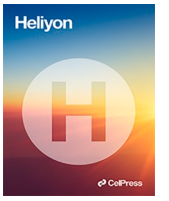
Heliyon 10 (2024) e37279

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

The preventive effects of native probiotic and postbiotic on inflammation and oxidative stress in DSS-induced colitis with normal diet: Which of these agents may offer greater advantages?

Niloofar Rezaie [a,1], Shadi Aghamohammad [a,1], Elham Haj Agha Gholizadeh Khiavi [a], Shohreh Khatami [b], Aria Sohrabi [c], Mahdi Rohani [a,*]

[a] Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran
[b] Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
[c] Department of Epidemiology and Biostatistics, Research Centre for Emerging and Re-emerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran

ARTICLE INFO

Keywords:
Probiotic
Postbiotic
ROS imbalance
Inflammatory status
Normal diet

ABSTRACT

Background: Maintaining a well-rounded and healthy diet is essential to promote the well-being and optimal performance of the body, especially for those suffering from Inflammatory Bowel Disease (IBD). The objective of this study is to examine whether probiotics and postbiotics can modulate oxidative stress and inflammation, and to evaluate the properties of these compounds.
Methods: A total of eighty eight strains of *Lactobacillus* and *Bifidobacterium* were assessed for their antioxidant activities. C57BL/6 mice were allocated into four groups: normal diet (ND) + PBS, ND + DSS, ND + DSS + 10^9 cfu/ml of probiotics, and ND + DSS + 10^9 cfu/ml of postbiotics. Biochemical antioxidant assays, along with colitis indices, were evaluated. The ELISA assay was conducted to measure oxidant/antioxidant properties and cytokines. Additionally, the genes enrolled in NF-kB and Nrf2 signaling pathways was analyzed.
Results: In comparison to the groups exposed to DSS alone, mice that received our native agents in addition to DSS demonstrated an improvement in the negative effects induced by DSS on DAI and pathological scores, as well as on colon length and body weight. The levels of cytokines and antioxidant markers have also been normalized following the administration of our native agents, along with molecular markers. It should also be noted that our native postbiotic was able to develop more pronounced and significant anti-inflammatory and antioxidant effects in comparison to the probiotic strains.
Conclusion: In this study, our native postbiotic has demonstrated a more pronounced ability to exhibit antioxidant and anti-inflammatory effects. This finding is particularly important for individuals with impaired immune function, for whom the use of live bacteria could be risky. Therefore, the utilization of agents like probiotics and postbiotics, which come with minimal side effects in compared to chemical drugs, could be essential in managing symptoms in IBD patients.

* Corresponding author. Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran.
E-mail address: kia.rohani@gmail.com (M. Rohani).
[1] Contributed as co-first authors.

https://doi.org/10.1016/j.heliyon.2024.e37279
Received 7 June 2024; Received in revised form 28 August 2024; Accepted 30 August 2024
Available online 31 August 2024

1. Background

Maintaining a balanced and nutritious diet with the right proportions of carbohydrates, proteins, fats, and micronutrients (normal diet) is crucial for ensuring the health and proper bodily function [1]. Maintaining a regular diet is increasingly crucial for patients experiencing inflammatory conditions, such as Inflammatory Bowel Disease (IBD). IBD is a condition that may result from irregularities in the gut microbiota, the immune system, and the function of the epithelial barrier, all of which can be influenced by dietary choices [2]. Probiotics are one of the most important choices that could help IBD patients for having healthier diet with beneficial anti-inflammatory, immunomodulatory, and gut-microbial balancing as complementary therapeutic agents [3,4]. Probiotics are beneficial bacteria that, as stated by the Food and Agriculture Organization of the United Nations and the World Health Organization in 2001, can provide health benefits when consumed in appropriate amounts [5]. Nonetheless, the potential risks of probiotic use, which are live bacteria, poses challenges especially for specific individuals, such as immunocompromised patients [6]. Therefore, the recent emphasis has transitioned towards the non-living probiotics, specifically the metabolites released by probiotics, known as postbiotics [7]. Factors such as short chain fatty acids (SCFAs), enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, endo- and exo-polysaccharides, cell surface proteins, vitamins, plasmalogens, and organic acids fall under the category of postbiotics, which are capable of providing physiological advantages [8]. Besides the beneficial aforementioned properties of probiotics and postbiotics, another remarkable feature of both these agents is their antioxidant activity [9,10].

Oxidative stress, a condition in the body resulting from an imbalance between oxidants, including reactive oxygen species (ROS) and antioxidants, is associated with a range of abnormalities such as inflammation and IBD [11]. IBD, as mentioned above, is an inflammatory condition with multiple causative factors. Prolonged oxidative stress is one of these factors that make a substantial impact on the worsening of inflammation. The heightened presence of ROS in the intestinal mucosae and reduced effectiveness of antioxidant mechanisms in individuals with IBD may result in alterations in intestinal permeability, which is itself a key factor in the escalation of inflammation [12]. Moreover, oxidative stress has the potential to result in the upregulation of genes associated with inflammation. The Nrf2-ARE transcriptional pathway, which is crucial in regulating genes responsible for detoxification and elimination of ROS, is interconnected with inflammatory signaling pathways such as NF-kB. The over activation of *NF-kB* was observed in *Nrf2* knockout mice, resulting in the generation of inflammatory cytokines [13].

IBD presents a range of challenges and limitations for individuals affected by it. Numerous patients experience symptoms such as diarrhea, fatigue, abdominal discomfort, incontinence, rectal bleeding, as well as psychological issues like anxiety and depression [14]. Conversely, the conventional treatment choices come with a range of complications such as side effects, eventually leading to the discontinuation of treatment. Hence, the development of new approaches to manage or reduce the symptoms of IBD is essential for patients [15]. In attention with this point, probiotics and postbiotics could be assumed as beneficial and appropriate choices for controlling the symptoms of IBD due to having beneficial properties, including antioxidant activities and also having the least side effects [16]. In this study, our aim was to examine the antioxidant properties of our native agents in DSS-induced mice. Additionally, we aimed to compare these two agents to determine which one could provide more beneficial properties in mice on a normal diet but experiencing inflammation.

2. Materials and methods

2.1. Bacterial isolation, postbiotic preparation, and evaluation the antioxidant activity

A total of eighty eight probiotic strains, including *Lactobacillus* and *Bifidobacterium*, were extracted from the specimens of healthy adults and were cultivated under conditions that were previously outlined [17,18]. Six strains, including *L. reuteri* RP100, *L. plantarum* RP42, *L. plantarum* RP119, *L. plantarum* RP155, *B. bifidum* RP1001, and *B. longum* RP1044 were ultimately chosen based on their high antioxidant activity through the results of biochemical antioxidant assessments, which included DPPH, ABTS, superoxide anion, hydroxyl radical, reducing power, and lipid peroxidation inhibition tests (See Supplementary file 1). To prepare the postbiotics, live cells were cultured at 37 °C for 18 h. An equal amount of bacterial suspension (10^9 CFU/mL) was then added to MRS broth and anaerobically incubated at 37 °C for 96 h. The supernatant was subsequently obtained through centrifugation at 12,000 rpm for 5 min at 4 °C, and filtered through a 0.22 μm filter. The postbiotic cocktail was divided into aliquots and stored at −80 °C until needed.

2.2. Animal experiment

Twenty male wild-type C57BL/6 mice were acquired at 4–6 weeks old from the Pasteur Institute of Iran and placed in three cages for the acclimation phase. The mice were kept under the same conditions (12 h of light, 22–23 °C, and 50 % humidity) with unrestricted access to a standard normal diet (ND) and water, both during acclimation and the duration of the study. Daily weight measurements were taken for all the mice. This study followed institutional guidelines for the care and ethical use of laboratory animals, ensuring that all animal experiments were conducted with measures in place to reduce the mice's suffering. The study's protocol was approved by the Animal Experimentation Committee of the Pasteur Institute of Iran (IR.PII.REC.1400.061).To assess the impact of our native probiotic and postbiotic, we divided our mice into four different groups. All groups were provided with a normal diet. The initial group received a daily intake of 200 μL PBS. In the second group, colitis was induced experimentally by administering 200 μL 2 % DSS orally (Sigma, LOT#BCCD5523). The third group was given a mixture of 200 μl containing 2 % DSS and 10^9 cfu/ml of our native probiotic cocktail which includes six strains, including *L. reuteri* RP100, *L. plantarum* RP42, *L. plantarum* RP119, *L. plantarum* RP155, *B. bifidum* RP1001, and *B. longum* RP1044, while the fourth group received postbiotics derived from mentioned probiotic strains (1 ×

10^9 CFU/mL) along with DSS daily via oral gavage The time of the gavage for all four groups was 28 days andall mice in every four groups received a daily intake of PBS, DSS, our native probiotic strains and our native postbiotic.

2.3. The analysis of disease activity index (DAI) and histopathological score

The colon was cleansed by gently washing it with PBS to remove stool. Subsequently, the colon was sectioned into 2–3 fragments and preserved at −80 °C for RNA extraction. The mice were observed daily for changes in body weight, presence of diarrhea, rectal conditions, and stool bleedingDAI scores were determined by considering weight loss, stool consistency, and bleeding, following the method outlined by Kwon et al. [19].

The histopathological analysis involved examining colon tissues that were fixed in paraformaldehyde, impanted in paraffin, and sectioned into 4 μm thick slices. The histological score was subsequently determined based on criteria outlined in a previous study [20].

2.4. Phenotypic assessments for the anti-inflammatory and antioxidant activities of our native probiotic and postbiotic

the amounts of antioxidant enzymes, including malondialdehyde (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX), were measured in both serum and sections of the distal intestinal tissue using the methods outlined by Navand Salamat, Iran. Additionally, the concentrations of pro-inflammatory cytokines (IL-1β and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10) in serum were analyzed following the protocols established by Karmania Pars Gene, Iran.

2.5. The molecular evaluation for the anti-inflammatory and antioxidant activities of our native agents

RNA was obtained from the colonic tissue of mice with the RNeasy Mini Kitt from Favorgen Biotech Corp in Taiwan, following the manufacturer's protocol. The cDNA synthesis kit from Yekta Tajhiz Azma Co in Iran was used. The quantitative PCR (qPCR) for evaluationg the transcriptional activity of genes associated with Nrf2 and NF-kB pathways was performed on the ABI Prism 7900HT instrument with 2x SYBR-Green, specifically the RealQ Plus Master Mix Green from Amplicon A/S in Denmark. Primer sequences are detailed in Table 1.

2.6. Statistical analysis

The *gapdh* gene, a housekeeping gene, was used for normalization. The $2-\Delta\Delta Ct$ method was employed to calculate the relative measurement of specific gene expression. Gene expression changes were analyzed using GraphPad Prism 8.0 (GraphPad Software Inc,

Table 1
The primers used in the current study.

Genes	Primer Sequence (5' > 3')	Product size
Nrf2MF	TAGATGACCATGAGTCGCTTGC	153bp
Nrf2MR	GCCAAACTTGCTCCATGTCC	
Keap1 MF	TCGAAGGCATCCACCCTAAG	135bp
Keap1MR	CTCGAACCACGCTGTCAATCT	
NQO1MF	AGGATGGGAGGTACTCGAATC	127bp
NQO1MR	TGCTAGAGATGACTCGGAAGG	
HO-1MF	GGTGATGGCTTCCTTGTACC	155bp
HO-1MR	AGTGAGGCCCATACCAGAAG	
Trx-1MF	CTTTTGCCCGTCTCTCAATCA	181bp
Trx-1MR	AGGGTATTTCACACTTAGGTCCT	
SOD2MF	CAGACCTGCCTTACGACTATGG	113bp
SOD2MR	CTCGGTGGCGTTGAGATTGTT	
CATMF	GGAGGCGGGAACCCAATAG	102bp
CATMR	GTGTGCCATCTCGTCAGTGAA	
Gpx1MF	CCACCGTGTATGCCTTCTCC	105bp
Gpx1MR	AGAGAGACGCGACATTCTCAAT	
COX-2(PTGS2) MF	TGCACTATGGTTACAAAAGCTGG	271bp
COX-2(PTGS2) MR	TCAGGAAGCTCCTTATTTCCCTT	
NF-kBp65(Rela) MF	TGACCCCTGTCCTCTCACATCCG	94bp
NF-kBp65(Rela) MR	CAGCTCCCAGAGTTCCGGTT	
NF-KBIA(IkBa)MF	TGAAGGACGAGGAGTACGAGC	127bp
NF-KBIA(IkBa)MR	TGCAGGAACGAGTCTCCGT	
Ikka (Chuk)MF	GAGAGCGATGGTGCCATGAA	136bp
Ikka (Chuk)MR	CCAGAACAGTACTCCATTGCCAGA	
Ikkb (IKBKB)MF	AAGTACACCGTGACCGTTGAC	91bp
Ikkb (IKBKB)MR	GCTGCCAGTTAGGGAGGAA	
GAPDHMF	TGGCCTTCCGTGTTCCTAC	
GAPDHMR	GAGTTGCTGTTGAAGTCGCA	178bp

CA, USA). Group comparisons were performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test, and the Kruskal-Wallis test was applied for non-normal data. Results are presented as mean ± SD from at least three replicates, with a significance level set at 0.05.

3. Results

3.1. The results of the colitis indices comparison in mice fed with ND and administered with our native agents

The results of impacting of our native probiotics and postbiotics on mice with with normal diet and treated with DSS is seen in Fig. 1. Mice in the control group, which received a normal diet, showed a rise in body weight of approximately 2 g. Conversely, mice treated with DSS experienced a notable decrease in weight of up to 8 g when contrasted with the control group. Administration of our native probiotic strains did not produce a notable change in body weight, whereas treatment with our postbiotic led to a substantial a weight increase of close to 1 g in relation to the probiotic strains utilized in the study (see Fig. 1A). Similar outcomes were observed concerning DAI (Fig. 1B), colon measurements (Fig. 1C), and pathological evaluation (Fig. 1D and E). Administration of DSS led to a notable rise in both DAI and pathological assessments, along with a decrease in colon length ($p < 0.0001$).Our native agents showcased remarkable anti-inflammatory capabilities. Both interventions led to a substantial drop in DAI and pathological scores, while also increasing colon length ($p < 0.0001$). When comparing the effects of our native probiotics and postbiotics, the latter demonstrated more pronounced impacts on DAI ($p < 0.05$), pathological assessment ($p < 0.001$), and colon dimensions ($p < 0.01$), effectively aligning the conditions more closely with those of the control group.

3.2. The outcomes of our native agents on phenotypic assessments

The effects of DSS and our native probiotics and postbiotics on the level of antioxidant/oxidant markers is demonstrated in Figs. 2 and 3. The occurrence of inflammation induced by DSS has been associated with a notable reduction in various antioxidant indicators in serum and gut, such as SOD, CAT, GPX, and GSH ($p < 0.0001$), along with an elevation in MDA as an oxidative marker ($p < 0.0001$). On the contrary, our native agents s enhanced the levels of antioxidant markers ($p < 0.0001$) while diminishing MDA ($p < 0.0001$)

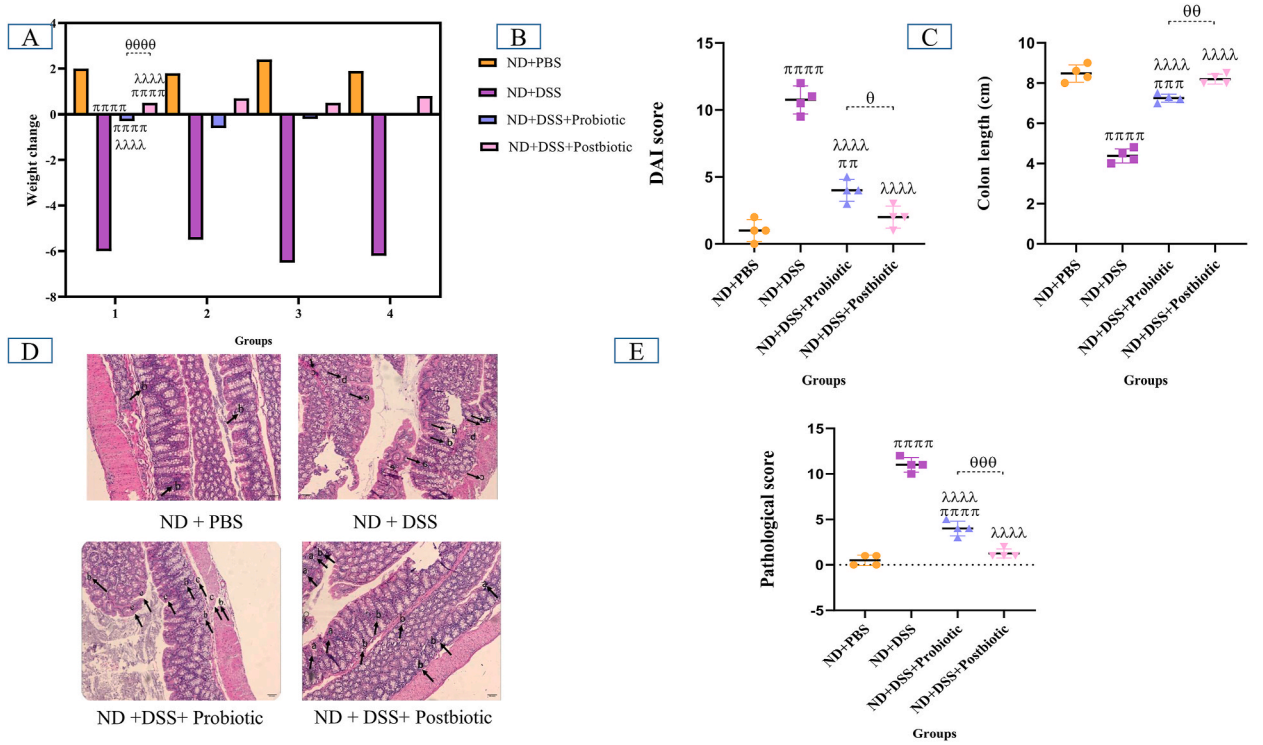


Fig. 1. Effects of probiotics and postbiotics mixture on disease severity in DSS-induced colitis mice. A) Body weight changes, B) DAI score, C) Colon length, D) H&E staining of colon section of mice (a: crypts architecture, b: inflammation, c: muscle thickness, d: goblet cells depletion, and e: crypts abscesses, The scale bar is 100 pixels. E) histopathological score. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

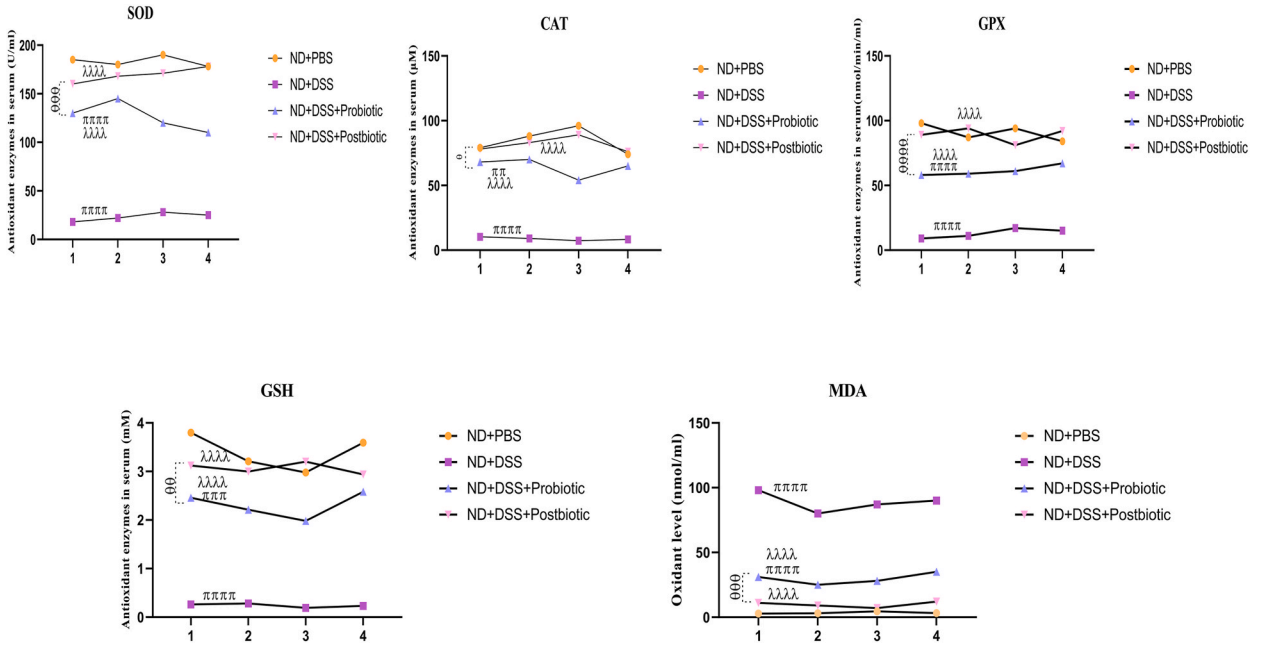


Fig. 2. The levels of SOD, CAT, GSH, GPX (antioxidant enzymes), and MDA oxidant enzyme in serum. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

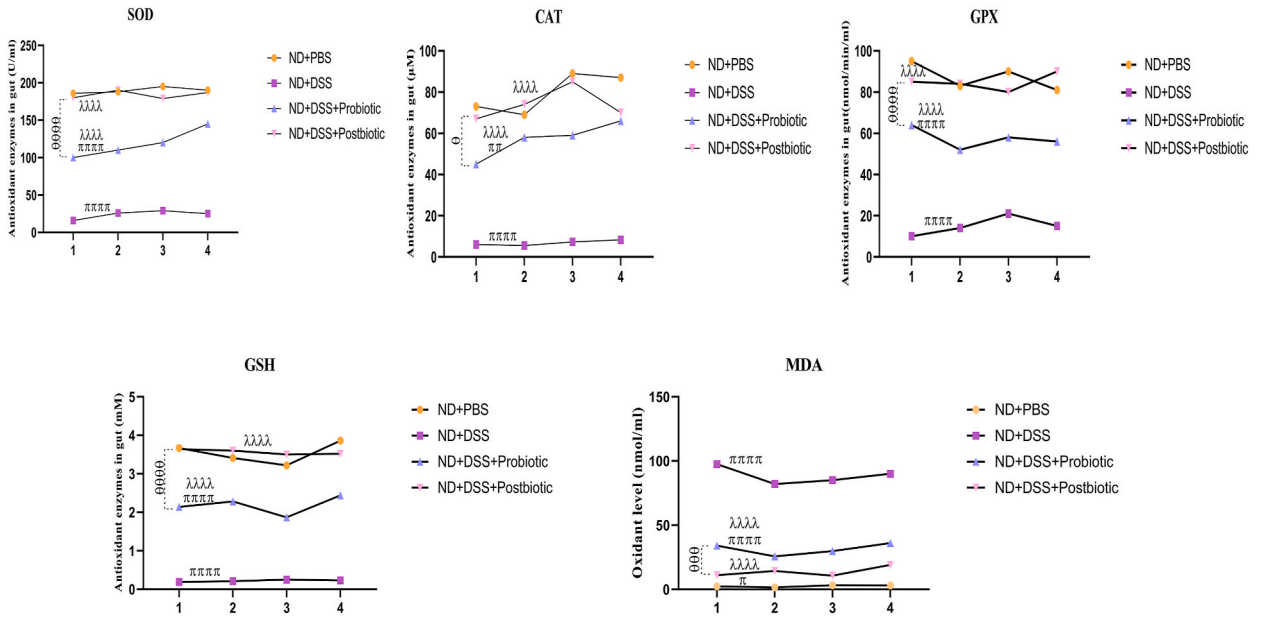


Fig. 3. The levels of SOD, CAT, GSH, GPX (antioxidant enzymes), and MDA oxidant enzyme in gut. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

significantly. Furthermore, our postbiotics exhibited superior and more pronounced antioxidant properties compared to probiotic strains, thereby creating a situation similar to the control cohorts devoid of inflammation ($p < 0.05$).

The evaluation of the impacts of our native agents on pro/inflammatory cytokines showed the remarkable anti-inflammatory properties exhibited by our native probiotics and postbiotics, see Fig. 4. It was noted that exposure to DSS led to a substantial rise

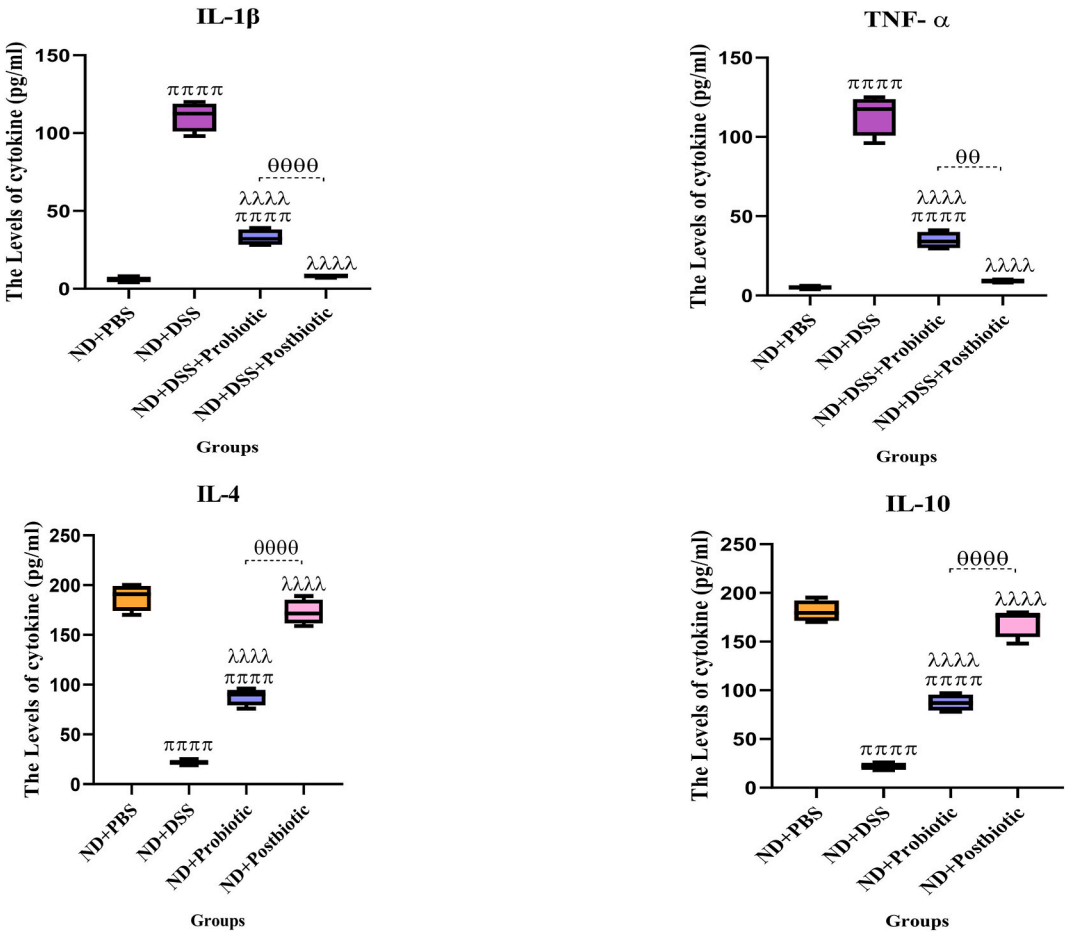


Fig. 4. The Levels of IL-1β, TNF-α (Inflammatory cytokines) and, IL-4, IL-10 (anti-inflammatory cytokines), in serum. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

in the levels of IL-1β and TNF-α ($p < 0.0001$), accompanied by a notable reduction in the quantities of IL-4 and IL-10 ($p < 0.0001$). Conversely, the application of our native probiotic strains and postbiotics showed a significant ability to lower the levels of pro-inflammatory cytokines ($p < 0.0001$) while simultaneously enhancing the levels of anti-inflammatory cytokines ($p < 0.0001$). Upon conducting a comparative analysis between our native probiotics and postbiotics, once again, it became evident that the latter showcased superior and more advantageous anti-inflammatory effects. In direct comparison with probiotic strains, our postbiotics exhibited a greater ability to decrease the levels of IL-1β ($p < 0.0001$) and TNF-α ($p < 0.01$), and increase the levels of IL-4 ($p < 0.0001$) and IL-10 ($p < 0.0001$) to such an extent that it could effectively eliminate the inflammatory conditions similar to those observed in the control group.

3.3. The results of our native probiotics and postbiotics on molecular assay

In order to evaluate the molecular antioxidant and anti-inflammatory impacts of our native probiotics and postbiotics, an analysis was conducted on the pathways involved, which encompass *NF-kB* and *Nrf2*, as illustrated in Figs. 5 and 6. The findings from our research indicated that apart from the phenotypic tests carried out, The native probiotic strains and postbiotics demonstrated the ability to invoke antioxidant and anti-inflammatory effects by modulating specific molecular pathways. Following DSS administration, a decrease in the expression levels of genes related to the Nrf2 pathway was observed ($p < 0.0001$). However, treatment with our native probiotic strains and postbiotics led to a significant increase in the expression levels compared to the DSS group ($p < 0.0001$). Across all the genes related to *Nrf2* that were examined, our native postbiotics demonstrated a greater impact on enhancing the expression levels compared to our native probiotic strains ($p < 0.0001$).

In relation to the NF-kB genes, it was observed that the exposure to DSS led to a notable and statistically significant rise in the expression levels of the genes under study ($p < 0.0001$). On the other hand, it was found that our native probiotic strains and post-biotics demonstrated a notable ability to significantly reduce the expression levels of the genes. ($p < 0.0001$). Furthermore, it was

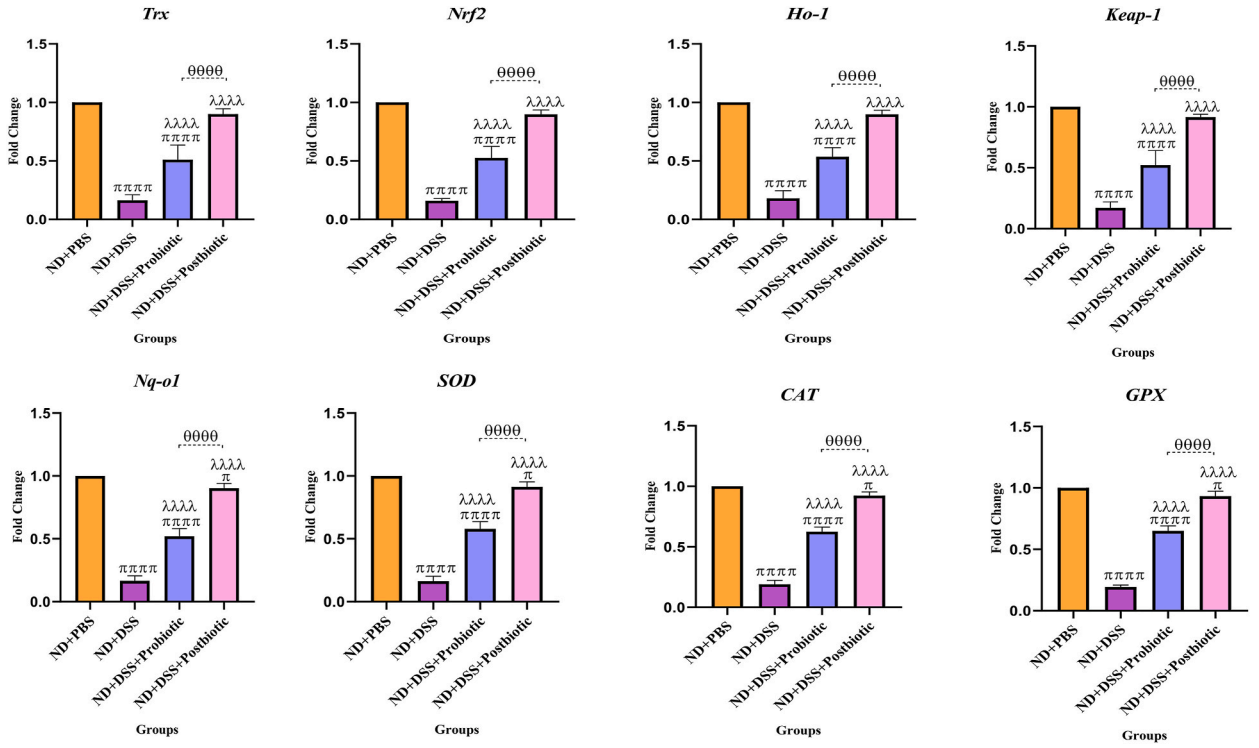


Fig. 5. Relative gene expression [mean fold change] of antioxidants and *Nrf2* related pathway genes expression in the different groups of treatments. Data were normalized with *gapdh*. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

noted that the postbiotic intervention yielded a more favorable impact in terms of lowering the gene expression levels in contrast to the probiotic strains ($p < 0.0001$) and could succeed in reinstating the gene expression levels to those similar to the group that did not undergo DSS administration.

Overall, the general trend of our results is shown in Fig. 7. From this figure, it can be seen that our phenotypic assessments, including the assessment of pro- and anti-inflammatory cytokines and oxidative/antioxidant markers, as well as the molecular investigation, revealed that both our native probiotic strains and the postbiotics were able to exert anti-inflammatory and antioxidant effects, and that our native postbiotics in particular behaved like our control group, as the colour tone (Fig. 7A) and also the trend of the graphs (Fig. 7B) were similar.

4. Discussion

Oxidative stress plays a significant role in the development and progression of Inflammatory Bowel Disease (IBD). Research findings highlight the significant involvement of oxidative stress in the tissue damage and pathology associated with this inflammatory condition [21]. One reason why oxidative stress contributes to the development and exacerbation of IBD is due to the excessive production of ROS overwhelming the antioxidant defenses, thereby disrupting the intestinal homeostasis and impairing the immune system [22]. On the other hand, it is crucial for patients with IBD to follow a regular diet that is free from nutritional deficiencies and imbalances, while also steering clear of fats and cholesterol, due to the impact of a high-fat diet on triggering oxidative stress [23–25]. Therefore, the utilization of any agents possessing antioxidant properties may be considered essential for individuals diagnosed with IBD. Furthermore, the administration of prolonged therapy in this patient population commonly results in adverse reactions; thus, the utilization of agents with minimal side effects is crucial for enhancing the overall quality of life for those affected by IBD [26]. Among the agents exhibiting minimal side effects, probiotics and postbiotics, in particular, possess elevated safety profiles, minimal side effects, and notable antioxidant capabilities [27,28]. The current study purposed to assess the anti-inflammatory and antioxidant properties of our native probiotics and postbiotics, as well as to compare the effectiveness of these two agents.

Our findings revealed the remarkable anti-inflammatory and antioxidant efficacy of our native probiotics and postbiotics. Based on our in vivo findings, both our substances were able to mitigate the negative impacts induced by DSS as the inflammatory factor. Upon comparing probiotics and postbiotics, our results indicated that the postbiotics could regulate the colitis parameters, such as body weight, DAI, and pathological scores, along with colon length in a manner that yielded outcomes similar to those of the control group

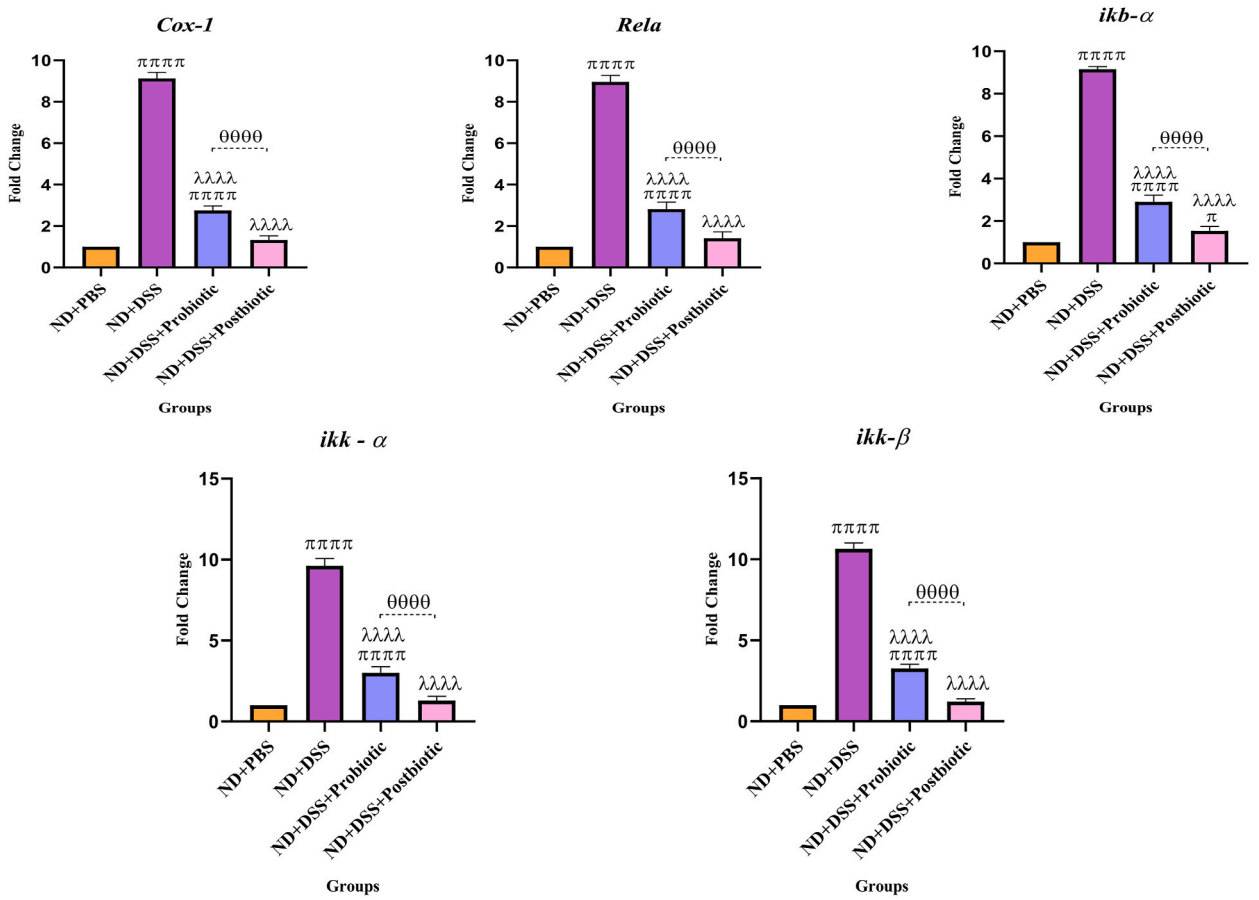


Fig. 6. Relative gene expression [mean fold change] of *NF-kB* related pathway genes expression in the different groups of treatments. Data were normalized with *gapdh*. Data are presented as the mean ± SD, N = 5 per group. Statistical significance was determined using the following symbols: π, $p < 0.05$; π π, $p < 0.01$; π π π, $p < 0.001$; π π π π, $p < 0.0001$ (ND + PBS vs. other groups), λ, $p < 0.05$; λ λ, $p < 0.01$; λ λ λ, $p < 0.001$; λ λ λ λ, $p < 0.0001$ (ND + DSS vs. Other groups), θ, $p < 0.05$; θ θ, $p < 0.01$; θ θ θ, $p < 0.001$; θ θ θ θ, $p < 0.0001$, The relatedness between ND + DSS + probiotic and ND + DSS + postbiotic groups.

without inflammation. In other words, our postbiotics have nearly achieved complete success in easing gastrointestinal symptoms triggered by inflammation. The phenotypical assessments used in this study, such as examining antioxidant markers in serum and gut, as well as cytokines, confirmed the beneficial effects of our native probiotics and postbiotics, with a particular emphasis on the latter. Similar trends were observed in the regulation of pro- and anti-inflammatory cytokines, highlighting the strong efficacy of our native substances, particularly our postbiotics in exerting anti-inflammatory effects. In other reports, similar findings were observed. Xu et al. stated that probiotic consortia and their metabolites could have anti-inflammatory properties by influencing DSS indices such as DAI and histological scores. According to their results, the probiotic consortia group relieved the shortened colon induced by DSS. Furthermore, this group exhibited a moderate weight change trend. The probiotic consortia administration notably decreased weight loss in comparison to the control group. Additionally, the probiotic consortia group showed a notably lower DAI score than the control group [29]. Based on the research carried out by Abrehame et al., it was found that the cell-free fermentation supernatants of *Ligilactobacillus salivarius* P1, *Lactobacillusgasseri* P12, and *Limosilactobacillus reuteri* G7 had an impact on the growth and proliferation of intestinal epithelial cells. This resulted in strengthening the intestinal barrier, reducing the DAI, and increasing the length of the colon [30]. The studies carried out by Dashtbanei et al. validated our findings on the antioxidant marker levels. Their findings revealed that the use of *L. rhamnosus*, *L. helveticus*, and *L. casei* in combating cadmium-induced oxidative stress resulted in elevated SOD and CAT levels, while reducing MDA levels [31]. According to Humam et al., it was observed that postbiotics derived from *L. plantarum* have the potential to markedly enhance the plasma activity of CAT and GSH, while concurrently reducing the level of MDA in the postbiotic group when compared to the control group [32].

Our molecular analyses could confirm the outcomes obtained from our phenotypic assays. Both of our native agents were able to greatly influence the regulation of genes involved in *Nrf2* and *NF-kB* signaling pathways, with a stronger focus on our native postbiotics, resulting in expression levels similar to those of the control group. These reports are also consistent with the others'. As per the findings from Chen et al., It was demonstrated that *L. plantarum* Lp2 can reduce LPS-induced liver damage by activating the Nrf2-HO-1/CYP2E1 pathway and inhibiting the TLR-4/MAPK/NFκB pathway [33]. As per the research conducted by Kim et al., it was found

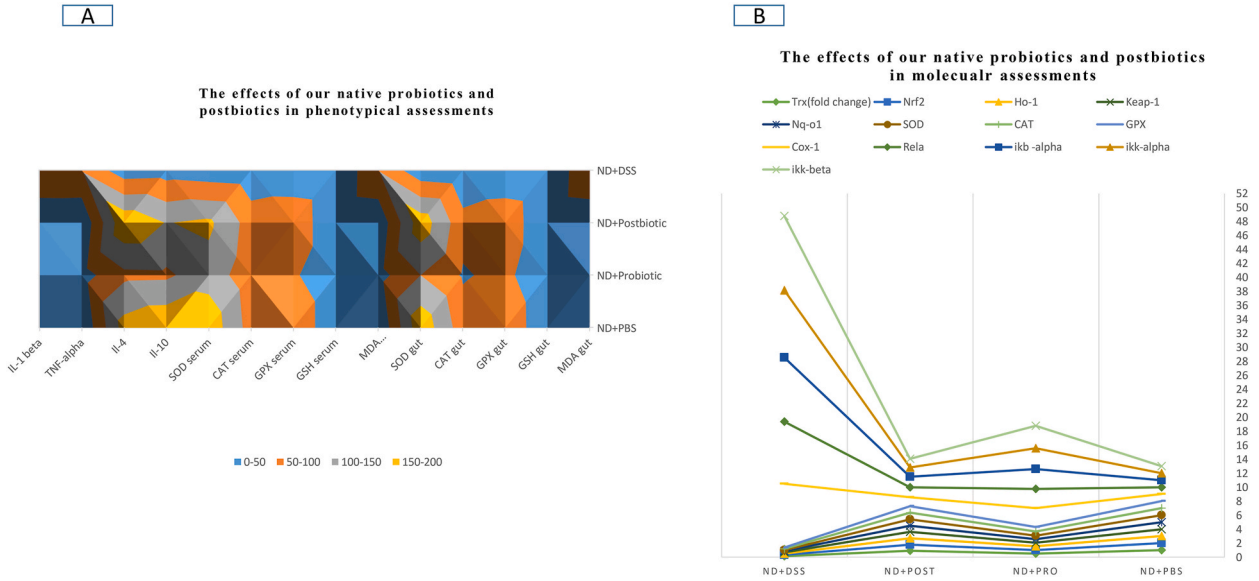


Fig. 7. The general tendency of the present study is as follows: A) the comprehensive outcomes of our phenotypical assessments, B) The comprehensive outcomes of our molecular analysis.

that the products of fermentation by *Lactobacillus* could effectively display antioxidant and anti-inflammatory properties by influencing the levels of TNF-α and IL-1β, the nuclear translocation of NF-κB (p65) induced by LPS, the production of ROS induced by LPS, and the expression of nuclear factor erythroid 2–related factor 2 and heme oxygenase 1 [34]. By compiling this data, it is possible to validate the significance of probiotic utilization and its derivatives in the reduction of inflammation.

5. Conclusion

This research aims to provide a comprehensive analysis of both physical and molecular attributes. The goal is to show that inflammatory conditions can occur even with a normal diet and that probiotics and postbiotics can effectively reduce inflammation due to their inherent antioxidant properties. Within this present study, our native postbiotic has shown the ability to manifest antioxidant and anti-inflammatory effects in a more pronounced manner. Since, according to various studies, probiotics as live bacteria can cause sepsis and fungemia in people with weakened immune systems, the administration of probiotics in these patients must be carefully considered [35,36]. Using postbiotics instead, holds particular significance for individuals facing compromised immune systems, where the utilization of live bacteria could pose risks. Consequently, the use of agents such as probiotics and postbiotics, which entail minimal side effects, could prove crucial in managing symptoms among patients afflicted with IBD.

Consent for publication

Not applicable.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by the by Pasteur Institute of Iran as Ph.D. thesis (B-9740) and Iran National Science Foundation (INSF) (Grant number: 4003890).

CRediT authorship contribution statement

Niloofar Rezaie: Formal analysis. **Shadi Aghamohammad:** Conceptualization. **Elham Haj Agha Gholizadeh Khiavi:** Validation, Conceptualization. **Shohreh Khatami:** Project administration. **Aria Sohrabi:** Investigation. **Mahdi Rohani:** Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37279.

References

[1] H. Cena, P.C. Calder, Defining a healthy diet: evidence for the role of contemporary dietary patterns in health and disease, Nutrients 12 (2) (2020) 334.
[2] J.H. de Vries, M. Dijkhuizen, P. Tap, B.J. Witteman, Patient's dietary beliefs and behaviours in inflammatory bowel disease, Dig. Dis. 37 (2) (2019) 131–139.
[3] S. Aghamohammad, A. Sepehr, S.T. Miri, S. Najafi, M. Rohani, M.R. Pourshafiea, The effects of the probiotic cocktail on modulation of the NF-kB and JAK/STAT signaling pathways involved in the inflammatory response in bowel disease model, BMC Immunol. 23 (1) (2022) 1–10.
[4] F. Cristofori, V.N. Dargenio, C. Dargenio, V.L. Miniello, M. Barone, R. Francavilla, Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body, Front. Immunol. 12 (2021) 578386.
[5] C. De Simone, The unregulated probiotic market, Clin. Gastroenterol. Hepatol. 17 (5) (2019) 809–817.
[6] P.A. Cohen, Probiotic safety—no guarantees, JAMA Intern. Med. 178 (12) (2018) 1577–1578.
[7] A. Mayorgas, I. Dotti, A. Salas, Microbial metabolites, postbiotics, and intestinal epithelial function, Mol. Nutr. Food Res. 65 (5) (2021) 2000188.
[8] J. Aguilar-Toalá, R. Garcia-Varela, H. Garcia, V. Mata-Haro, A. González-Córdova, B. Vallejo-Cordoba, A. Hernández-Mendoza, Postbiotics: an evolving term within the functional foods field, Trends in food science & technology 75 (2018) 105–114.
[9] B. Păcularu-Burada, G.-E. Bahrim, Extraction and antioxidant activity assessment of postbiotic exopolysaccharides produced by selected lactic acid bacteria, Innovat. Rom. Food Biotechnol. (20) (2021).
[10] A. Hoffmann, P. Kleniewska, R. Pawliczak, Antioxidative activity of probiotics, Arch. Med. Sci.: AMS 17 (3) (2021) 792.
[11] M. Krzystek-Korpacka, R. Kempiński, M.A. Bromke, K. Neubauer, Oxidative stress markers in inflammatory bowel diseases: systematic review, Diagnostics 10 (8) (2020) 601.
[12] Y. Hu, D. Chen, P. Zheng, J. Yu, J. He, X. Mao, B. Yu, The bidirectional interactions between resveratrol and gut microbiota: an insight into oxidative stress and inflammatory bowel disease therapy, BioMed Res. Int. 2019 (2019).
[13] K. Lingappan, NF-κB in oxidative stress, Current opinion in toxicology 7 (2018) 81–86.
[14] C. Byron, N. Cornally, A. Burton, E. Savage, Challenges of living with and managing inflammatory bowel disease: a meta-synthesis of patients' experiences, J. Clin. Nurs. 29 (3–4) (2020) 305–319.
[15] J.J. Ashton, Z. Green, V. Kolimarala, R.M. Beattie, Inflammatory bowel disease: long-term therapeutic challenges, Expet Rev. Gastroenterol. Hepatol. 13 (11) (2019) 1049–1063.
[16] R. Tan, W.M. Loke, Gut oxidative modulation of polyphenol, prebiotic, probiotic, and postbiotic in vitro, Current Research in Nutrition and Food Science Journal 10 (1) (2022) 56–70.
[17] M. Eshaghi, M.H. Bibalan, M. Rohani, M. Esghaei, M. Douraghi, M. Talebi, M.R. Pourshafie, Bifidobacterium obtained from mother's milk and their infant stool; A comparative genotyping and antibacterial analysis, Microb. Pathog. 111 (2017) 94–98.
[18] M. Rohani, N. Noohi, M. Talebi, M. Katouli, M.R. Pourshafie, Highly heterogeneous probiotic Lactobacillus species in healthy Iranians with low functional activities, PLoS One 10 (12) (2015) e0144467.
[19] J. Kwon, C. Lee, S. Heo, B. Kim, C.-K. Hyun, DSS-induced colitis is associated with adipose tissue dysfunction and disrupted hepatic lipid metabolism leading to hepatosteatosis and dyslipidemia in mice, Sci. Rep. 11 (1) (2021) 5283.
[20] R. Ghanavati, A. Akbari, F. Mohammadi, P. Asadollahi, A. Javadi, M. Talebi, M. Rohani, Lactobacillus species inhibitory effect on colorectal cancer progression through modulating the Wnt/β-catenin signaling pathway, Mol. Cell. Biochem. 470 (2020) 1–13.
[21] La Kruidenier, H. Verspaget, Oxidative stress as a pathogenic factor in inflammatory bowel disease—radicals or ridiculous? Alimentary pharmacology & therapeutics 16 (12) (2002) 1997–2015.
[22] E. Alemany-Cosme, E. Sáez-González, I. Moret, B. Mateos, M. Iborra, P. Nos, J. Sandoval, B. Beltrán, Oxidative stress in the pathogenesis of Crohn's disease and the interconnection with immunological response, microbiota, external environmental factors, and epigenetics, Antioxidants 10 (1) (2021) 64.
[23] J.A. Fitzpatrick, S.L. Melton, C.K. Yao, P.R. Gibson, E.P. Halmos, Dietary management of adults with IBD—the emerging role of dietary therapy, Nat. Rev. Gastroenterol. Hepatol. 19 (10) (2022) 652–669.
[24] M.M. de Castro, L.B. Pascoal, K.M. Steigleder, B.P. Siqueira, L.P. Corona, MdLS. Ayrizono, M. Milanski, R.F. Leal, Role of diet and nutrition in inflammatory bowel disease, World J. Exp. Med. 11 (1) (2021) 1.
[25] B.L. Tan, M.E. Norhaizan, Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function, Nutrients 11 (11) (2019) 2579.
[26] K. Dziąbowska-Grabias, M. Sztanke, P. Zając, M. Celejewski, K. Kurek, S. Szkutnicki, P. Korga, W. Bulikowski, K. Sztanke, Antioxidant therapy in inflammatory bowel diseases, Antioxidants 10 (3) (2021) 412.
[27] Y. Tong, Hn Guo, Z. Abbas, J. Zhang, J. Wang, Q. Cheng, S. Peng, T. Yang, T. Bai, Y. Zhou, Optimizing postbiotic production through solid-state fermentation with Bacillus amyloliquefaciens J and Lactiplantibacillus plantarum SN4 enhances antibacterial, antioxidant, and anti-inflammatory activities, Front. Microbiol. 14 (2023) 1229952.
[28] V. Mishra, C. Shah, N. Mokashe, R. Chavan, H. Yadav, J. Prajapati, Probiotics as potential antioxidants: a systematic review, J. Agric. Food Chem. 63 (14) (2015) 3615–3626.
[29] L. Xu, B. Liu, L. Huang, Z. Li, Y. Cheng, Y. Tian, G. Pan, H. Li, Y. Xu, W. Wu, Probiotic consortia and their metabolites ameliorate the symptoms of inflammatory bowel diseases in a colitis mouse model, Microbiol. Spectr. 10 (4) (2022) e00657, 00622.
[30] S. Abrehame, M.-Y. Hung, Y.-Y. Chen, Y.-T. Liu, Y.-T. Chen, F.-C. Liu, Y.-C. Lin, Y.-P. Chen, Selection of fermentation supernatant from probiotic strains exhibiting intestinal epithelial barrier protective ability and evaluation of their effects on colitis mouse and weaned piglet models, Nutrients 16 (8) (2024) 1138.
[31] S. Dashtbanei, Z. Keshtmand, A Mixture of Multi-Strain Probiotics (Lactobacillus Rhamnosus, Lactobacillus Helveticus, and Lactobacillus Casei) had anti-inflammatory, anti-apoptotic, and anti-oxidative effects in oxidative injuries induced by cadmium in small intestine and lung, Probiotics and antimicrobial proteins 15 (2) (2023) 226–238.
[32] A.M. Humam, T.C. Loh, H.L. Foo, W.I. Izuddin, E.A. Awad, Z. Idrus, A.A. Samsudin, N.M. Mustapha, Dietary supplementation of postbiotics mitigates adverse impacts of heat stress on antioxidant enzyme activity, total antioxidant, lipid peroxidation, physiological stress indicators, lipid profile and meat quality in broilers, Animals 10 (6) (2020) 982.
[33] Y. Chen, W. Guan, N. Zhang, Y. Wang, Y. Tian, H. Sun, X. Li, Y. Wang, J. Liu, Lactobacillus plantarum Lp2 improved LPS-induced liver injury through the TLR-4/MAPK/NFκB and Nrf2-HO-1/CYP2E1 pathways in mice, Food Nutr. Res. 66 (2022).

[34] S.-L. Kim, H.S. Choi, Y.-C. Ko, B.-S. Yun, D.-S. Lee, 5-hydroxymaltol derived from beetroot juice through lactobacillus fermentation suppresses inflammatory effect and oxidant stress via regulating NF-kB, MAPKs pathway and NRF2/HO-1 expression, Antioxidants 10 (8) (2021) 1324.
[35] E.B.-M. Daliri, B.H. Lee, D.H. Oh, Safety of probiotics in health and disease. The Role of Functional Food Security in Global Health, 2019, pp. 603–622.
[36] V. Stadlbauer, Immunosuppression and probiotics: are they effective and safe? Benef. Microbes 6 (6) (2015) 823–828.