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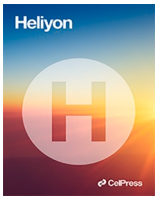

Research article

Helix pomatia mucin alleviates DSS-induced colitis in mice: Unraveling the cross talk between microbiota and intestinal chemokine

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ABSTRACT

Gut microbiota imbalance and alterations in the chemokine-chemokine receptor interactions are pivotal in the initiation and advancement of ulcerative colitis (UC). The current UC treatments are prolonged, exhibit high recurrence rates, and may lead to colorectal cancer. So, this study explores the efficacy of *Helix pomatia* (*H. pomatia*) mucin in preventing DSS-induced UC. This research focuses on investigating the underlying mechanisms, such as oxidative stress, inflammation, and alterations in gut microbiota and chemokine-chemokine receptor interactions, to understand the anti-inflammatory and antioxidant characteristics of the mucin. Using 4 % DSS in drinking water, UC was induced in C57BL/6 mice. For seven days, mice were given oral doses of either *H. pomatia* mucin or sulfasalazine. The study assessed changes in oxidative stress, gut microbiota, and histopathology, along with expression of IL-6, CXCR4, CCR7, CXCL9, and CXCL10. The *H. pomatia* mucin exhibited unique contents, including high glycolic acid (200 ± 2.08 mg/L), collagen (88 ± 2.52 mg/L), allantoin (20 ± 2 mg/L), and concentrated vitamins and minerals. Treatment with *H. pomatia* mucin in high dose demonstrated reduction in DAI, an increase in fecal *Firmicutes*, and elevated expression of colonic CCR7, CXCL9, and CXCL10, accompanied by enhanced CXCR4 (75 %) and diminished IL-6 (1.33 %) immunostaining. It also alleviated oxidative stress, reduced fecal *Bacteroidetes*, and mitigated inflammation, indicating its potential efficacy against DSS-induced UC. In conclusion, *H. pomatia* mucin is a promising candidate that could be an effective adjuvant in the management and prophylaxis of UC.

1. Introduction

Ulcerative colitis (UC) is a persistent, general inflammatory condition that specifically targets the intestines that requires strength and persistence to overcome. It is distinguished by frequent episodes of diarrhea, the existence of pus and blood in the stool, and

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abdominal pain [1]. Regretfully, due to dietary and lifestyle modifications, the number of UC patients worldwide has been sharply rising in recent years [2]. It is becoming recognized as one of the most challenging clinical conditions globally. Consequently, it is imperative to initiate treatment promptly [3]. UC development is affected by complicated interactions of genetic, microbiota alteration, immunological, and environmental factors [4]. Yet, not all of the precise mechanisms behind this process are completely understood. Despite the fact that the global prevalence and severity of UC continue to rise, a definitive medical cure for UC remains elusive, and only limited therapeutic options with anti-inflammatory and immunomodulatory properties are available [5]. In addition, the current treatments for UC are protracted and cannot effectively manage the disease course, with a high risk of remission and progression into colorectal cancer [6].

Intriguingly, the intestinal microbiota, referred to as the biological barrier of the gut, provides an essential part in preserving the immunological balance of the host and synthesizing advantageous metabolites. However, there is a connection between changes in the microbiota and the emergence of ulcerative colitis (UC) [7]. It is stated that whereas pathogenic microorganisms cause intestinal harm by interacting with immune cells or via their metabolites, they also cause the generation of inflammatory cytokines. The beneficial microbes control the activities of the host immune cells [8]. The dysbiosis or imbalance between commensal and pathogenic microorganisms aids to the initiation of intestinal disorders [9]. Moreover, the alteration of the intestinal microbial diversity disrupts the microbial barrier, leading to a reduction in diversity among microbes. This can cause an overgrowth of Gram-negative bacteria, which in turn generates large amounts of lipopolysaccharides. Consequently, there is an overproduction of reactive oxygen species (ROS) [10]. The surplus of ROS has a detrimental impact on both colon epithelial cells and immune cells, compromising the immunological barrier and exacerbating inflammation [11]. Numerous studies that used DSS-induced UC in mice models revealed that the *Bacteroidetes* phylum elevated while the *Firmicutes* phylum diminished [12–14].

Besides immune cells, fibroblasts, endothelial cells, and epithelial cells are among the cell types that invade the intestinal wall in inflammatory bowel disease (IBD), all of which have the ability to produce different chemokines. Chemokines and their receptors are crucial in the inflammatory process as they induce leukocytes' migration and retention to the specific location of inflammation in a manner that is specific to the tissue and selective to immune cells [15]. Furthermore, it has been shown that proinflammatory cytokines stimulate the synthesis of CXCL9, CXCL10, and CXCL11 in the cells that line the intestines [16]. The C-C chemokine receptor 7 (CCR7) has been detected on the surface of activated dendritic cells and naïve T lymphocytes, suggesting its function is to control these cells' movement to the lymph nodes [17]. However, subsequent research has revealed that activated T cells regain the expression of CCR7, which guides their migration from peripheral organs to afferent lymphatics [18]. Studies have shown that the level of CCR7 expression elevates throughout the active phase of ulcerative colitis (UC) [19]. Furthermore, Uo et al. [20] have discovered that plasma cells expressing CXCR4 facilitate the activation of macrophages which potentially resulting in the development of UC.

Lately, snail mucin has been blended with authorized treatments to enhance the potency of the drugs in addressing conditions like stomach ulcers. Moreover, it is being employed in the creation of skincare products, wound-healing solutions, and surgical adhesives [21]. Helix sp., a kind of gastropod, has been used for millennia for its culinary purposes. In ancient times, Hippocrates and Pliny acknowledged the therapeutic characteristics of snail mucus in healing injuries, abscesses, and other disorders [22]. Nowadays, skin regeneration, acne therapy, wound healing, and anti-aging are some of the cosmetic claims made with snail mucus, which is used in both its pure and commercial cream forms [23]. *Helix pomatia* (*H. pomatia*), a member of the *Helix* sp. family, has essential components which possess a bronchodilator action that results from a substantial rise in the prostaglandin E2 [24]. The previously described biological properties of snail mucus are mostly ascribed to its essential constituents, namely elastin, collagen, glycolic acid, and allantoin, each of which plays a function in mitigating the damage caused by oxidative damage and inflammation [25].

Thus far, there has been no prior study undertaken to examine the impact of *H. pomatia* mucin on preventing the initiation of ulcerative colitis. Moreover, there have been no previous studies conducted to assess the regulatory effects of *H. pomatia* mucin on the levels of *Firmicutes* and *Bacteroidetes* bacteria in fecal matter, as well as the interactions between chemokines and chemokine receptors that take place throughout the progression of ulcerative colitis. The objective of this research was to assess the effectiveness of *H. pomatia* mucin in the treatment of UC caused by DSS and also investigate its mechanisms of action, particularly focusing on oxidative stress, alterations in the gut microbiota, and modifications in the interactions between chemokines and chemokine receptors.

2. Materials and methods

2.1. Chemicals and reagents

Sulfasalazine (SSZ), specifically the brand Colosalazine-EC®, was obtained from The Arab Company for gelatin and pharmaceutical items in Alexandria, Egypt. The batch number for this purchase is 622400588113. The Dextran sulfate sodium (DSS) was purchased from a Biodiagnostic Company in Giza, Egypt. The DSS was sourced from CHEM-LAB in Zedelgem, Belgium and its CAS number is 26.4361710. Biodiagnostic Company also supplied all of the analytical kits used in the study.

2.2. Collection of mucin

Snails of the species *H. pomatia* were collected from several areas within the Giza Governorate. Plastic cages with perforated lids were made in the laboratory, and sterilized dirt was added as a bottom layer [26]. For one week, the snails were placed in these cages under controlled laboratory settings, given fresh lettuce leaves, and sprayed with water. The snails were allowed to fast for three days before collecting their mucin [25]. Subsequently, each snail was thoroughly cleansed using distilled water to remove any dirt or fecal matter. To stimulate the formation of mucin, the pedal glands in the foot area of the snails were delicately stimulated using a sterile

cotton swab tip [27]. At a temperature of −80 °C, the mucin was extracted and kept in a sterile 50 mL falcon tube. Before manipulating the mucin, it went through a series of filtering steps through three distinct filters with progressively smaller particle sizes (10 μL, 1 μL, and 0.22 μL). Subsequently, it was maintained at 4 °C.

2.3. Physical and chemical characterization of mucin

The physical characteristics of mucin were determined according to **Horwitz et al.** [28]. The allantoin and glycolic acid contents were chemically assessed with high-performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan, and CLASS-M10A) [29]. The Bradford technique was used to quantify the total protein content, with bovine serum albumin serving as the reference standard [30]. Elastin was quantified using the Fastin Elastin assay [31], while collagen was evaluated with the Sircol Collagen assay [32]. The levels of all-trans-retinol, 13-cis-retinol, as well as vitamins A and E, were assessed by **Lee et al.** [33]. The quantification of vitamin C was performed using spectrophotometry, according to the procedure outlined by **Jagota et al.** [34]. The acid hydrolysis technique was used to evaluate the concentrations of vitamins B1 and B2 [35], However, the technique described by **Jagota and Dani** [36] was used to assess the amounts of vitamins B3, B6, and B12.

The mineral concentration of the mucus was determined using atomic absorption spectroscopy. At first, 7 mL of a solution containing 70 % nitric acid (HNO3) was introduced into the sample, which was held in a test tube. Additionally, a cooling agent was also included. The mixture was subjected to temperatures of 50, 90, and 140 °C for a period of 20 min each. Subsequently, the temperature was raised to 200 °C and maintained for 40 min to allow for the condensation of the gases produced during the mineralization process. Following the mineralization process, all the organic molecules underwent conversion into H_2O and CO_2, and all of the metals changed into soluble nitrate salts. Following that, one mL of 40 % H_2O_2 was added to fully oxidize the organic constituents, where the sample was preserved at 200 °C for 20 min, constituting the stripping process. Finally, the specimen was retrieved in a 20 mL flask filled with milliQ water, filtered, and analyzed using atomic adsorption spectroscopy [37].

2.4. Microbiological characterization

Petri dishes with solidified Tryptic Soy agar (Biomerieux, Italy) were inoculated with 100 μL of *H. pomatia* mucin to detect microbial contamination. After a period of 24–48 h of incubation at a temperature of 37 °C, the Petri dishes were examined for any bacterial growth [37]. The presence of contaminating fungi was assessed by inoculating 100 μL of *H. pomatia* mucin into Sabouraud dextrose agar medium (Biomerieux, Italy), and then observing the fungal growth after incubation period from 3 to 7-day at 28 °C.

2.5. Experimental animals

The male C57BL/6 mice were acquired from the animal house of the Theodor Bilharz Research Institute (TBRI) in Giza, Egypt. The mice weighed 20–25 g and were 6–8 weeks old. The animals were housed in conditions with a humidity range of 50 %–60 %, a day/night cycle of 12 h, and a steady 25 °C temperature throughout the experiment. Food and water were available to them at all times.

2.6. Experimental design

A total of thirty mice were distributed at random into five groups, with each group consisting of six mice. Group 1: Normal mice were administered 2 % Cremophore-El as the treatment vehicle to for a week. Group 2: For seven days, 4 % (w/v) solution of DSS was mixed into the mice's drinking water [38]. Groups 3–5 comprised of mice with ulcerative colitis produced by DSS. These animals were given orally with SSZ at a dosage of 200 mg/kg [39] or with *H. pomatia* mucin at doses of 7.5 and 15 mL/kg, respectively [40]. Daily assessments were made to determine body weight, stool consistency, and bleeding. On the eighth day of the experiment, feces samples were collected in a sterile manner for assessment of fecal *Firmicutes* and *Bacteriodetes* contents. The mice were weighed and promptly beheaded while under mild anesthesia caused by thiopental (50 mg/kg, i.p.), and thereafter, a colon dissection was performed. Subsequently, the colon was meticulously extracted for further examination. A morphometric assessment of inflammation was undertaken by quantifying the distance between the ileocecal junction and the anal border. After being cut into 5 μm-thick sections, the distal portion of the colon was preserved in a 4 % formalin solution. A separated portion of the colon was excised, cleansed with an ice-cold saline solution, dried, weighted, and homogenized to provide a 10 % homogenate for biochemical analysis and RNA extraction.

2.7. Disease activity index (DAI)

Three criteria were combined to determine the DAI: diarrhea (0, normal; 2, loose stools; 4, watery diarrhea); blood in the stool (0, no bleeding; 2, minor bleeding; 4, significant bleeding); and body weight loss (0, ≤1 %; 1, 1–5%; 2, 5–10 %; 3, 10–15 %; 4, >15 %) [41].

2.8. Colonic oxidative stress markers assessment

The levels of reduced glutathione (GSH), catalase (CAT), malondialdehyde (MDA), and nitric oxide (NO) were quantified in colon homogenates using spectrophotometrically kits.

Table 1
Sequences of primers for quantitative real-time PCR.

Target gene(s)	Amplicon length (bp)	Primer sequence
Firmicutes	126	Forward primer: 5- GAGYATGTGGTTTAATTCGAAGCA-3 Reverse primer: 5- AGCTGACGACAACCATGCAC-3
Bacteroidetes	136	Forward primer: 5-GAGAGGAAGGTCCCCCAC-3 Reverse primer: 5-CGCTACTTGGCTGGTTCAG-3
Universal bacteria	118	Forward primer: 5′GGGAATGGGTCAGAAGGACT-3′ Reverse primer: 5′-CTTCTCCATGTCGTCCCAGT-3′

2.9. Quantification of Firmicutes and Bacteriodetes contents in fecal samples

The fecal *Firmicutes* and *Bacteriodetes* contents were assessed by isolating bacterial DNA from the fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen Hidden®, Germany) following the manufacturer's instructions. A total amount of 20 μL was produced, including 5 pmoles of each primer, 10 μL of 2x SYBR Green PCR Master Mix (Power upTM SYBRTM Green Master Mix, Thermo Scientific, USA), and 5 μL of the extracted DNA. Table 1 lists the primer sequences for *Bacteriodetes* and *Firmicutes*. The levels of *Firmicutes* and *Bacteroidetes* in the feces were determined and adjusted based on the standard gene (Universal bacteria).

2.10. Histopathological examination of colon tissue

The distal colon tissues of mice were preserved in 10 % buffered formalin. Afterward, following their immersion in paraffin, the tissues were sectioned that were 5 nm thick, and then treated with hematoxylin and eosin (H&E) stain. At × 200 magnification, the sections were evaluated using Zeiss Germany's Axiovision version 4.8 computerized image processing system. According to the inflammation level and the existence of edema and/or ulceration, the colonic tissue was microscopically graded for damage.

2.11. Immunohistochemical examination (IHC) of IL-6 and CXCR4

The paraffin slices underwent deparaffinization, followed by rehydration and rinsing in Tris-buffered saline after being deparaffinized in xylene. The inherent peroxidase activity was suppressed through exposing it to a 5-min incubation in a solution of 3 % hydrogen peroxide in methanol. For 20 min, the non-specific binding sites were blocked using the non-serum Protein Block (DAKO, Carpinteria, CA). Sections were microwaved in citrate buffer for 15 min to extract antigens for monoclonal anti-IL-6 and CXCR4 antibodies (Santa Cruz Biotechnology, USA). A further 60 min of administration of a secondary antibody (Agilent Dako, CA, USA) followed the PBS rinse. The reaction was examined using the chromagen 3,3′-diaminobenzidine (Agilent Dako, CA, USA). After staining with hematoxylin, the slides were mounted and examined thereafter. For every sample, 10 areas were measured consecutively at a magnification of ×400 to ascertain the percentages of cytoplasmic IL-6 and CXCR4 positive cells.

2.12. CCR7, CXCL9, and CXCL10 genes expression analysis

Total RNA was extracted from colon tissues using the RNeasy Mini kit (Qiagen, Germany), under the manufacturer's instructions. The RNA's purity and concentration were evaluated using Thermo Fisher Scientific's NanoDrop 2000 spectrophotometer. Next, using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit, the RNA was reverse-transcribed. 10 μl 2 × PoweUpTM SYBRTM Green/ROX PCR Master Mix (Applied Biosystems, USA) was used to conduct the PCR reactions. Ready-made primers for CCR7, CXCL9, and CXCL10 are available from Qiagen in the United States. To evaluate the relative expression, the comparative cycle threshold (C_t) ($2^{-\Delta\Delta CT}$) approach was used [42]. As an endogenous control, the genes under investigation were regulated by the βeta-actin expression.

2.13. Statistical analysis

The data was presented using the mean ± SEM. SPSS, version 16.0 (Chicago, IL, USA), was used to conduct a one-way ANOVA test and a Tukey post-hoc test. The purpose was to determine whether the groups under investigation had mean values that differed statistically significantly. P values of less than 0.05 determined that the findings were statistically significant.

3. Results and discussion

The current research was to assess the effectiveness of *H. pomatia* mucin in the treatment of DSS-induced UC and to explore the underlying processes, specifically related to oxidative stress, gut microbiota composition, and changes in chemokine-chemokine receptor interactions. In fact, the components of *H. pomatia* make it a one-of-a-kind organism that cannot be substituted by synthetic substances. Table 2 provides an in-depth explanation of the chemical composition and microbiological status of *H. pomatia* mucin in the present study. The findings revealed that *H. pomatia* mucin possessed distinct characteristics, including elevated glycolic acid and collagen concentration, in addition to allantoin and elastin. The results of the microbiological investigation revealed that the mucin

Table 2
Physical, chemical and microbiological characteristics of *Helix pomatia* mucin.

Characteristics	Value (Mean ± SEM)
Aspect	Clear
Color	Pale Yellow
Smell	Odourless
Ph	4.80 ± 0.05
Density	1.10 ± 0.10
Dry residual (g/ml)	3.20 ± 0.25
Yield (%)	0.19
Proteins (mg/L)	240 ± 1.53
Glycolic acid (mg/L)	200 ± 2.08
Allantoin (mg/L)	21 ± 2
Polyphenols (mg/L)	77 ± 3
Elastin (g/100g)	0.094 ± 0.005
Sugars (g/L)	0.031 ± 0.001
Collagen (mg/L)	88 ± 2.52
Gram + (CFU)	–
Gram – (CFU)	–
Fungi (CFU)	–
All-trans-retinol (μg/100g)	11 ± 2
13-cis-retinol (μg/100g)	8 ± 0.76

Table 3
Vitamin content of *Helix pomatia* mucin.

Vitamin	Concentration (Mean ± SEM)
E (mg/kg)	0.15 ± 0.01
C (mg/kg)	0.13 ± 0.006
A (μg/mL)	0.87 ± 0.01
B1 (μg/mL)	3.02 ± 0.11
B2 (μg/mL)	0.65 ± 0.04
B3 (μg/100g)	9 ± 0.85
B6 (μg/mL)	18.31 ± 0.15
B12 (μg/100g)	9 ± 0.49

Table 4
Minerals and heavy metals content of *Helix pomatia* mucin.

Minerals and heavy metals	Value (Mean ± SEM)
Ca (mg/kg)	1285 ± 1.53
Mg (mg/kg)	534 ± 3
Fe (mg/kg)	16.51 ± 0.1
Na (mg/kg)	902 ± 1.99
P (mg/kg)	1055 ± 0.58
K (mg/kg)	815 ± 1.52
Cr (mg/kg)	0.008 ± 0.002
Cu (mg/kg)	5.01 ± 0.01
Hg (μg/kg)	0.22 ± 0.025
Cd (μg/kg)	0.014 ± 0.002
Co (μg/kg)	0.001 ± 0.001
Ni (μg/kg)	0.83 ± 0.001
Zn (mg/mL)	1.12 ± 0.01
Mn (mg/mL)	0.54 ± 0.025

was sterile, as it was devoid of bacterial and fungal growth.

According to our investigation, vitamin B6 (the vitamin with the greatest concentration) was present in 100 g of *H. pomatia* mucus, as well as noteworthy concentrations of vitamins B3 and B12, besides significant levels of vitamins B1, A, B2, E, and C (Table 3). All of these vitamins have been proven to have key roles in various physiological functions of the human body [43].

The distinctive defensive features of *H. Pomatia* are believed to be attributed to its mineral components. *H. pomatia* had a high mineral concentration, as shown in Table 4. The concentration of calcium was the highest, followed by potassium, phosphorus, sodium, magnesium, and iron. In addition, Cr, Cu, Hg, Cd, Co, Ni, Zn, and Mn were found in *H. pomatia* mucin (Table 4).

In 2023, the global estimated cases of UC were 5 million, and there is a rising incidence worldwide [44]. UC is believed to manifest in individuals who have a genetic predisposition after exposure to certain environmental factors. There is substantial evidence linking

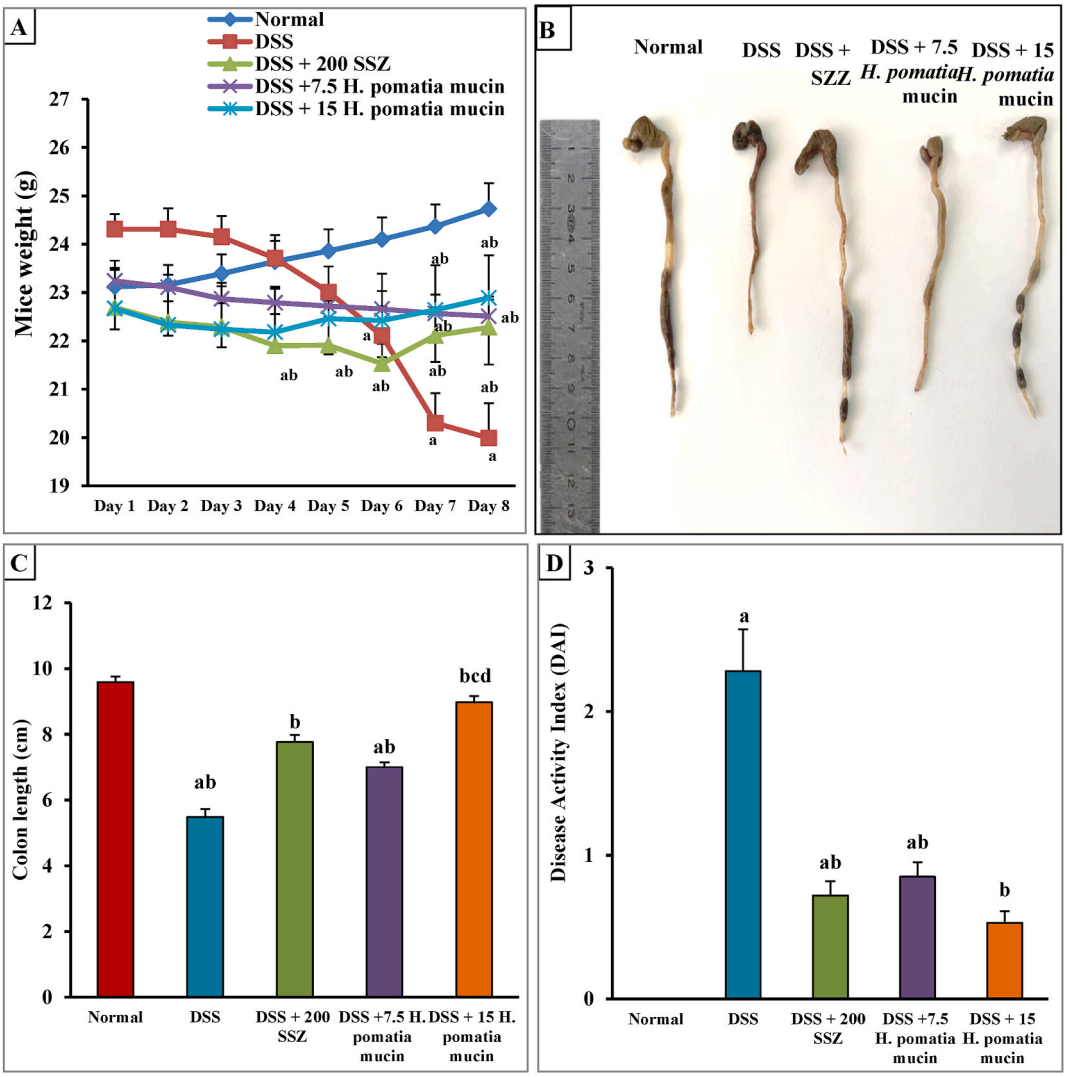

Fig. 1. Effect of *H. pomatia* mucin on clinical symptoms in the studied groups of mice. Daily body weight variations (A), colon length representative images (B), colon length quantitative analysis (C), and DAI were evaluated on the eighth day of the study (D).

the involvement of variables such as dysbiosis, immune response disorder, and abnormalities in the gut epithelial barrier [44]. Recent research has emphasized the significance of microbial dysbiosis and abnormal gut immunity as crucial elements that trigger the inflammatory responses in UC [45]. Modifying the interaction between gut microbiota and intestinal immunity is proposed as a vital strategy for alleviating UC [45]. Finding a safe therapeutic strategy that may improve intestinal immunity and alter gut microbial dysbiosis is thus crucial for the efficient UC management. In this research, ulcerative colitis (UC) was successfully generated in mice by providing a 4 % (w/v) dextran sulfate sodium (DSS) solution by drinking. This approach mimicked the pathological alterations observed in individuals with ulcerative colitis, including severe diarrhea, loose stool, and obvious blood in the feces, leading to substantial weight loss and infiltration of inflammatory cells [46,47]. According to multiple investigations, giving DSS orally to UC mice may closely resemble the pathophysiology and histological characteristics of UC in humans [48,49]. The ongoing work has effectively confirmed the development of acute colitis in mice by the administration of DSS. This is supported by the existence of characteristic signs of ulcerative colitis, which include weight loss, reduction in colon length ulceration, and bloody diarrhea. Throughout the 7-day trial, the mice that were not subjected to any special conditions exhibited a consistent and gradual rise in their body weight. DSS, in contrast, significantly decreased the body weight as opposed to the mice in the normal control group ($p < 0.001$) (Fig. 1A). As opposed to the DSS-induced mice, the groups treated with SSZ and either dose of the *H. pomatia* mucin showed significant restoration of their body weight ($p < 0.05$) (Fig. 1**A**). Furthermore, DSS administration led to a marked colon shortening indicating inflammation in the colon as opposed to the normal control group ($p < 0.001$) (Fig. 1 B&C). A 7.5 mL/kg dose of *H. pomatia* mucin treatment significantly increased colon shortening. SSZ or *H. pomatia* mucin (15 mL/kg) treatment was shown to significantly improve colon shortening recovery (Fig. 1 B&C). AI scores, which indicate the severity of colitis, significantly increased in mice fed DSS alone

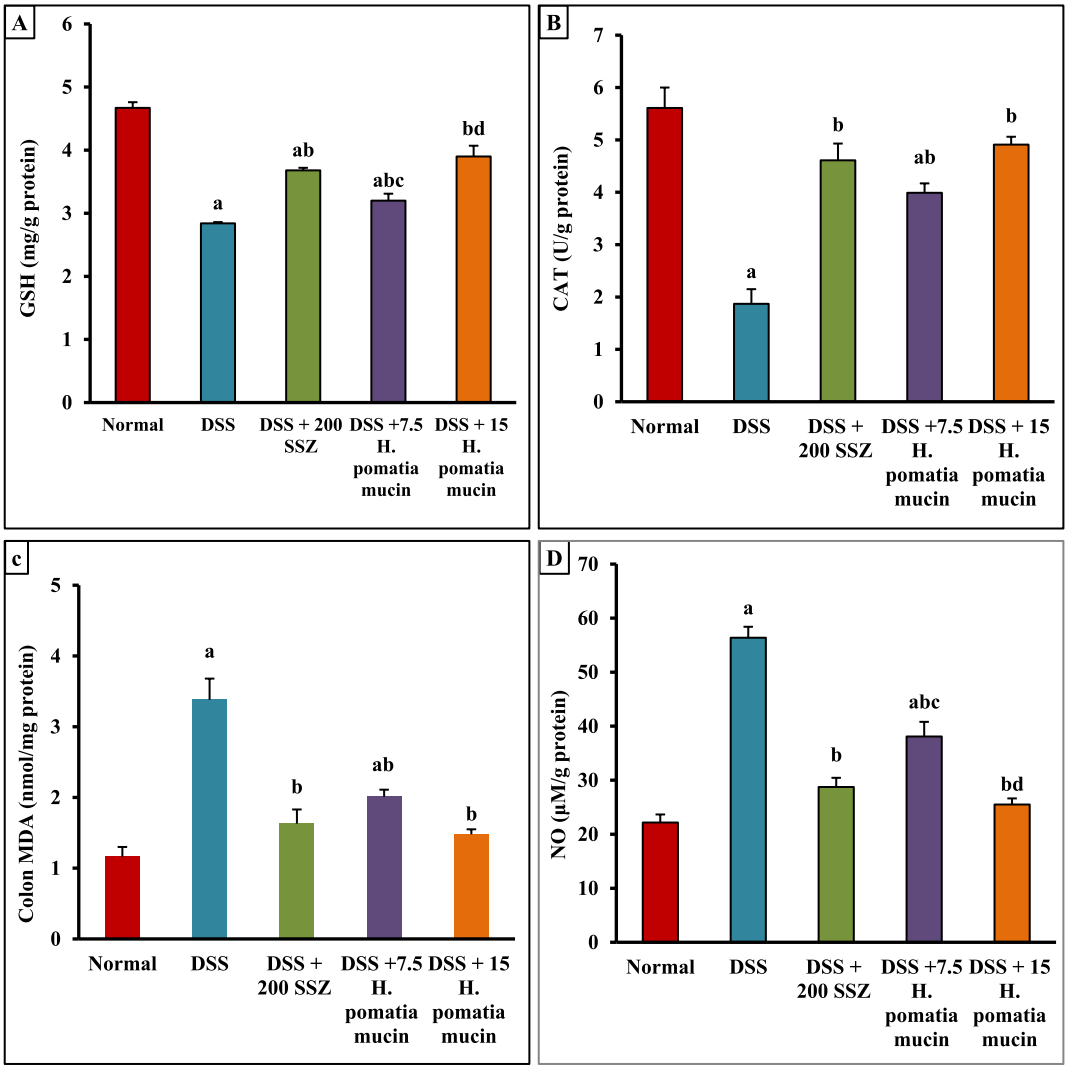

Fig. 2. Effect of *H. pomatia* mucin on colonic reduced glutathione (GSH) (A), catalase (CAT) (B), malondialdehyde (MDA) (C), and nitric oxide (NO) (D) contents in the studied groups of mice.

($p < 0.05$) (Fig. 1 D). Nevertheless, the DAI scores were considerably ($p < 0.05$) decreased with the injection of either SSZ or *H. pomatia* mucin (7.5 mL/kg) (Fig. 1 D). As opposed to other treatments, *H. pomatia* mucin (15 mL/kg) improved DAI scores more significantly in mice.

The primary contributing factors to IBD include oxidative stress and impaired scavenging of free radicals that enhance the generation of ROS and inducible nitric oxide synthase (iNOS) [50]. The increase in ROS and iNOS levels leads to a reduction in the levels of catalase and glutathione antioxidants, initiating a cascade of reactive oxygen metabolites and ultimately causing lipid peroxidation [51]. Damage is caused by the oxidation of lipids, proteins, and DNA when reactive oxygen species (ROS) are overproduced. The present research observed a notable decrease ($p < 0.001$) in colonic GSH (Fig. 2 A) and CAT (Fig. 2 B) levels in mice with DSS-induced UC. GSH, a tri-peptide molecule, is an essential antioxidant found inside cellular structures due to its sulfhydryl (-SH) group. Under situations of aberrant oxidative stress, GSH plays a vital role in counteracting free radicals by converting them into their oxidized form (GSSG). Additionally, catalase (CAT), an essential antioxidant enzyme, is responsible for effectively eliminating O^{2-} from the biological system. MDA (Fig. 2C) and nitric oxide (NO) (Fig. 2 D) concentrations showed an elevated level ($p < 0.001$). This increase indicates lipid peroxidation, a damaging process occurring inside the phospholipid compartments of cellular membranes. Additionally, peroxynitrite anion ($ONOO^{-}$), an extremely powerful cytotoxic molecule, is formed when excessive quantities of nitric oxide (NO) react with superoxide anions (O^{2-}). Increased production of inducible nitric oxide synthase (iNOS) in colon tissue may explain the higher levels of nitric oxide (NO) observed in this research. These results were consistent with previous studies [52]. Conversely, colonic GSH and CAT levels were significantly elevated in mice given 7.5 mL/kg of *H. pomatia* mucin, whereas colonic MDA and NO levels were significantly decreased. This signifies a revival of the ability to readicate free radicals. Significantly, the removal of damaging free radicals from the colon of mice with UC was more significantly restored after receiving SSZ + *H. pomatia* mucin (15

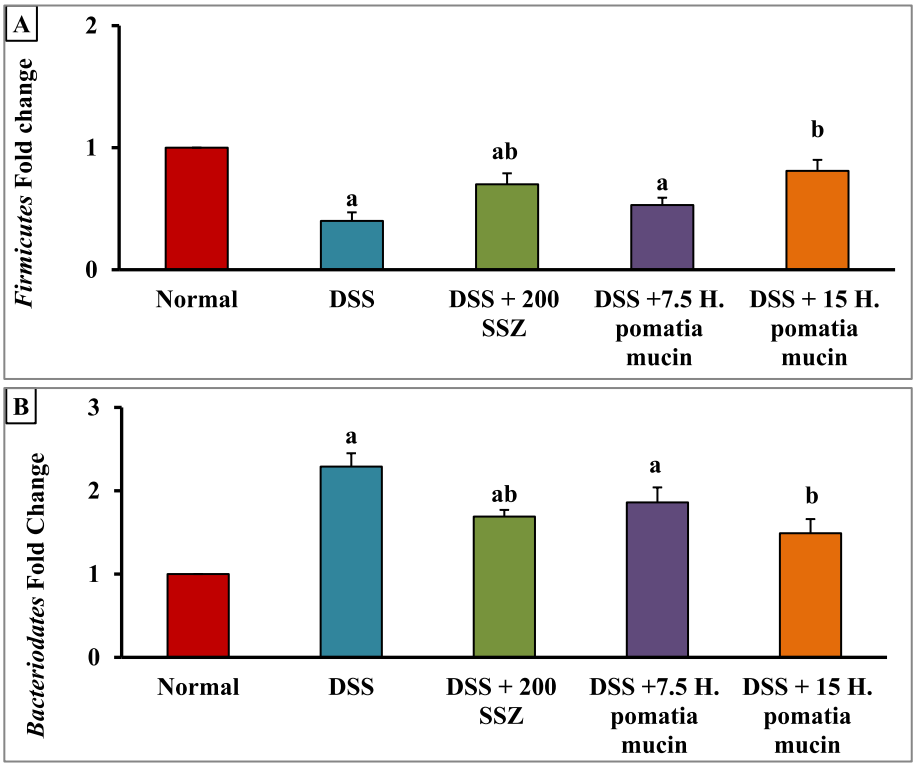

Fig. 3. Effect of *H. pomatia* mucin on fecal *Firmicutes* and *Bacteriodetes* abundance expressions in the studied groups of mice.

mL/kg).

The host creates a vital nutrient-rich environment for the gut microbiota, and in return, the microbiota provides essential vitamins, neurotransmitters, and short-chain fatty acids (SCFAs), establishing a mutually beneficial relationship [53]. It's worth noting that individuals with IBD often exhibit reduced gut microbial diversity and dysbiosis as distinctive features. Pathogens cause imbalances in the gut microbiota, resulting in variations in its composition, diversity, and abundance, ultimately triggering a compromised intestinal barrier and the progression of ulcerative colitis (UC) [54]. The balance between Firmicutes and Bacteroidetes, the most predominant gut microbiota phyla, is linked to maintaining physiological equilibrium, and alterations in this ratio can give rise to various pathological conditions. Therefore, the manipulation of gut microbiota may potentially be applied as a therapeutic strategy for the UC treatment. The DSS-induced UC group exhibited a notable reduction ($p < 0.001$) in the abundance of *Firmicutes* in fecal samples, while there was a substantial elevate ($p < 0.001$) in the number of *Bacteroidetes* as opposed to the normal control group. These results suggest the existence of dysbiosis in the microbiota (Fig. 3A and B). Bacteria belonging to the phylum Firmicutes have firm or semi-rigid cell walls. These bacteria are mostly found in the genera Bacillus, Clostridium, Enterococcus, Lactobacillus, and Ruminicoccus. These results are consistent with the study carried out by **Nagao-Kitamoto et al.** [55], which concluded that the levels of Firmicutes and Bacteroidetes are notably lower in individuals with inflammatory bowel disease (IBD). The treatment of mice with the low dose of *H. pomatia* mucin didn't have a substitutable effect on the fecal *Firmicutes* and *Bacteroidetes* abundance. Yet, the SZZ caused a noticeable elevate in fecal *Firmicutes* and a considerable decline in the high prevalence of *Bacteroidetes* ($p < 0.05$) which primarily produce elevated acetate levels may have harmful effects. Notably, high dose (15 mL/kg) of *H. pomatia* mucin markedly improved the dysbiosis as demonstrated by the normalization of fecal *Firmicutes* and *Bacteroidetes* abundance (Fig. 3A and B). Our results showed that *Helix pomatia* mucin may enhance the growth of *Firmicutes*, which primarily produce butyrate, thereby increasing its levels in the colon. Butyrate aids in the integrity of the stomach barrier by regulating Claudin-1 and limiting pro-inflammatory cytokines. The gut microbiota also metabolizes main bile acids into secondary bile acids. Butyrate enhances the gut barrier's integrity by promoting the synthesis of mucus, which serves as a lubricant and physical barrier against germs, toxins, and acidic conditions that arise during digestion. These results align with the histopathological examinations in Fig. (4-6). Therefore, the mechanism of action underlying the use of *H. pomatia* mucin in mitigating colitis severity may be related to its capacity to alter the gut microbiota's composition, which can be attributed to its unique constituents. The use of collagen peptides resulted in notable alterations in the gut microbiota and might have the ability to be used as an additional therapeutic agent to avoid the onset of obesity [56]. Enhancing intestinal barrier function and preserving immune system normalcy are two benefits of vitamin C and E's ability to modify the composition and metabolic activity of the gut microbiota [57]. Additionally, a significant association was shown to exist between the prevalence of the *Firmicutes* phylum and vitamin B1 [58]. The endogenous production of Vitamin B6 in the gastrointestinal tract is inadequate to satisfy the daily nutritional needs of the host. This deficiency leads to significant alterations in intestinal metabolite levels, as evidenced by the results in rats

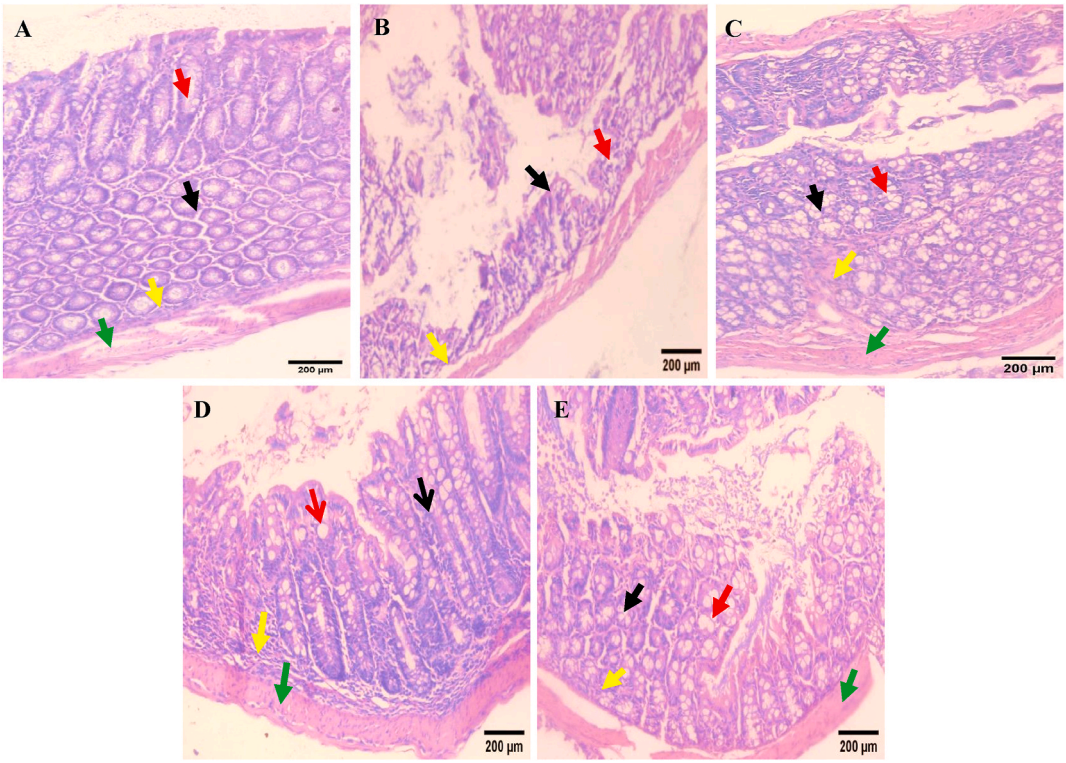

Fig. 4. Effect of *H. pomatia* mucin on colon histopathological changes (H & E; ×200) in normal control (A), 4 % (w/v) DSS-induced UC (B), DSS-induced UC treated with SSZ (200 mg/kg) (C), DSS-induced UC treated with a low dose of *H. pomatia* mucin (7.5 mL/kg) (D), and DSS-induced UC treated with a high dose of *H. pomatia* mucin (15 mL/kg) (E). The mucosal epithelial lining cells are shown by black arrows, the goblet cells by red arrows, the lamina propria by yellow arrows, and the muscle layer by green arrows.

opposed to the control groups [59]. Likewise, a lack of vitamin B12 in the diet is likely to affect the development of gut bacteria. Furthermore, the administration of vitamin B12 to people increased the proportion of Prevotella, while causing a reduction in the quantity of *Bacteroidetes* [59–61].

The colonic mucosa sections from the normal mice revealed a typical mucosal epithelial lining architecture with regular profile and arrangement of glands and preserved goblet cells, as well as a preserved lamina propria, and muscle layer with rare inflammatory cells (Fig. 4 A). The colonic mucosa sections from the group treated with 4 % (w/v) DSS exhibited significant epithelial atrophy, characterized by the lack of glands and flattening of the mucosa. Additionally, the mucosa was infiltrated with both acute and chronic inflammatory cells, and the lamina propria was replaced by collagen and inflammatory cells (Fig. 4 B). However, the SSZ-treated group colonic sections showed intact colon architecture and regular profile and regular arrangement glands with preserved goblet cells and mild inflammatory infiltrate in the lamina propria (Fig. 4C). Administering *H. pomatia* mucin at a dosage of 7.5 mL/kg almost restored the normal mucosa architecture, with regular gland profile and preserved goblet cells. The lamina propria was infiltrated with dispersed inflammatory cells while the muscular layer seemed normal (Fig. 4 D). The treatment of mice with *H. pomatia* mucin at a dosage of 15 mL/kg resulted in normal colonic mucosa architecture with typical regular profile and arrangement of glands and preserved goblet cells, with normal appearance of the lamina propria and muscle layer (Fig. 4 E).

Mucosal immune cells are involved in the creation and release of interleukin-6 (IL-6). This cytokine has the ability to initiate inflammatory responses, maintain gastric homeostasis, and facilitate the regeneration of the intestine following damage [62]. It is released in response to an elevation in bacterial pathogen species and disruptions in the microbiome balance and implicated in severe inflammatory responses and detrimental reactions [62]. In the current investigation, a negative expression of IL-6 was noticed in the epithelial cells and lamina propria in the colon sections from normal mice (Fig. 5 A). Nevertheless, the group of mice with UC had a substantial rise (47.50 %) in the presence of IL-6 in the cytoplasm of the cells lining the atrophic colonic mucosa. There was a less noticeable presence of IL-6 in the mononuclear cells of the lamina propria (Fig. 5 B & F). This outcome is consistent with **Zhang et al.** [63] who indicated that interleukin-6 (IL-6) was shown to be excessively produced in a mouse model of ulcerative colitis (UC) and was implicated in the advancement of the disorders. Conversely, treatment with either SSZ or *H. pomatia* mucin (7.5 mL/kg) lowered the expression of IL-6 (29.17 % and 21.67 %, epithelial cells and lamina propria mononuclear cells (Fig. 5C and D & F). Still, excellent recovery of colon IL-6 was noticed in the DSS-induced mice following the treating with *H. pomatia* mucin (15 mL/kg) as an almost normal level of IL-6 (1.33 %) (Fig. 5E and F) was detected in the colon epithelial cells.

Intestinal epithelial cells demonstrate distinct expression patterns of various chemokines and chemokine receptors. In addition to facilitating immune cell infiltration and activation, these receptor-ligand interactions also trigger intestinal epithelial cells' survival

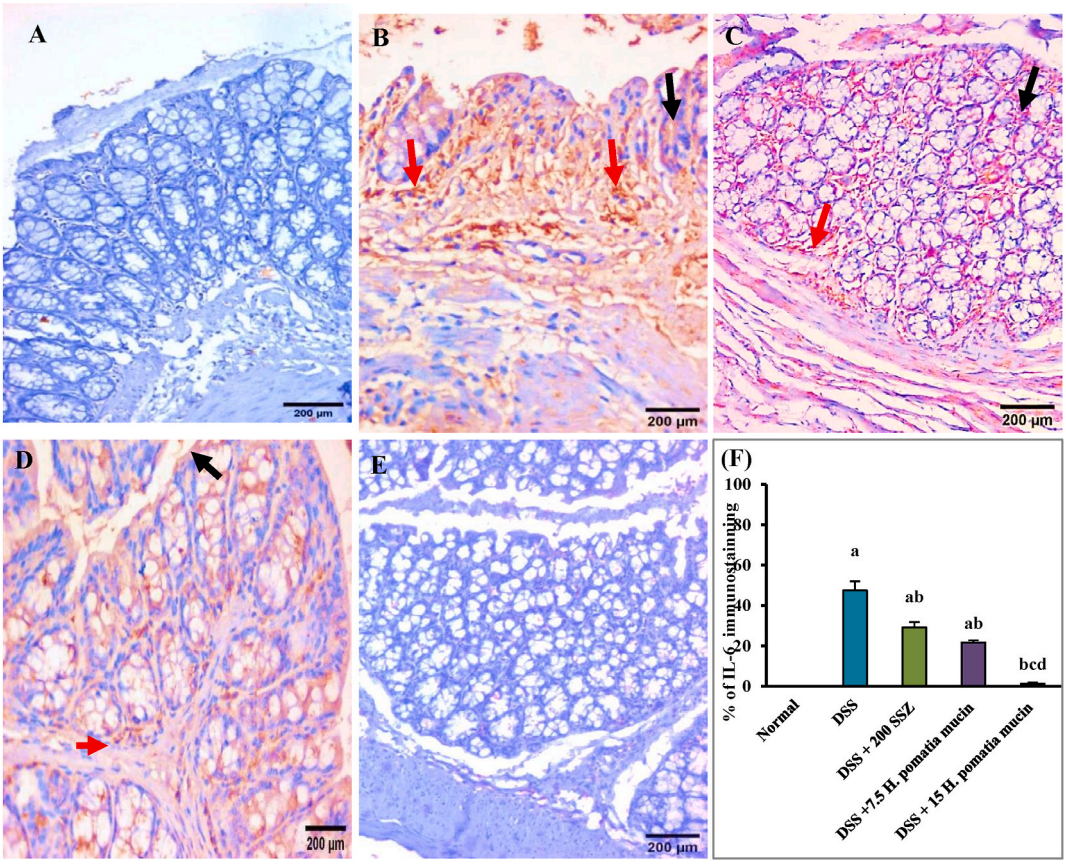

Fig. 5. Effect of *H. pomatia* mucin on colonic IL-6 immunostaining changes (DAB, IHC, IL-6, ×400) in normal control (A), 4 % (w/v) DSS-induced UC (B), DSS-induced UC treated with SSZ (200 mg/kg) (C), DSS-induced UC treated with a low dose of *H. pomatia* mucin (7.5 mL/kg) (D), DSS-induced UC treated with a high dose of *H. pomatia* mucin (15 mL/kg) (E), and (F) % of IL-6 immunostaining. Red arrows represent lamina propria mononuclear cells, while black arrows represent mucosal epithelial lining cells.

pathways [64]. In this study, a marked expression of CXCR4 (67 %) was detected in the colon epithelial cells and lamina propria of normal mice (Fig. 6 A & F). Meanwhile, the colonic mucosa of the DSS-induced UC group showed moderate expression of CXCR4 in the lamina propria mononuclear cells and modest cytoplasmic brownish expression in the atrophic mucosal epithelial lining cells (8 %) (Fig. 6 B & F). In contrast, treatment with either SSZ or *H. pomatia* mucin (7.5 mL/kg) resulted in a substantial elevation in the CXCR4 cytoplasmic expression (28 % and 51 %, respectively) in both the colon epithelial and lamina propria mononuclear cells (Fig. 6C and D & F respectively). Nevertheless, a perfect recovery of colonic CXCR4 was noticed in UC mice after being treated with *H. pomatia* mucin at a dose of 15 mL/kg, where a pronounced expression of CXCR4 (75 %) was detected in both the colon epithelial and lamina propria mononuclear cells (Fig. 6E and F).

As well, the DSS caused a substantial up-regulation ($p < 0.001$) in the expression of colonic CCR7, CXCL9, and CXCL10. Conversely, mice treated with SSZ or *H. pomatia* (7.5 mL/kg) demonstrated a significant decline in colonic CCR7, CXCL9, and CXCL10 expression. However, the oral administration of *H. pomatia* (15 mL/kg) normalized the expression of colonic CCR7, CXCL9, and CXCL10 (Fig. 7A and B & C). These results align with the histopathological examinations in Fig. (4-6).

In this study, *H. pomatia* mucin was found to contain unique components, including abundant mineral and vitamin concentrations, glycolic acid, collagen, and allantoin. Many of these components may have a vital function in the anti-inflammatory activity of *H. pomatia* mucin, particularly through interactions with chemokine receptors to modulate inflammatory responses in UC. Earlier research found that glycolic acid inhibits the levels of mRNA and protein of nuclear factor kappa B-dependent inflammatory mediators produced by solar ultraviolet B [65]. Moreover, T-cell migration and their capacity to eradicate cancer cells may be impacted by collagen in the tumor microenvironment, suggesting that bone collagen peptides could modulate immune response and inflammatory processes [66,67]. Additionally, *H. pomatia* mucin is rich in vitamins such as vitamins E, C, B1, B3, and B6. Immune cell membranes contain polyunsaturated fatty acids that are susceptible to oxidative damage from high metabolic activity and pathogen defense; vitamin E's preventive impact against this oxidation is one reason for its reputation for modulating immune function [68]. It has been shown that vitamin E supplements affect inflammation and the immune system via several methods, such as changes in signal transduction, membrane integrity, and the regulation of inflammatory mediators and cell cycles [69]. Numerous aspects of neutrophil activity, including phagocytosis and migration in response to inflammatory mediators (chemotaxis), are hypothesized to be impacted

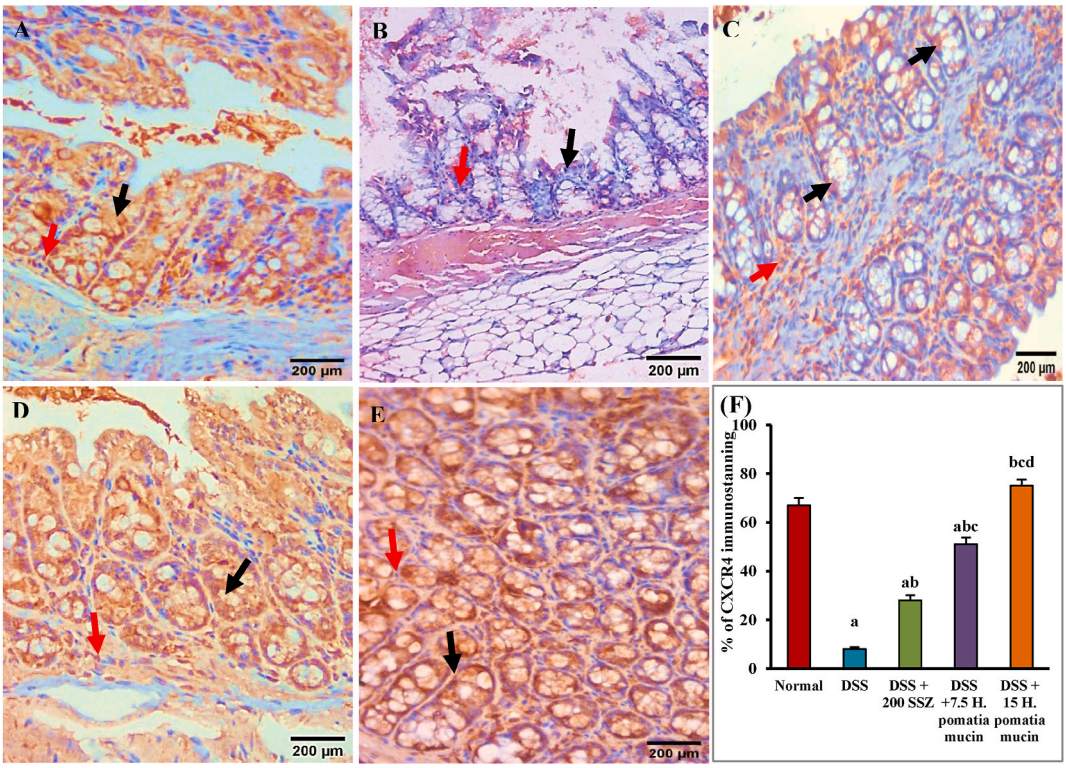

Fig. 6. Effect of *H. pomatia* mucin on colonic CXCR4 immunostaining changes (DAB, IHC, CXCR4, ×400) in normal control (A), 4 % (w/v) DSS-induced UC (B), DSS-induced UC treated with SSZ (200 mg/kg) (C), DSS-induced UC treated with a low dose of *H. pomatia* mucin (7.5 mL/kg) (D), DSS-induced UC treated with a high dose of *H. pomatia* mucin (15 mL/kg) (E), and (F) % of CXCR4 immunostaining. The mucosal epithelial lining cells are shown by black arrows, whereas the lamina propria mononuclear cells are indicated by red arrows.

by vitamin C [70]. The administration of a retention enema containing vitamin B3 was shown to successfully stimulate the repair of the mucosal lining in individuals with ulcerative colitis. This beneficial effect is probably due to the suppression of proinflammatory gene expression and the decrease in colonic inflammatory cytokines [71]. Moreover, studies have shown that a large amount of vitamin B6 may effectively reduce inflammation caused by lipopolysaccharide in monocyte/macrophage cells. This is achieved by suppressing the production of several inflammatory substances [72]. C57BL/6 mice that are deficient in vitamin B12 are protected against DSS-induced inflammations [73]. In contrast, Previous studies have shown that rats with a vitamin B12 deficit have a diminishing in cell differentiation and intestinal barrier function [74]. Furthermore, individuals with a vitamin B12 deficiency exhibit a decrease in villus length and a lower villus/crypt ratio as opposed to the control group [75]. Additionally, it has been shown that taking vitamin B12 supplements during pregnancy and breastfeeding helps shield the body from pro-inflammatory cytokines and oxidative stress [76].

4. Conclusion

Based on our current understanding, this is the first proof-of-concept study revealing that *H. pomatia* mucin, particularly at a high dose, regulates the intestinal flora via increasing the fecal *Firmicutes* abundance while reducing the fecal *Bacteriodetes*. *H. pomatia* mucin was also found to improve the DAI, diminish oxidative stress, and revert the alterations in the hepatic architecture and chemokine-chemokine receptor interactions observed in the experimental UC. *H. pomatia* mucin constituents could bind directly to chemokines, neutralizing their activity and preventing them from interacting with their receptors. This can decrease the overall inflammatory response in UC. Therefore, *H. pomatia* mucin has great potential as a safe adjuvant for treating and preventing UC. Nevertheless, more investigation is required to comprehend the influence of *H. pomatia* mucin on the general composition, variety, and chemokine network of the gut microbiota. Also, exploring its impact on a wider range of chemokines and receptors could reveal new therapeutic approaches for managing UC. Furthermore, additional clinical studies are necessary to elucidate the precise function of *H. pomatia* mucin in alleviating UC, including a comprehensive examination of its bioavailability, metabolism, and pharmacokinetics in the gastrointestinal tract.

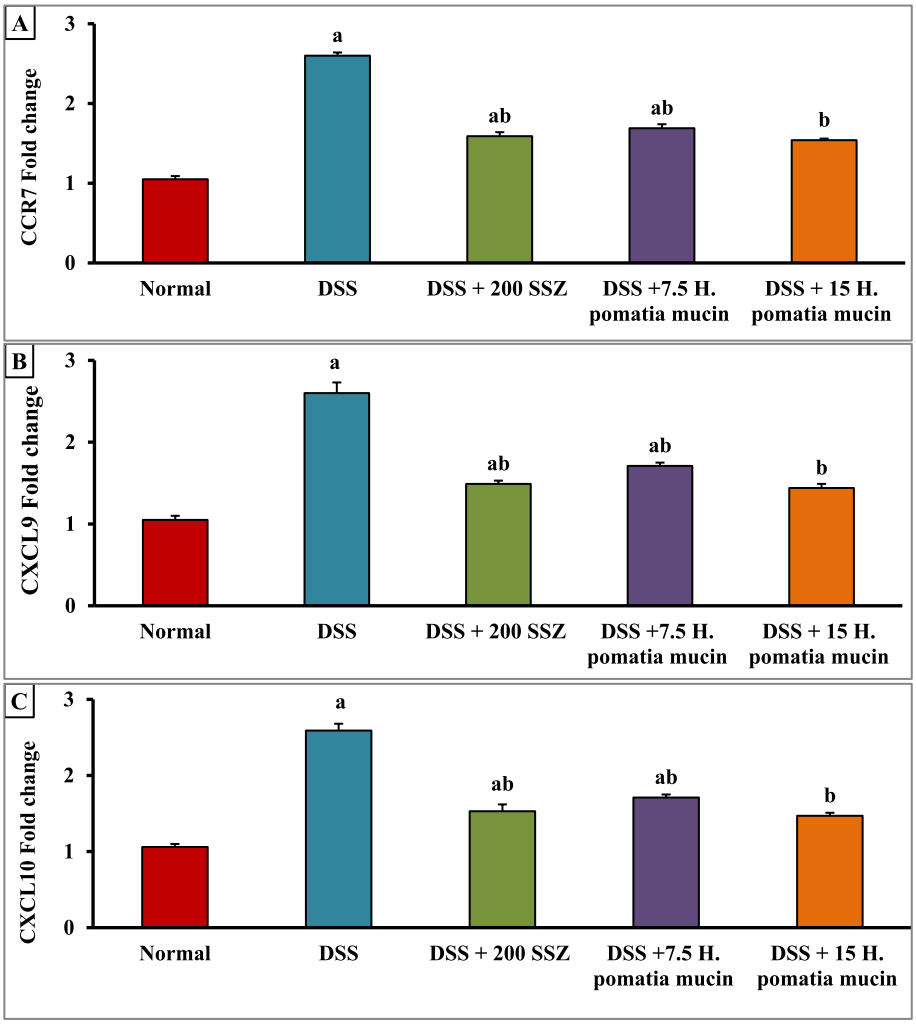

Fig. 7. Effect of *H. pomatia* mucin on colonic CCR7 (A), CXCL9 (B) CXCL10 (C) gene expression in the studied groups of mice.

Ethical declaration

All the animal procedures were performed in compliance with the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Medical Research Ethics Committee at the National Research Centre (FWA 00014747) with protocol number (2497082022). The study was published using the ARRIVE (Animal Research: Reporting of In Vivo Experiments) principles, which were created to enhance the reporting of research involving animals. The execution of all procedures adhered strictly to the relevant laws and regulations.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Maha B. Salem: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohamed Elzallat:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dina Mostafa Mohammed:** Writing – review & editing,

Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Olfat A. Hammam:** Methodology, Investigation. **Marwa Tamim A. Abdel-Wareth:** Writing – review & editing, Methodology. **Marwa Hassan:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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