group was administered with the vehicle (10 ml kg^{-1} per os). Visceral sensitivity was assessed 30 and 60 min after the acute administration of the extract at different doses or the vehicle. The dose dependence of the acute pain-relieving effect was studied.

3.4.2 | Assessment of visceral sensitivity by abdominal withdrawal response (AWR)

Visceral sensitivity to colorectal distension (CRD) was assessed via Abdominal Withdrawal Reflex (AWR) measurement using a semi-quantitative score as described previously in conscious animals (Lucarini et al., 2020). Briefly, rats were anesthetized with isoflurane (2%; VIRBAC S.r.l., Milan, Italy), by means of a Compact Gas Anesthesia System (UGO Basile, Varese, Italy), and a lubricated latex balloon (length: 4.5 cm), attached to polyethylene tubing, assembled to an embolectomy catheter and connected to a syringe filled with water were inserted through the anus into the rectum and descending colon of adult rats. The tubing was taped to the tail to hold the balloon in place. Then rats were allowed to recover from the anaesthesia for 15 min. AWR measurement consisted of visual observation of animal responses to graded CRD (0.5, 1, 2, and 3 ml) by a blinded observers who assigned a scores: No behavioral response to colorectal distention (0); Immobile during colorectal distention and occasional head clinching at stimulus onset (1); Mild contraction of the abdominal muscles but absence of abdomen lifting from the platform (2); Observed strong contraction of the abdominal muscles and lifting of the abdomen off the platform (3); Arching of the body and lifting of the pelvic structures and scrotum (4).

3.4.3 | Assessment of visceral sensitivity by Viscero-motor response

The objective measure of visceral sensitivity in animals involved assessing the visceromotor response (VMR) to CRD. Two electromyographic electrodes were surgically implanted into the external oblique abdominal muscle under deep anesthesia (isofluorane 2%; VIRBAC S.r.l., Milan, Italy), inhaled into animals by means of a Compact Gas Anesthesia System (UGO Basile, Varese, Italy) and externally positioned dorsally. The evaluation of visceromotor response took place under light anesthesia (isofluorane 1.5%). A lubricated latex balloon (length: 4.5 cm), attached to an embolectomy catheter and connected to a syringe filled with water, was employed for colorectal distension. The balloon was introduced into the colon, positioned 6.5 cm from the anus, and filled with incremental volumes of water (0.5, 1, 2, and 3 ml). The electromyographic electrodes were connected to a data acquisition system, and the corresponding signal generated by colorectal stimulation was recorded, amplified, and filtered (Animal Bio Amp; ADInstruments, Oxford, United Kingdom), digitized (PowerLab 4/35; ADInstruments), analyzed, and quantified using LabChart 8 (ADInstruments). To quantify the VMR magnitude at each distension volume, the area under the curve 30 s before the distension was subtracted from the area under the curve during the balloon distension (30 s), with responses expressed as a percentage increase from the baseline. The time interval between two consecutive distensions was set at 5 min.

3.5 | Statistical analysis

Animals were randomly assigned to the experimental groups. Behavioral measurements were performed on six animals for each treatment, performed in two experimental sets. The experimental procedures were performed by a researcher blinded to the treatment. Results from ex vivo and in vivo studies were expressed as means ± SEM. The analysis of variance (ANOVA) was performed by one-way ANOVA with Bonferroni's significant difference procedure used for post-hoc comparisons. p values of <0.05 were considered significant. Data were analyzed using the "Origin 9" software (OriginLab, Northampton, MA).

The number of animals randomized for each experimental group was calculated on the basis of the "Resource Equation" $N = (E + T)/T$ ($10 \leq E \leq 20$) (Charan and Kantharia, 2013).

4 | RESULTS AND DISCUSSION

4.1 | Toxicological and pharmacological studies

In the first set of experiments, we tested the effect of the ABGE (1–1000 μg/ml) on viability of normal HFF-1 fibroblast cells. Notably, ABGE exhibited no toxicity against normal HFF-1 cells, as shown in the dose response curve (Figure 2). These findings are consistent with a recent study of ours, showing that the same extract did not affect viability of cardiomyoblast (H9c2) cells in basal conditions (Recinella et al., 2023). Moreover, ABGE was tested on SW-480 cells for evaluating the effects on cell viability. In the present study, the aged black garlic extract (50–1000 μg/ml) was effective to exert a significant reduction in colon cancer SW-480 cell viability, in basal conditions (Figure 3). In this context, an aged garlic extract was previously shown

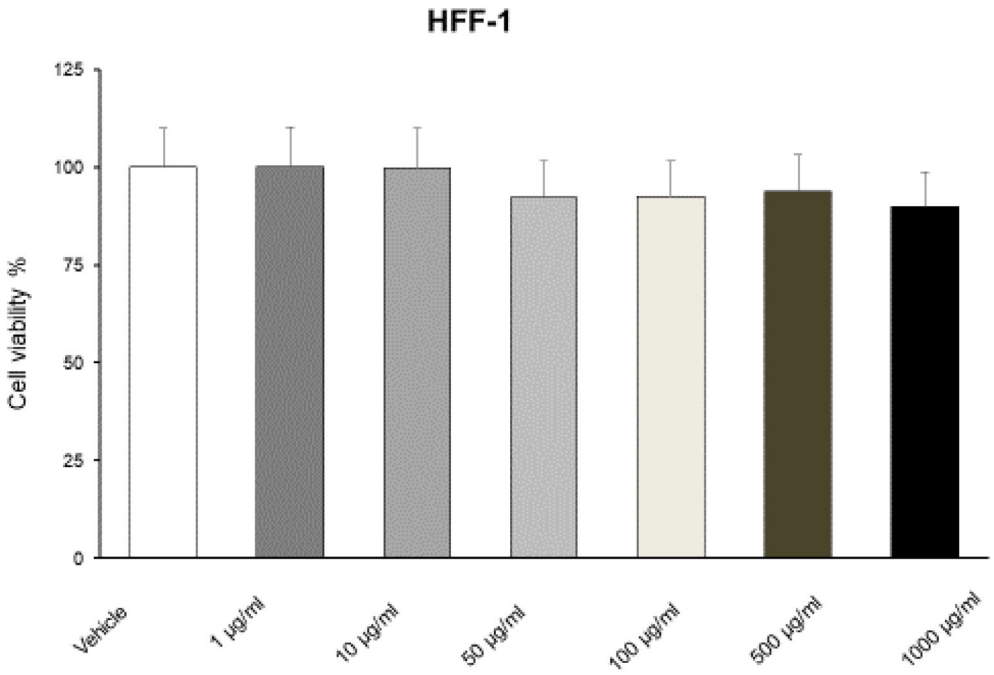

FIGURE 2 Effect of aged black garlic water extract (ABGE) on viability of normal HFF-1 fibroblast cells. Cell viability was assessed by MTT assay after incubation for 72 h of HFF-1 cells with ABGE (1–1000 µg/ml), or with vehicle (control). Data shown are the means ± SEM of two independent experiments with quintuplicate determinations.

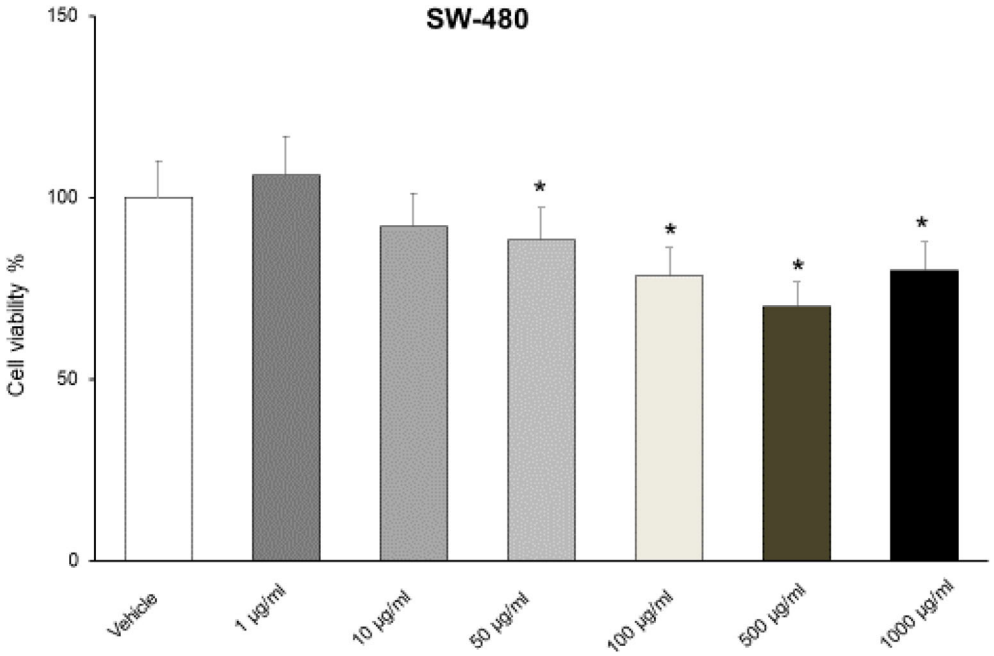

FIGURE 3 Effect of aged black garlic water extract (ABGE) on viability of colorectal cancer cell line SW-480. Cell viability was assessed by MTT assay after incubation for 72 h of SW-480 cell line with ABGE (1–1000 µg/ml), or with vehicle (control). Data shown are the means ± SEM of two independent experiments with quintuplicate determinations. ANOVA, $^*p < 0.05$ vs. vehicle.

to exert antiproliferative effects on colorectal carcinoma cells HT-29, SW-480 and SW-620, suggesting its potential role in chemoprevention of colorectal cancer (Matsuura et al., 2006). Previous studies also demonstrated the capability of catechins to induce apoptosis in SW-480 cells (Cordero-Herrera et al., 2013; Kim et al., 2012). Similarly, gallic acid was found able to inhibit proliferation and induce apoptosis of colon cancer HCT-116 and HT-29 cells (Lin et al., 2021). In particular, the beneficial activities induced by ABGE could be related to the pattern of polyphenolic composition, with particular regard to gallic acid and catechin.

A wide body of evidence suggested the critical role played by inflammation in ulcerative colitis (Kang et al., 2011b; Kruidenier et al., 2003).

In this context, we also evaluated the potential beneficial activities induced by ABGE (1–500 µg/ml) in rat colon specimens stimulated with LPS, which represents a validated model of ulcerative colitis (Recinella et al., 2020; Recinella et al., 2022). We aimed to investigate the effects of ABGE on pro-inflammatory mediators, such as TNF-α, IL-1β, IL-6, and NF-kB gene expression on isolated LPS-stimulated colon specimens, by RT-PCR analysis. In our ex-vivo model,

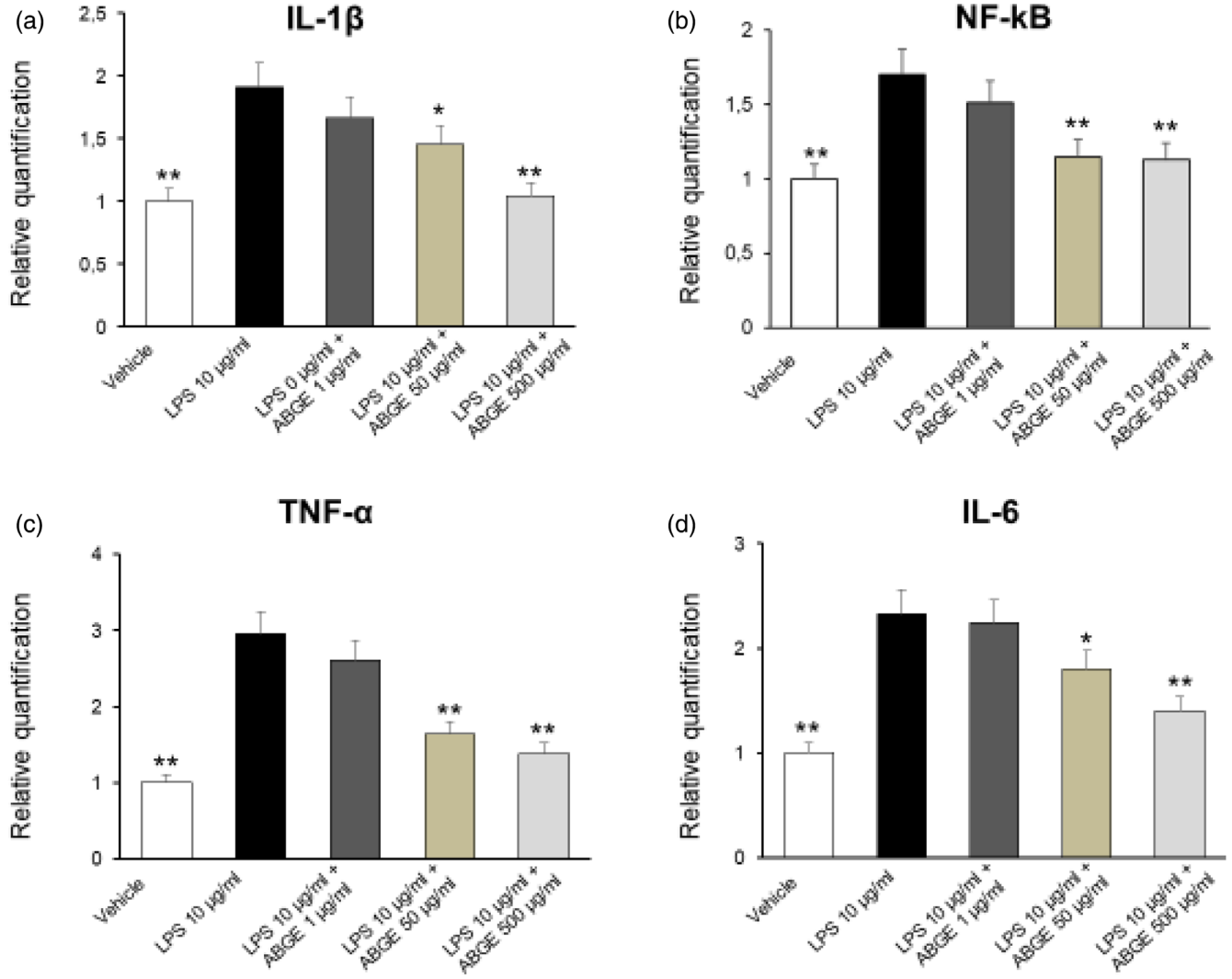

FIGURE 4 Effects of aged black garlic water extract (ABGE) (1, 50, and 500 μg/ml) on LPS-induced interleukin (IL)-1β (a), nuclear factor kB (NF-kB) (b), tumor necrosis factor-α (TNF-α) (c) and IL-6 (d) gene expression (RQ, relative quantification), in rat colon specimens (n = 6 for each experimental group). Data shown are the means ± SEM of two independent experiments with triplicate determinations. ANOVA, $^{*}p < 0.05$ and $^{**}p < 0.005$ vs. LPS.

we showed that ABGE (50 and 500 μg/ml) was able to reduce gene expression of all markers investigated without showing a dose-dependent relationship (Figure 4a–d).

IBD has been hypothesized to be related to the activation of NF-kB pathways, resulting in increased expression of pro-inflammatory markers, leading to abdominal pain, diarrhea, bleeding, along with various extra-intestinal manifestations (Laurindo et al., 2023). In this context, various phytochemicals, including catechins, have been shown to modulate various inflammation targets, such as TNF-α, COX-2, IL-1β, and IL-6 (Iqbal et al., 2022; Qazi et al., 2022). In particular, catechins were found able to revert histological lesions and decrease expression of cytokines, such as TNF-α and IL-6, as well as antioxidant markers, including SOD, GSH-Px, and CAT, and MDA in a mouse model of DSS-induced colitis (Zhang et al., 2016). Furthermore, our present findings are in agreement with a previous study of ours showing that a water garlic extract exerted anti-inflammatory and antioxidant effects on isolated mouse colon specimens treated ex vivo with LPS, which have been suggested to be related, at least in part, to its polyphenolic composition, with particular regards to catechin (Recinella et al., 2022). Interestingly, a wide body of evidence found that gallic acid was effective in inhibiting inflammatory process in animal models of experimental colitis (Li et al., 2019; Pandurangan et al., 2015; Shree et al., 2020; Zhu et al., 2019). In particular, Shree and collaborators (2019) suggested that gallic acid could revert 1,2-dimethylhydrazine-induced ulcerative colitis through inhibition of NF-κB signaling pathway. More recently, Yu et al. (2023) reported that gallic acid inhibited NLRP3 inflammasome in DSS-induced ulcerative colitis, in mice, further suggesting potential anti-inflammatory activity of herbal preparations containing gallic acid in ulcerative colitis. Accordingly, BenSaad et al. (2017) reported that gallic acid suppressed LPS-induced prostaglandin E_2 and IL-6 production in RAW264.7 cells. Taken together, our present findings suggest that the beneficial effects induced by the extract could be related to the pattern of

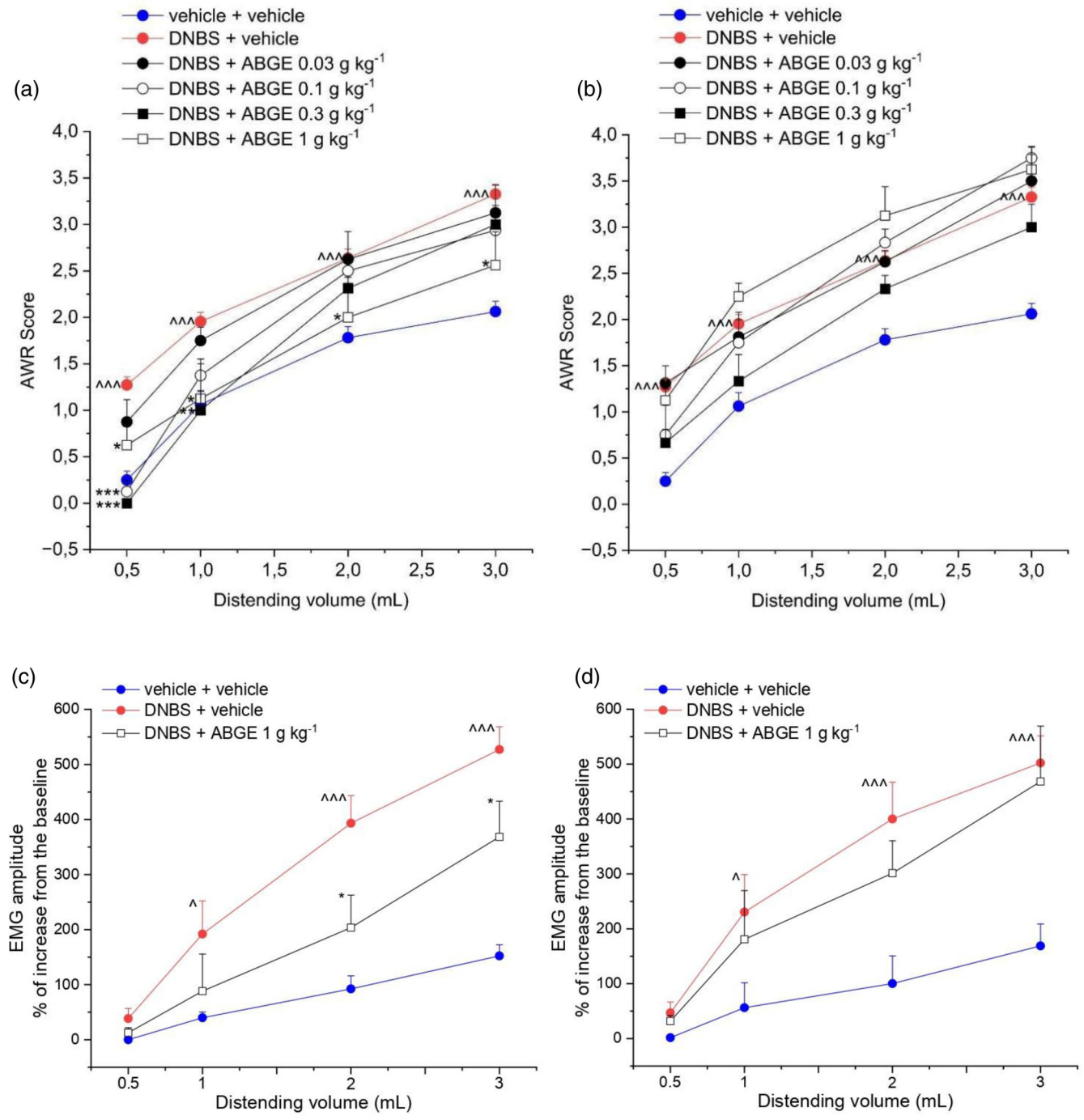

FIGURE 5 Effect of the acute administration of aged black garlic water extract (ABGE) on visceral pain induced by 2,4-Dinitrobenzenesulfonic acid (DNBS) in rats. Visceral pain was assessed by evaluating the Abdominal Withdrawal Reflex (AWR) score (0–4; a and b) and the Viscero-Motor Response (VMR) (c and d) of animals in response to Colo-Rectal Distension (CRD) (0.5–3 ml), 30 min (a and c) and 60 min (b and d) after the oral administration of the ABGE (0.03–1 g kg^{-1}). Behavioral tests were performed 14 days after DNBS injection. Each value is the mean ± SEM of six rats per each group, performed in two experimental sets. ANOVA, ^^^$p < 0.001$ vs. vehicle + vehicle treated animals. *$p < 0.05$ and ***$p < 0.001$ vs. DNBS + vehicle treated animals.

polyphenolic composition, with particular regard to gallic acid and catechin. However, various phytoconstituents, including phenolics, S-allyl cysteine (SAC), and hydroxycinnamic acid derivatives were also found in BG, with respect to raw garlic (Javed & Ahmed, 2022; Vinayagam et al., 2023).

On the basis of these results, we performed a second set of experiments, with the aim to investigate the effects of ABGE (0.03–1 g kg^{-1} p.o.) on visceral pain induced by colitis after DNBS injection.

4.2 | Anti-hyperalgesic efficacy of ABGE against colitis-associated visceral pain

The effect of the acute administration of ABGE on visceral pain induced by colitis was evaluated 14 days after DNBS injection (Figure 5), when colon hypersensitivity persists despite tissue healing (Lucarini et al., 2020). Visceral pain was monitored in the animals by assigning a score to their abdominal withdrawal response (AWR; 0–4) to colorectal distension (CRD; 0.5–3 ml). 14 days after colitis induction, the AWR elicited by the distension with 0.5–3 ml was significantly higher in DNBS-treated animals compared to the controls (vehicle + vehicle treated group; Figure 5a,b; $p < 0.001$ for each volume tested). The acute administration of black garlic aqueous extract (ABGE 0.03–1 g kg^{-1} p.o.) dose-dependently reduced visceral hypersensitivity induced by DNBS in rats. This effect peaked 30 min after administration (Figure 5a) and began to subside after 60 min (Figure 5b). In particular, the dose of 1 g kg^{-1} significantly reduced animals' AWR response to CRD back to the value of controls (vehicle + vehicle treated group; $p < 0.05$ for 0.5–3 ml; Figure 5a). ABGE (0.1–0.3 g kg^{-1}) was partially effective, significantly reducing the AWR only at the stimuli 0.5 ml ($p < 0.05$) and 1 ml ($p < 0.05$), while the lowest dose (0.03 g kg^{-1}) was ineffective (Figure 5a). The fully effective dose (1 g kg^{-1}) was also tested for the evaluation of EMG abdominal muscle response (VMR) in response to CRD. The entity of VMR resulted greater in animals treated with DNBS compared to healthy controls after the application of the same protocol of CRD (Figure 5c,d). 30 min after the injection, the VMR was overall lower in DNBS-treated animals administered with ABGE 1 g kg^{-1} in comparison to that of DNBS-treated animals receiving vehicle, though the effect resulted statistically significant at higher distension volumes (2 and 3 ml; $p < 0.05$; Figure 5c). The effect on VMR was no longer present 60 min after administration (Figure 5d).

The response to rectal distention testing in patients with irritable bowel syndrome have been demonstrated to be a predictive value of pain sensory thresholds (Bouin et al., 2002). Similarly, AWR and VMR assessment to colorectal distension provide a quantitative measure of animal's intestinal sensitivity and a faithful predictor of therapy efficacy at clinical level (Camilleri et al., 2017; Lucarini et al., 2020). The higher dose of ABGE (1 g kg^{-1}) was able to significantly reduce both AWR and VMR entity in response to CRD in DNBS-treated animals, thus it is expected that it might effectively relieve persistent visceral hypersensitivity in patients remitting from IBD. The management of post-inflammatory pain still represents an unmet clinical issue because of the lack of effective and safe therapeutic approaches. Although different types of approaches are currently used to manage pain in patients with different gut disease, most of interventions displayed a limited effectiveness in IBD patients (Baillie et al., 2023; Camilleri & Boeckxstaens, 2023; Swierczynski et al., 2023).

Several studies focused on the potential role of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, as well as NF-kB on pain processing and associated these cytokines with chronic pain conditions (Murphy et al., 1999; Woolf et al., 1997; Xu et al., 1997). Besides their role in modulation of neuronal expression of neuropeptides (Klein et al., 1997), there is evidence that certain cytokines, such as IL-6 and TNFα, are critically involved in the neuroplastic response of sensory neurons to inflammatory insult, as well as in microglial and astrocytic activation (Ramer et al., 1998). In agreement, a direct relationship between baseline tissue inflammation and systemic TNF-α and IL-6, with worsen of pain over 5 years, has been reported in clinical studies (Hosnijeh et al., 2015; Stannus et al., 2013).

Antinociceptive effect of garlic and its organosulphur compounds in preclinical models and clinical assays to treat different diseases have been recently reviewed, but direct evidence of pain-relieving effects of garlic on colitis-associated pain were not proven (Hernández-Cruz et al., 2022). Antinociceptive properties exerted by garlic were consistently demonstrated in chronic inflammatory diseases in humans, including knee rheumatoid arthritis and osteoarthritis (Dehghani et al., 2018; Moosavian et al., 2020). Regarding the mechanisms of action, the antioxidant and anti-inflammatory properties, including the ability to block the synthesis pro-inflammatory cytokines, are likely involved in effects of garlic constituents on pain. In particular, the anti-hyperalgesic activities induced by ABGE might be related to its polyphenolic content, with particular regards to catechin and gallic acid. Indeed, polyphenols, beyond to the therapeutic benefits on IBD pathophysiology (Fan et al., 2017), play beneficial effects on pain, as confirmed by clinical and pre-clinical studies (Hernández-Cruz et al., 2022; Sirše, 2022). A potential role of catechin in treatment and management of neuropathic pain has been recently suggested in rats, including an effective decrease of both hyperalgesia and allodynia. The same authors hypothesized that these effects could be related to inhibition of NF-kB activation and reduction of TNF-α, IL-6, and IL-β levels in the rat brain (Foudah et al., 2022). These results are in line with previous studies showing that catechin was able to suppress NF-kB (Suhail et al., 2022), and the expression of NF-kB-regulated pro-inflammatory cytokines (Noll et al., 2013; Vazquez Prieto et al., 2015). Anyway, gallic acid has been reported to exert anti-hyperalgesic effects (Sohrabi et al., 2021; Yang et al., 2021) in neuropathic pain conditions which are not responsive to anti-inflammatory drugs, suggesting further mechanisms of action. Indeed, recent evidence reported gallic acid efficacy against comorbid visceral pain and depression may be mediated by the negative modulation of P2X7 in the central nervous system (Wen et al., 2022), which has implication in both inflammation and pain (Hu et al., 2022).

Aside from its therapeutic uses in traditional medicine, garlic has a wide use as a spice in cooking, thus overall, garlic consumption is considered safe for humans, though some side effects have been reported after ingestion of raw garlic in high doses on an empty stomach by sensitive people (El-Saber Batiha et al., 2020). In the present work, any toxic effect was observed in rats treated with ABGE (0.03–1 g kg^{-1}), in a range of doses which might be translated in 0.3-1 g day in humans. The recommended daily dose for the therapeutic use of garlic is 2–4 g of crushed raw garlic or equivalent in alliin content (ESCOP MONOGRAPHS. *The Scientific Foundation for Herbal Medicinal Products*. ESCOP; Devon, PA: 2019. Allii sativi bulbus Garlic). In this regard, it is also important to consider that the manufacturing conditions of black garlic and the procedure of extraction change the

chemical composition of raw garlic, which has a major impact on its biological properties and on its toxicological profile. For instance, during the preparation of aged garlic extracts, the odorous, harsh, and irritating compounds of garlic are naturally transformed into stable and safe sulfur compounds (Ribeiro et al., 2021). Accordingly, different toxicological studies have confirmed the safety of aged garlic (Ansary et al., 2020; Miraghajani et al., 2018). Nevertheless, further studies are needed to assess the effectiveness and the safety of a repeated treatment with ABGE and to elucidate its pharmacodynamic profile.

In conclusion, our present findings showed that ABGE could represent a potential adjuvant strategy to defuse colitis-associated inflammatory process, as well as to manage resulting persistent visceral pain. The anti-inflammatory and anti-hyperalgesic effects induced by ABGE are likely attributable to its content in polyphenolic compounds, with particular regards to gallic acid and catechin, though other constituents in the extract might support ABGE beneficial properties.

AUTHOR CONTRIBUTIONS

Maria Loreta Libero: Conceptualization; data curation; methodology; resources; writing – original draft; writing – review and editing. **Elena Lucarini:** Data curation; formal analysis; investigation; methodology; resources; visualization; writing – original draft. **Lucia Recinella:** Conceptualization; data curation; funding acquisition; methodology; project administration; resources; writing – original draft; writing – review and editing. **Clara Ciampi:** Formal analysis; investigation. **Serena Veschi:** Formal analysis; investigation. **Anna Piro:** Formal analysis; investigation. **Annalisa Chiavaroli:** Formal analysis; investigation. **Alessandra Acquaviva:** Formal analysis; investigation. **Nilofar Nilofar:** Formal analysis; investigation. **Giustino Orlando:** Formal analysis; investigation; software; validation. **Daniele Generali:** Formal analysis; investigation. **Carla Ghelardini:** Formal analysis; investigation. **Lorenzo di Cesare Mannelli:** Formal analysis; investigation; writing – original draft. **Antonio J. Montero-Hidalgo:** Formal analysis; investigation. **Raúl M. Luque:** Formal analysis; investigation. **Claudio Ferrante:** Formal analysis; investigation; software; validation. **Luigi Menghini:** Formal analysis; investigation; software; validation. **Simonetta Cristina di Simone:** Formal analysis; investigation. **Luigi Brunetti:** Methodology; project administration; resources; writing – original draft. **Sheila Leone:** Conceptualization; data curation; funding acquisition; methodology; project administration; resources; supervision; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no financial/commercial conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Achitei, D., Ciobica, A., Balan, G., Gologan, E., Stanciu, C., & Stefanescu, G. (2013). Different profile of peripheral antioxidant enzymes and lipid peroxidation in active and non-active inflammatory bowel disease patients. *Digestive Diseases and Sciences*, *58*(5), 1244–1249.

Ansary, J., Forbes-Hernández, T. Y., Gil, E., Cianciosi, D., Zhang, J., Elexpuru-Zabaleta, M., Simal-Gandara, J., Giampieri, F., & Battino, M. (2020). Potential health benefit of garlic based on human intervention studies: A brief overview. *Antioxidants (Basel)*, *9*(7), 619.

Baillie, S., Norton, C., Saxena, S., & Pollok, R. (2023). Chronic abdominal pain in inflammatory bowel disease: A practical guide. *Frontline Gastroenterology*, *15*(2), 144–153.

Bakshi, N., Hart, A. L., Lee, M. C., Williams, A. C. C., Lackner, J. M., Norton, C., & Croft, P. (2021). Chronic pain in patients with inflammatory bowel disease. *Pain*, *162*(10), 2466–2471.

BenSaad, L. A., Kim, K. H., Quah, C. C., Kim, W. R., & Shahimi, M. (2017). Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A&B isolated from Punica granatum. *BMC Complementary and Alternative Medicine*, *17*(1), 47.

Biasi, F., Astegiano, M., Maina, M., Leonarduzzi, G., & Poli, G. (2011). Polyphenol supplementation as a complementary medicinal approach to treating inflammatory bowel disease. *Current Medicinal Chemistry*, *18*(31), 4851–4865.

Bouin, M., Plourde, V., Boivin, M., Riberdy, M., Lupien, F., Laganière, M., Verrier, P., & Poitras, P. (2002). Rectal distention testing in patients with irritable bowel syndrome: Sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology*, *122*(7), 1771–1777.

Camilleri, M., & Boeckxstaens, G. (2023). Irritable bowel syndrome: Treatment based on pathophysiology and biomarkers. *Gut*, *72*(3), 590–599.

Camilleri, M., Lembo, A., & Katzka, D. A. (2017). Opioids in gastroenterology: Treating adverse effects and creating therapeutic benefits. *Clinical Gastroenterology and Hepatology*, *15*(9), 1338–1349.

Charan, J., & Kantharia, N. D. (2013). How to calculate sample size in animal studies?. *Journal of Pharmacology & Pharmacotherapeutics*, *4*(4), 303–306.

Choi, C. H., Moon, W., Kim, Y. S., Kim, E. S., Lee, B.-I., Jung, Y., Yoon, Y. S., Lee, H., Park, D. I., Han, D. S., & IBD Study Group of the Korean Association for the Study of Intestinal Diseases. (2017). Second Korean guidelines for the management of ulcerative colitis. *Intestinal Research*, *15*(1), 7–37.

Chung, H.-L., Yue, G.-G.-L., To, K.-F, Su, Y.-L., Huang, Y., & Ko, W.-H. (2007). Effect of Scutellariae radix extract on experimental dextran-sulfate sodium-induced colitis in rats. *World Journal of Gastroenterology*, *13*(42), 5605–5611.

Coates, M. D., Johri, A., Gorrepati, V. S., Maheshwari, P., Dalessio, S., Walter, V., Stuart, A., Koltun, W., Bernasko, N., Tinsley, A., Williams, E. D., & Clarke, K. (2021). Abdominal pain in quiescent inflammatory bowel disease. *International Journal of Colorectal Disease*, *36*(1), 93–102.

Colombel, J.-F., Shin, A., & Gibson, P. R. (2019). AGA clinical practice update on functional gastrointestinal symptoms in patients with inflammatory bowel disease: Expert review. *Clinical Gastroenterology and Hepatology*, *17*(3), 380–390.e1.

Cordero-Herrera, I., Martín, M. A., Bravo, L., Goya, L., & Ramos, S. (2013). Epicatechin gallate induces cell death via p53 activation and stimulation of p38 and JNK in human colon cancer SW480 cells. *Nutrition and Cancer*, *65*(5), 718–728.

Das, M., & Das, D. K. (2010). Resveratrol and cardiovascular health. *Molecular Aspects of Medicine*, *31*(6), 503–512.

Dehghani, S., Alipoor, E., Salimzadeh, A., Yaseri, M., Hosseini, M., Feinle-Bisset, C., & Hosseinzadeh-Attar, M. J. (2018). The effect of a garlic supplement on the pro-inflammatory adipocytokines, resistin and tumor necrosis factor-alpha, and on pain severity, in overweight or obese women with knee osteoarthritis. *Phytomedicine*, *48*, 70–75.

El-Saber Batiha, G., Magdy Beshbishy, A., Wasef, L. G., Elewa, Y. H. A., Al-Sagan, A. A., Abd El-Hack, M. E., Taha, A. E., Abd-Elhakim, Y. M., & Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. *Nutrients*, *12*(3), 872.

Fan, F. Y., Sang, L. X., & Jiang, M. (2017). Catechins and their therapeutic benefits to inflammatory bowel disease. *Molecules*, *22*(3), 484.

Foudah, A. I., Alqarni, M. H., Devi, S., Singh, A., Alam, A., Alam, P., & Singh, S. (2022). Analgesic action of catechin on chronic constriction injury-induced neuropathic pain in Sprague-Dawley rats. *Frontiers in Pharmacology*, *13*, 895079.

Fujisawa, H., Suma, K., Origuchi, K., Kumagai, H., Seki, T., & Ariga, T. (2008a). Biological and chemical stability of garlic-derived allicin. *Journal of Agricultural and Food Chemistry*, *56*(11), 4229–4235.

Fujisawa, H., Suma, K., Origuchi, K., Seki, T., & Ariga, T. (2008b). Thermostability of allicin determined by chemical and biological assays. *Bioscience, Biotechnology, and Biochemistry*, *72*(11), 2877–2883.

Harisa, G. E. I., Abo-Salem, O. M., El-Sayed, E.-S. M., Taha, E. I., & El-Halawany, N. (2009). L-arginine augments the antioxidant effect of garlic against acetic acid-induced ulcerative colitis in rats. *Pakistan Journal of Pharmaceutical Sciences*, *22*(4), 373–380.

Hernández-Cruz, E. Y., Silva-Islas, C. A., Maldonado, P. D., Pedraza-Chaverri, J., & Carballo-Villalobos, A. I. (2022). Antinociceptive effect of garlic, garlic preparations and derivative compounds. *European Journal of Pain*, *26*(5), 947–964.

Hosnijeh, F. S., Runhaar, J., van Meurs, J. B. J., & Bierma-Zeinstra, S. M. (2015). Biomarkers for osteoarthritis: Can they be used for risk assessment? A systematic review. *Maturitas*, *82*(1), 36–49.

Hu, S. Q., Hu, J. L., Zou, F. L., Liu, J. P., Luo, H. L., Hu, D. X., Wu, L. D., & Zhang, W. J. (2022). P2X7 receptor in inflammation and pain. *Brain Research Bulletin*, *187*, 199–209.

Iqbal, N., Zubair, H. M., Almutairi, M. H., Abbas, M., Akhtar, M. F., Aleya, L., Kamel, M., Saleem, A., Jabeen, Q., Noreen, S., Baig, M. M. F. A., & Abdel-Daim, M. M. (2022). Hepatoprotective effect of Cordia rothii extract against CCl4-induced oxidative stress via Nrf2-NFκB pathways. *Biomedicine & Pharmacotherapy*, *156*, 113840.

Javed, M., & Ahmed, W. (2022). Black garlic: A review of its biological significance. *Journal of Food Biochemistry*, *46*(12), e14394.

Jeong, Y. Y., Ryu, J. H., Shin, J.-H., Kang, M. J., Kang, J. R., Han, J., & Kang, D. (2016). Comparison of anti-oxidant and anti-inflammatory effects between fresh and aged black garlic extracts. *Molecules*, *21*(4), 430.

Kang, J., Kugathasan, S., Georges, M., Zhao, H., & Cho, J. H. (2011a). Improved risk prediction for Crohn's disease with a multi-locus approach. *Human Molecular Genetics*, *20*(12), 2435–2442.

Kang, N. J., Shin, S. H., Lee, H. J., & Lee, K. W. (2011b). Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. *Pharmacology & Therapeutics*, *130*(3), 310–324.

Kim, D., Mollah, M. L., & Kim, K. (2012). Induction of apoptosis of SW480 human colon cancer cells by (−)-epicatechin isolated from Bulnesia sarmienti. *Anticancer Research*, *32*(12), 5353–5361.

Kim, J.-S., Kang, O.-J., & Gweon, O.-C. (2013). Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. *Journal of Functional Foods*, *5*(1), 80–86.

Klein, M. A., Möller, J. C., Jones, L. L., Bluethmann, H., Kreutzberg, G. W., & Raivich, G. (1997). Impaired neuroglial activation in interleukin-6 deficient mice. *Glia*, *19*(3), 227–233.

Kodera, Y., Suzuki, A., Imada, O., Kasuga, S., Sumioka, I., Kanezawa, A., Taru, N., Fujikawa, M., Nagae, S., Masamoto, K., Maeshige, K., & Ono, K. (2002). Physical, chemical, and biological properties of s-allylcysteine, an amino acid derived from garlic. *Journal of Agricultural and Food Chemistry*, *50*(3), 622–632.

Kruidenier, L., Kuiper, I., Lamers, C. B., & Verspaget, H. W. (2003). Intestinal oxidative damage in inflammatory bowel disease: Semi-quantification, localization, and association with mucosal antioxidants. *The Journal of Pathology*, *201*(1), 28–36.

Laurindo, L. F., Santos, A. R. O. D., Carvalho, A. C. A., Bechara, M. D., Guiguer, E. L., Goulart, R. A., Vargas Sinatora, R., Araújo, A. C., & Barbalho, S. M. (2023). Phytochemicals and regulation of NF-kB in inflammatory bowel diseases: An overview of in vitro and in vivo effects. *Metabolites*, *13*(1), 96.

Lenoir, L., Joubert-Zakeyh, J., Texier, O., Lamaison, J.-L., Vasson, M.-P., & Felgines, C. (2012). Aloysia triphylla infusion protects rats against dextran sulfate sodium-induced colonic damage. *Journal of the Science of Food and Agriculture*, *92*(7), 1570–1572.

Li, Y., Xie, Z., Gao, T., Li, L., Chen, Y., Xiao, D., Liu, W., Zou, B., Lu, B., Tian, X., Han, B., Guo, Y., Zhang, S., Lin, L., Wang, M., Li, P., & Liao, Q. (2019). A holistic view of gallic acid-induced attenuation in colitis based on microbiome-metabolomics analysis. *Food & Function*, *10*(7), 4046–4061.

Lin, X., Wang, G., Liu, P., Han, L., Wang, T., Chen, K., & Gao, Y. (2021). Gallic acid suppresses colon cancer proliferation by inhibiting SRC and EGFR phosphorylation. *Experimental and Therapeutic Medicine*, *21*(6), 638.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, *25*(4), 402–408.

Lucarini, E., di Pilato, V., Parisio, C., Micheli, L., Toti, A., Pacini, A., Bartolucci, G., Baldi, S., Niccolai, E., Amedei, A., Rossolini, G. M., Nicoletti, C., Cryan, J. F., O'Mahony, S. M., Ghelardini, C., & di Cesare Mannelli, L. (2022). Visceral sensitivity modulation by faecal microbiota transplantation: The active role of gut bacteria in pain persistence. *Pain*, *163*(5), 861–877.

Lucarini, E., Parisio, C., Branca, J. J. V., Segnani, C., Ippolito, C., Pellegrini, C., Antonioli, L., Fornai, M., Micheli, L., Pacini, A., Bernardini, N., Blandizzi, C., Ghelardini, C., & di Cesare Mannelli, L. (2020). Deepening the mechanisms of visceral pain persistence: An evaluation of the gut-spinal cord relationship. *Cells*, *9*(8), 1772.

Mathew, B., & Biju, R. (2008). Neuroprotective effects of garlic a review. *Libyan Journal of Medicine*, *3*(1), 23–33.

Matsuura, N., Miyamae, Y., Yamane, K., Nagao, Y., Hamada, Y., Kawaguchi, N., Katsuki, T., Hirata, K., Sumi, S.-I., & Ishikawa, H. (2006). Aged garlic extract inhibits angiogenesis and proliferation of colorectal carcinoma cells. *The Journal of Nutrition*, *136*(3 Suppl), 842S–846S.

McGrath, J. C., & Lilley, E. (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): New requirements for publication in BJP. *British Journal of Pharmacology*, *172*, 3189–3193.

Miraghajani, M., Rafie, N., Hajianfar, H., Larijani, B., & Azadbakht, L. (2018). Aged garlic and cancer: A systematic review. *International Journal of Preventive Medicine*, *9*, 84.

Moosavian, S. P., Paknahad, Z., Habibagahi, Z., & Maracy, M. (2020). The effects of garlic (Allium sativum) supplementation on inflammatory biomarkers, fatigue, and clinical symptoms in patients with active rheumatoid arthritis: A randomized, double-blind, placebo-controlled trial. *Phytotherapy Research*, *34*(11), 2953–2962.

Murphy, P. G., Ramer, M. S., Borthwick, L., Gauldie, J., Richardson, P. M., & Bisby, M. A. (1999). Endogenous interleukin-6 contributes to

hypersensitivity to cutaneous stimuli and changes in neuropeptides associated with chronic nerve constriction in mice. *The European Journal of Neuroscience*, *11*(7), 2243–2253.

Najman, K., Sadowska, A., & Hallmann, E. (2021). Evaluation of bioactive and physicochemical properties of white and black garlic (Allium sativum L.) from conventional and organic cultivation. *Applied Sciences*, *11*(2), 874.

Noll, C., Lameth, J., Paul, J.-L., & Janel, N. (2013). Effect of catechin/epicatechin dietary intake on endothelial dysfunction biomarkers and proinflammatory cytokines in aorta of hyperhomocysteinemic mice. *European Journal of Nutrition*, *52*(3), 1243–1250.

Pandurangan, A. K., Mohebali, N., Esa, N. M., Looi, C. Y., Ismail, S., & Saadatdoust, Z. (2015). Gallic acid suppresses inflammation in dextran sodium sulfate-induced colitis in mice: Possible mechanisms. *International Immunopharmacology*, *28*(2), 1034–1043.

Qazi, N. G., Khan, A.-U., Abbasi, S. W., Shah, F. A., Rasheed, F., Ali, F., Hassan, S. S. U., & Bungau, S. (2022). Pharmacological basis of Rumex hastatus D. Don in gastrointestinal diseases with focusing effects on H+/K+-ATPase, calcium channels inhibition and PDE mediated signaling: Toxicological evaluation on vital organs. *Molecules*, *27*(18), 5919.

Ramer, M. S., Murphy, P. G., Richardson, P. M., & Bisby, M. A. (1998). Spinal nerve lesion-induced mechanoallodynia and adrenergic sprouting in sensory ganglia are attenuated in interleukin-6 knockout mice. *Pain*, *78*(2), 115–121.

Recinella, L., Chiavaroli, A., Masciulli, F., Fraschetti, C., Filippi, A., Cesa, S., Cairone, F., Gorica, E., De Leo, M., Braca, A., Martelli, A., Calderone, V., Orlando, G., Ferrante, C., Menghini, L., Di Simone, S. C., Veschi, S., Cama, A., Brunetti, L., & Leone, S. (2021). Protective effects induced by a hydroalcoholic Allium sativum extract in isolated mouse heart. *Nutrients*, *13*(7), 2332.

Recinella, L., Chiavaroli, A., Orlando, G., Ferrante, C., Marconi, G. D., Gesmundo, I., Granata, R., Cai, R., Sha, W., Schally, A. V., Brunetti, L., & Leone, S. (2020). Antinflammatory, antioxidant, and behavioral effects induced by administration of growth hormone-releasing hormone analogs in mice. *Scientific Reports*, *10*(1), 732.

Recinella, L., Chiavaroli, A., Orlando, G., Menghini, L., Ferrante, C., Di Cesare Mannelli, L., Ghelardini, C., Brunetti, L., & Leone, S. (2019). Protective effects induced by two polyphenolic liquid complexes from olive (Olea europaea, mainly cultivar Coratina) pressing juice in rat isolated tissues challenged with LPS. *Molecules*, *24*(16), 3002.

Recinella, L., Gorica, E., Chiavaroli, A., Fraschetti, C., Filippi, A., Cesa, S., Cairone, F., Martelli, A., Calderone, V., Veschi, S., Lanuti, P., Cama, A., Orlando, G., Ferrante, C., Menghini, L., Di Simone, S. C., Acquaviva, A., Libero, M. L., Nilofar, N., Brunetti, L., & Leone, S. (2022). Anti-inflammatory and antioxidant effects induced by Allium sativum L. extracts on an ex vivo experimental model of ulcerative colitis. *Foods*, *11*(22), 3559.

Recinella, L., Libero, M. L., Citi, V., Chiavaroli, A., Martelli, A., Foligni, R., Mannozzi, C., Acquaviva, A., Di Simone, S., Calderone, V., Orlando, G., Ferrante, C., Veschi, S., Piro, A., Menghini, L., Brunetti, L., & Leone, S. (2023). Anti-inflammatory and vasorelaxant effects induced by an aqueous aged black garlic extract supplemented with vitamins D, C, and B12 on cardiovascular system. *Food*, *12*(7), 1558.

Ribeiro, M., Alvarenga, L., Cardozo, L. F. M. F., Chermut, T. R., Sequeira, J., de Souza Gouveia Moreira, L., Teixeira, K. T. R., Shiels, P. G., Stenvinkel, P., & Mafra, D. (2021). From the distinctive smell to therapeutic effects: Garlic for cardiovascular, hepatic, gut, diabetes and chronic kidney disease. *Clinical Nutrition*, *40*(7), 4807–4819.

Sasmaz, H. K., Sevindik, O., Kadiroglu, P., Adal, E., Erkin, Ö. C., Selli, S., & Kelebek, H. (2022). Comparative assessment of quality parameters and bioactive compounds of white and black garlic. *European Food Research and Technology*, *248*(9), 2393–2407.

Shin, J. H., Lee, C. W., Oh, S. J., Yun, J., Kang, M. R., Han, S.-B., Park, H., Jung, J. C., Chung, Y. H., & Kang, J. S. (2014). Hepatoprotective effect of aged black garlic extract in rodents. *Toxicology Research*, *30*(1), 49–54.

Shree, A., Islam, J., Vafa, A., Mohammad Afzal, S., & Sultana, S. (2020). Gallic acid prevents 1,2-dimethylhydrazine induced colon inflammation, toxicity, mucin depletion, and goblet cell disintegration. *Environmental Toxicology*, *35*(6), 652–664.

Singh, M., Arseneault, M., Sanderson, T., Murthy, V., & Ramassamy, C. (2008). Challenges for research on polyphenols from foods in Alzheimer's disease: Bioavailability, metabolism, and cellular and molecular mechanisms. *Journal of Agricultural and Food Chemistry*, *56*(13), 4855–4873.

Sirše, M. (2022). Effect of dietary polyphenols on osteoarthritis-molecular mechanisms. *Life (Basel)*, *12*(3), 436.

Sohrabi, F., Dianat, M., Badavi, M., Radan, M., & Mard, S. A. (2021). Gallic acid suppresses inflammation and oxidative stress through modulating Nrf2-HO-1-NF-κB signaling pathways in elastase-induced emphysema in rats. *Environmental Science and Pollution Research International*, *28*(40), 56822–56834.

Srinath, A. I., Walter, C., Newara, M. C., & Szigethy, E. M. (2012). Pain management in patients with inflammatory bowel disease: Insights for the clinician. *Therapeutic Advances in Gastroenterology*, *5*(5), 339–357.

Stannus, O. P., Jones, G., Blizzard, L., Cicuttini, F. M., & Ding, C. (2013). Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: A prospective cohort study. *Annals of the Rheumatic Diseases*, *72*(4), 535–540.

Suhail, M., Rehan, M., Tarique, M., Tabrez, S., Husain, A., & Zughaibi, T. A. (2022). Targeting a transcription factor NF-κB by green tea catechins using in silico and in vitro studies in pancreatic cancer. *Frontiers in Nutrition*, *9*, 1078642.

Swierczynski, M., Makaro, A., Grochowska, A., & Salaga, M. (2023). Pharmacological approaches to treat intestinal pain. *Expert Review of Clinical Pharmacology*, *16*(4), 297–311.

Takahashi, K., Khwaja, I. G., Schreyer, J. R., Bulmer, D., Peiris, M., Terai, S., & Aziz, Q. (2021). Post-inflammatory abdominal pain in patients with inflammatory bowel disease during remission: A comprehensive review. *Crohns Colitis 360*, *3*(4), otab073.

Vazquez Prieto, M. A., Bettaieb, A., Rodriguez Lanzi, C., Soto, V. C., Perdicaro, D. J., Galmarini, C. R., Haj, F. G., Miatello, R. M., & Oteiza, P. I. (2015). Catechin and quercetin attenuate adipose inflammation in fructose-fed rats and 3T3-L1 adipocytes. *Molecular Nutrition & Food Research*, *59*(4), 622–633.

Veschi, S., De Lellis, L., Florio, R., Lanuti, P., Massucci, A., Tinari, N., De Tursi, M., di Sebastiano, P., Marchisio, M., Natoli, C., & Cama, A. (2018). Effects of repurposed drug candidates nitroxoline and nelfinavir as single agents or in combination with erlotinib in pancreatic cancer cells. *Journal of Experimental & Clinical Cancer Research*, *37*(1), 236.

Vinayagam, R., Eun Lee, K., Ambati, R. R., Gundamaraju, R., Fawzy Ramadan, M., & Gu Kang, S. (2023). Recent development in black garlic: Nutraceutical applications and health-promoting phytoconstituents. *Food Reviews International*, *39*(6), 3534–3554.

Wen, L., Tang, L., Zhang, M., Wang, C., Li, S., Wen, Y., Tu, H., Tian, H., Wei, J., Liang, P., Yang, C., Li, G., & Gao, Y. (2022). Gallic acid alleviates visceral pain and depression via inhibition of P2X7 receptor. *International Journal of Molecular Sciences*, *23*(11), 6159.

Woolf, C. J., Allchorne, A., Safieh-Garabedian, B., & Poole, S. (1997). Cytokines, nerve growth factor and inflammatory hyperalgesia: The contribution of tumour necrosis factor alpha. *British Journal of Pharmacology*, *121*(3), 417–424.

Xu, X. J., Hao, J. X., Andell-Jonsson, S., Poli, V., Bartfai, T., & Wiesenfeld-Hallin, Z. (1997). Nociceptive responses in interleukin-6-deficient mice to peripheral inflammation and peripheral nerve section. *Cytokine*, *9*(12), 1028–1033.

Yang, R., Li, Z., Zou, Y., Yang, J., Li, L., Xu, X., Schmalzing, G., Nie, H., Li, G., Liu, S., Liang, S., & Xu, C. (2021). Gallic acid alleviates neuropathic pain behaviors in rats by inhibiting P2X7 receptor-mediated NF-κB/STAT3 signaling pathway. *Frontiers in Pharmacology*, *12*, 680139.

Yu, T.-Y., Feng, Y.-M., Kong, W.-S., Li, S.-N., Sun, X.-J., Zhou, G., Xie, R.-F., & Zhou, X. (2023). Gallic acid ameliorates dextran sulfate sodium-

induced ulcerative colitis in mice via inhibiting NLRP3 inflammasome. *Frontiers in Pharmacology*, *14*, 1095721.

Zeitz, J., Ak, M., Müller-Mottet, S., Scharl, S., Biedermann, L., Fournier, N., Frei, P., Pittet, V., Scharl, M., Fried, M., Rogler, G., Vavricka, S., & Swiss IBD Cohort Study Group. (2016). Pain in IBD patients: Very frequent and frequently insufficiently taken into account. *PLoS One*, *11*(6), e0156666.

Zhang, H., Deng, A., Zhang, Z., Yu, Z., Liu, Y., Peng, S., Wu, L., Qin, H., & Wang, W. (2016). The protective effect of epicatechin on experimental ulcerative colitis in mice is mediated by increasing antioxidation and by the inhibition of NF-κB pathway. *Pharmacological Reports*, *68*(3), 514–520.

Zhu, L., Gu, P., & Shen, H. (2019). Gallic acid improved inflammation via NF-κB pathway in TNBS-induced ulcerative colitis. *International Immunopharmacology*, *67*, 129–137.

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