Research Paper

The Potential Anti-inflammatory Effect of *Spirulina Platensis* on an in Vitro Model of Celiac Disease

Somayeh Jahani-Sherafat[1], Samira Alipour[2], Ensiyeh Olamafar[2], Mahtab Jahdkaran[3], Mona Zamanian Azodi[4], Mahmood Khodadoost[5], Mohammad Rostami-Nejad[6*]

1. Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Department of Traditional Medicine, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
6. Celiac Disease and Gluten Related Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Citation: Jahani-Sherafat S, Alipour S, Olamafar E, Jahdkaran M, Zamanian Azodi M, Khodadoost M, et al. The Potential Anti-inflammatory Effect of Spirulina Platensis on an in Vitro Model of Celiac Disease. International Journal of Medical Toxicology and Forensic Medicine. 2023; 13(4):E42998. https://doi.org/10.32598/ijmtfm.v13i4.42998

https://doi.org/10.32598/ijmtfm.v13i4.42998

Article info:
Received: 15 Aug 2023
First Revision: 15 Sep 2023
Accepted: 01 Oct 2023
Published: 13 Dec 2023

Keywords:
Celiac disease, Gluten-free diet, Spirulina, Therapy

ABSTRACT

Background: Celiac disease (CD) is a prevalent autoimmune enteropathy triggered by the ingestion of gluten. The management of CD involves adhering to a gluten-free diet (GFD). Recent studies have been actively exploring potential supplementary or alternative therapies for individuals with CD. The primary objective of the present study was to assess the effectiveness of *Spirulina platensis* in regulating the intestinal barrier-related gene expression and alleviating inflammation and oxidative stress associated with CD in PT-gliadin-triggered Caco-2 cells.

Methods: *S. platensis* extracts and a pepsin/trypsin (PT) digest of gliadin were prepared and exposed to the human colon carcinoma Caco-2 cell line. Cell viability was assessed. Total RNA was extracted from Caco-2 cells and cDNA synthesis was performed. A quantitative real-time polymerase chain reaction (qRT-PCR) assay was conducted to evaluate the mRNA levels of interleukin (IL)-6, transforming growth factor beta (TGF-β), COX-2, nuclear factor kappa B (NF-κB), ZO-1, and occludin.

Results: Treating Caco-2 cells with *S. platensis* alone (P=0.01 for both) or in combination with PT-gliadin (P=0.004 and P=0.02, respectively) resulted in decreased IL-6 expression and increased occludin mRNA expression. Additionally, *S. platensis* extract enhanced Zo-1 mRNA levels (P=0.002) and reduced NF-κB mRNA expression (P=0.02). The combination of gliadin and *S. platensis* led to decreased mRNA expression of COX-2 (P=0.03) and NF-κB (P=0.04). No significant differences were observed in TGF-β mRNA expression between the studied groups (P>0.05).

Conclusion: Additional investigation is needed to examine the influence of interactions between *S. platensis* and gliadin regarding the comprehensive response of CD to gliadin, encompassing the activation of gluten-sensitive immune cells.

*** Corresponding Author:**
Mohammad Rostami Nejad, PhD.
Address: *Celiac Disease and Gluten Related Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Tel: *+98 (21) 22432525*
E-mail: *m.rostamii@gmail.com*

1. Introduction

Celiac disease (CD) is a prevalent autoimmune enteropathy affecting approximately 1% of the world's population [1]. CD, triggered by the consumption of gluten, a protein found in wheat, barley, and rye, leads to a cascade of immune responses that target the small intestine [2]. Gluten, comprising two main subunits known as glutenins and gliadins, is rich in glutamine (Gln) and proline (Pro) residues [3]. This protein triggers zonulin release, leading to increased intestinal permeability accompanied by autoimmune responses and inflammation [4]. Zonulin acts by disassembling the tight junction proteins (including ZO-1 and occludin) leading to the passage of undigested peptides to the lamina propria, causing proinflammatory cascades and changes in levels of cytokines, such as interleukin (IL)-6, IL-10, and transforming growth factor beta (TGF-β) [5]. Exposure to gliadin has been associated with intracellular oxidative imbalance. Oxidative stress in CD may contribute to the progression of intestinal damage and the development of associated complications. It can further exacerbate inflammation, disrupt the integrity of the intestinal barrier, and interfere with the absorption of nutrients. Increased oxidative stress results in the activation of nuclear factor kappa B (NF-κB), an important transcription factor involved in regulating immune and inflammatory responses and plays central roles in oxidative stress [6]. NF-κB may serve as a marker for oxidative stress and increases the transcription of enzymes, including cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase 2 (NOS2) [7].

The management of CD involves adhering to a strict, lifelong gluten-free diet (GFD) [8]. Nonetheless, alternative therapies with anti-inflammatory and antioxidant properties have been explored over time. Recent studies have been actively exploring potential components that possess the ability to reduce the production of harmful oxidants and support the integrity of the intestinal barrier. The ultimate aim is to determine whether these components can be recommended as supplementary or alternative therapies for individuals with CD [9]. At present, there is no model of a CD that replicates the intricate complexity of a CD. However, in vitro models using various cell lines, including Caco-2 cells are widely recognized as a suitable model, which is frequently employed to study the toxicity of gluten-containing cereals and are widely used in this type of study [7].

Spirulina platensis, is a type of blue-green algae with a filamentous structure composed of multiple cells. It is derived from *Arthrospira platensis*, a microscopic cyanobacterium that thrives in fresh and marine waters around the world through photosynthesis [10]. In addition to protein (up to 70%), *S. platensis* is rich in vitamins, particularly B12 and provitamin A (beta-carotene), minerals, like iron, and compounds, such as phenolic acids, tocopherols, and gamma-linolenic acid. Notably, C-phycocyanin (C-PC), as a pigment-binding protein found in *S. platensis*, has been reported to possess antioxidant properties, scavenging free radicals, inhibiting cyclooxygenase-2 selectively, and exhibiting anti-inflammatory and anticancer effects [11, 12]. Numerous researchers have recommended *S. platensis* as a safe food source and algae supplement for human consumption without any significant side effects [12, 13].

Our study aimed to evaluate the effects of *S. platensis* extract on oxidative stress, inflammation, and tight junction-related genes in Caco-2 cells exposed to gliadin.

2. Materials and Methods

Investigations were carried out at the Celiac Disease and Gluten Related Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Preparation of *S. Platensis* extract

A total of 40 grams of *S. platensis* powder underwent homogenization in a mixture of solvents consisting of methanol, acetone, and hexane, in a volume of 200 mL. The homogenization process involved stirring the mixture for 2 hours. Subsequently, the resulting supernatants were collected. The solvents were retained using a rotary evaporator, while the remaining residues were stored at a temperature of 4°C for subsequent analysis [14].

In vitro digestion of gliadins

A pepsin/trypsin (PT) digest of gliadin from wheat (Sigma) (to mimic normal intestinal degradation) was prepared to be exposed to the human colon carcinoma Caco-2 cell line as described [15]. In brief, the process began by dissolving gliadin in 0.2 N HCL and subjecting it to a two-hour incubation at 37°C with pepsin. The resulting peptic digest was then treated with trypsin after adjusting the pH to 7.4 using 2 N NaOH. The solution was further incubated at 37°C for four hours with vigorous agitation. To deactivate the enzymes, the mixture was boiled for 30 minutes and subsequently stored at -20°C. This resulting product is referred to as PT-gliadin.

Cell culture conditions

Caco-2 cells were cultivated in minimum essential medium (MEM), supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 0.1% penicillin-streptomycin, sodium bicarbonate, and sodium pyruvate. The cells were maintained at 37°C in an atmosphere with 5% CO_2. The culture medium was replaced every three days until the cells reached 80% confluency. Untreated Caco-2 cells were used as negative controls and lipopolysaccharide (LPS)-treated Caco-2 cells were used as positive.

MTT assay

Caco-2 cells were cultured in a 96-well plate with 62.5×10^3 cells/well and incubated overnight. The cells underwent a 24-hour treatment with extracts dissolved in a fresh medium. Accordingly, *S. platensis* extracts were added from a stock diluted to concentrations ranging from 5 to 100 mg/mL. Afterward, the cells were exposed to 0.5 mg/mL MTT for 4 hours. To dissolve the formazan produced by viable cells, the culture medium was replaced with 100 µL of DMSO. Subsequently, absorbance readings were performed at 570 nm with a reference wavelength of 630 nm. Cell viability was assessed using the formula described previously [16]. As a negative control, cells were incubated solely in the culture medium.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

The YTA total RNA purification mini kit (Yekta Tajhiz Azma, Tehran, Iran) was employed to extract total RNA from Caco-2 cells. The concentration and purity of the extracted RNA were measured using the NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Reverse transcription was carried out using the 2-step 2X qRT-PCR Premix (Taq) kit (BioFact™, Daejeon, Republic of Korea).

A quantitative real-time PCR assay was conducted using the Rotor-Gene Q MDx instrument to evaluate the levels of IL-6 and TGF-β as cytokines associated with inflammation, COX-2 and NF-κB as agents related to oxidative stress, and *ZO-1* and *occludin* as genes related to tight junctions. Primer sequences were designed using Gene Runner software version 6.0, and their specificity was confirmed through NCBI Primer-BLAST and PCR experiments, followed by 1.5% agarose gel electrophoresis (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) served as the reference housekeeping gene, and the relative quantitation (RQ) for each gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The data analysis was performed using Prism software, version 6.04 (GraphPad, La Jolla, CA, USA). Data were presented as Mean±SD, and differences in means between groups were assessed using one-way ANOVA. Statistical significance was determined at a $P<0.05$.

3. Results

Viability assessment

In order to assess the potential toxicity of *S. platensis* extract, Caco-2 cells were subjected to escalating concentrations of the compound for 48 hours. Subsequently, the MTT cell proliferation assay was conducted to evaluate the impact of *S. platensis* extract at different concentrations. The findings from the assay indicated that the differences in cell proliferation rates following exposure to different concentrations of *S. platensis* extract were not statistically significant ($P>0.05$), except for instances where the concentrations reached 80 and 100 mg/mL. These two concentrations exhibited notable decreases in cell proliferation rates compared to the untreated control group (Figure 1). Based on the results obtained from the MTT analysis, the concentration of 50 mg/mL was selected for further analysis.

The expression of pro- and anti-inflammatory, stress oxidative, and tight junction-related genes

Our study examined the influence of *S. platensis* on mRNA expression levels of IL-6 (pro-inflammatory cytokine) and TGF-β (anti-inflammatory cytokine). The results revealed that treating Caco-2 cells with S. platensis alone ($P=0.01$) or in combination with PT-gliadin ($P=0.004$) led to a decrease in IL-6 mRNA expression compared to cells treated with PT-gliadin alone. The expression of IL-6 mRNA considerably increased following PT-gliadin treatment relative to the untreated cells ($P=0.03$) (Figure 2A).

There were no significant differences observed in TGF-β mRNA expression between the different studied groups ($P>0.05$). However, a gradual decline in the expression of TGF-β mRNA expression was observed in cells treated with PT-gliadin in comparison to the untreated group, and the administration of *S. platensis* slightly enhanced its expression ($P>0.05$) (Figure 2B).

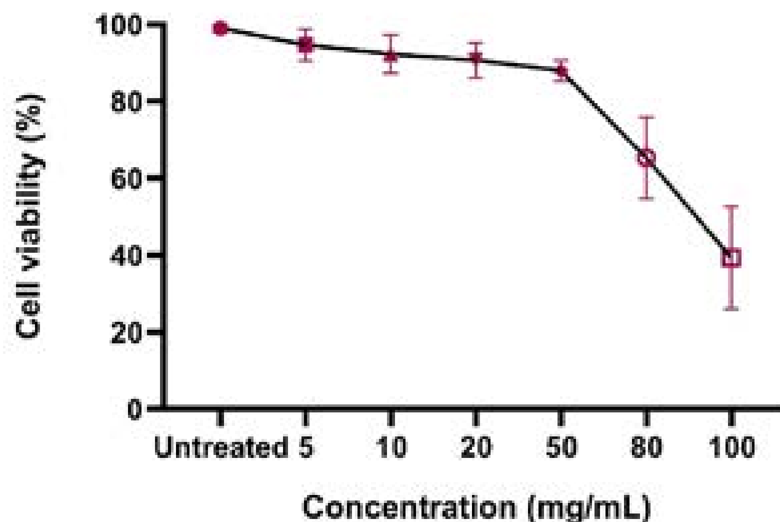
Table 1. Primers used in qRT-PCR

Gene Symbol	Primer Sequence	Reference
IL-6	F: 5′-CTGGATTCAATGAGGAGACTTGC-3′ R: 5′-TCAAATCTGTTCTGGAGGTACTCTAGG-3′	Molaaghaee-Rouzbahani (2023) [32]
TGF-β	F: 5′-CAATTCCTGGCGATACCTCAG-3′ R: 5′-GCACAACTCCGGTGACATCAA-3′	Molaaghaee-Rouzbahani (2023) [32]
COX-2	F: 5′-CCCTGAGCATCTACGGTTTG-3′ R: 5′-ACAACTGCTCATCACCCCAT-3′	This study
NF-κB	F:5′- ATGTGGCCGAGGACTTTGATT -3′ R:5′- AGTGGGGTGGCTTTTAGGATG -3′	Hajinabi (2022) [33]
ZO-1	F: 5′-TCACCTACCACCTCGTCGT-3′ R: 5′-CGCAGGGGTGTGTGTTTAC-3′	This study
Occludin	F: 5′-AACTTCGCCTGTGGATGACTT-3′ R: 5′-CTCATCACAGGACTCGCCG-3′	This study
GAPDH	F: 5′-TCTGACTTCAACAGCGACAC-3′ R: 5′-TACTCCTTGAGGCCATGT-3′	Molaaghaee-Rouzbahani (2023) [32]

International Journal of
Medical Toxicology & Forensic Medicine

Furthermore, our study demonstrated that the combination of PT-gliadin and *S. platensis* led to a decrease in the mRNA expression of *COX-2* (P=0.03) and *NF-κB* (P=0.04) genes associated with oxidative stress. Treating cells with *S. platensis* alone also resulted in reduced mRNA expression of NF-κB (P=0.02). The expression of these genes was increased following PT-gliadin treatment in comparison to the untreated cells (P>0.05) (Figure 3).

Regarding genes associated with tight junctions, there was a notable rise in the mRNA levels of occludin (P=0.01 and P=0.02, respectively) when Caco-2 cells were treated with *S. platensis* alone or a combination of PT-gliadin and *S. platensis*. Additionally, *S. platensis* enhanced the mRNA levels of Zo-1 (P=0.002). However, the stimulation of PT-gliadin resulted in a decrease in the expression of these two genes (P>0.05) (Figure 4).



International Journal of
Medical Toxicology & Forensic Medicine

Figure 1. MTT assay results of the *S. platensis* effects on cell viability of Caco-2 cells

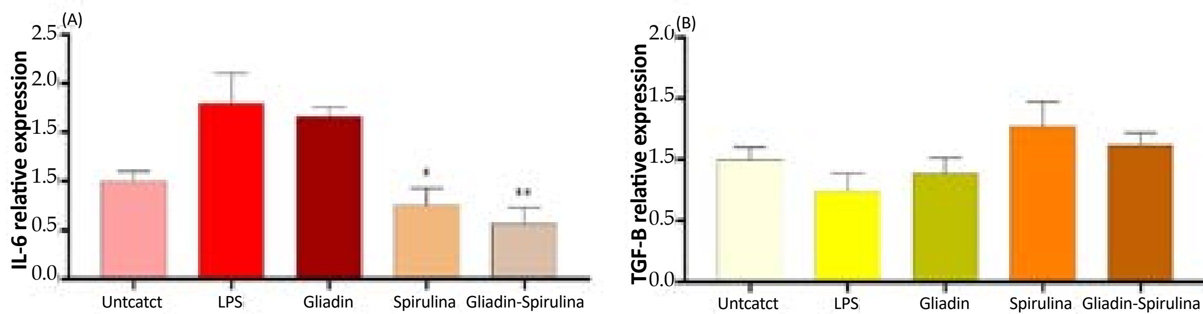


Figure 2. The relative expression of (A) *IL-6* and (B) *TGF-B* in Caco-2 cells

International Journal of
Medical Toxicology & Forensic Medicine

Results are expressed as Mean±SD.

*P≤0.05, **P≤0.01.

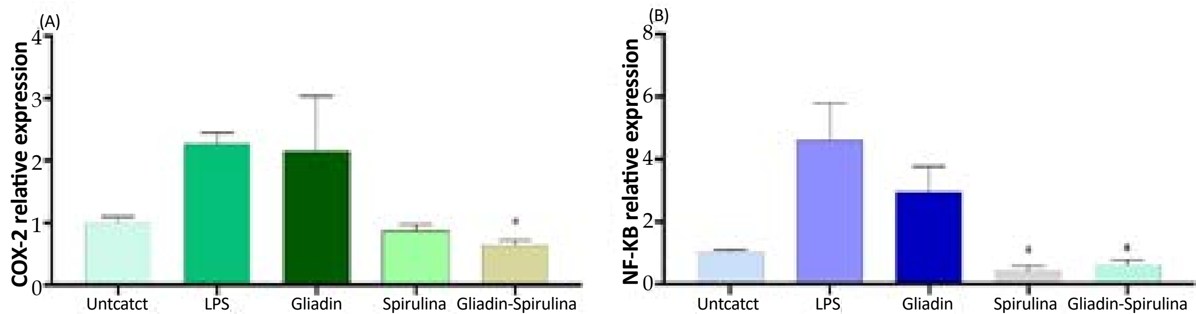


Figure 3. The relative expression of (A) *COX-2* and (B) *NF-KB* in Caco-2 cells

International Journal of
Medical Toxicology & Forensic Medicine

Results are expressed as the Mean±SD.

*P≤0.05.

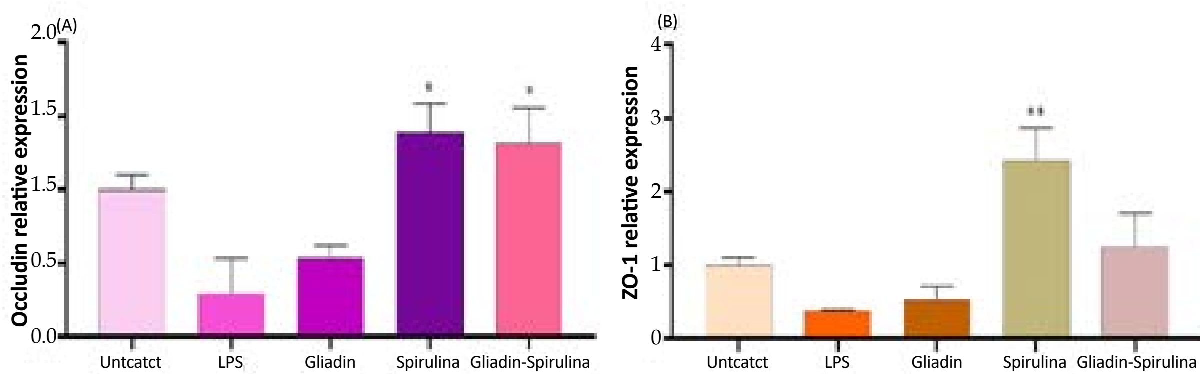


Figure 4. The relative expression of (A) *Occludin* and (B) *ZO-1* in Caco-2 cells

International Journal of
Medical Toxicology & Forensic Medicine

Results are expressed as Mean±SD.

*P≤0.05, **P≤0.01.

4. Discussion

CD is a multifaceted condition, in which the pathophysiological mechanisms are not very well known. However, it is recognized that various factors, including oxidative stress, inflammation, and disassembly of tight junctions play significant roles in its development [17]. The gliadin sequence has regions with an important cytotoxic or immunomodulatory activity in this disease and the gliadin administration significantly alters the paracellular permeability and induces oxidative stress and inflammation in Caco-2 cells [7]. Our results confirmed the literature and the stimulation of Caco-2 cells with PT-gliadin increased the levels of IL-6, COX-2, and NF-κB, and a reduction in the levels of occludin and ZO-1 mRNA expressions. Drago et al. [18] observed that the exposure of Caco-2 cells to gliadin resulted in the rearrangement of the cell cytoskeleton. This rearrangement led to the loss of occludin, a crucial protein involved in maintaining cellular tight junctions, ultimately resulting in an increase in cell permeability. Furthermore, numerous in vitro investigations have documented redox imbalance and increased free radical levels subsequent to gliadin exposure [19-22]. Ramedani et al. [23] found that PT-gliadin had the ability to enhance the production of pro-inflammatory cytokines by Caco-2 cells while simultaneously decreasing the production of anti-inflammatory cytokines by these cells.

The central role of NF-κB has been reported in the persistence of inflammation in CD [7]. Gliadin exposure has been reported to potentially induce the activation of NF-κB through the generation of free radicals [7]. Our results showed that Caco-2 cell treatment with *S. platensis* and a mixture of gliadin and *S. platensis* reduced mRNA expression of NF-κB by Caco-2 cells. Furthermore, the activation of NF-κB by PT-gliadin is considered to play a role in increasing the permeability of the barrier. This is achieved through the down-regulation of genes involved in tight junctions, such as *ZO-1*, and by causing alterations in the positioning of these junctions [24]. According to the current study, the administration of *S. platensis* alone or in combination with PT-gliadin to Caco-2 cells protected tight junction integrity and increased the mRNA expression of ZO-1 and occludin.

COX-2, an additional gene targeted by NF-κB, is activated by inflammatory cytokines in the inflamed area. The expression of *COX-2* is controlled by growth factors and various cytokines, like IL-6 [25-27]. Our study demonstrated that the mRNA expression levels of COX-2 and IL-6 were reduced following treating Caco-2 cells with *S. platensis* alone or in combination with PT-digested gliadin. These data confirmed the anti-inflammatory and anti-oxidant effects of *S. platensis* and demonstrated its protective effects on tight junction integrity.

We have chosen this model of study for CD because previous research has reported numerous favorable effects of *S. platensis* and its active component C-phycocyanin. These include immune-modulatory, anti-inflammatory, kidney-protective, liver-protective, anti-diabetic, neuroprotective, anti-cancer, anti-hypertensive, and antigenotoxic functions [28]. In this regard, Yu et al. showed that *S. platensis* had beneficial effects on gut health in rats fed a high-fat diet. It reduced body weight, improved the composition of the gut microbiota, lowered inflammation, and restored normal gut permeability [28]. Phycocyanin exhibited immune-regulatory and anti-inflammatory effects by preserving the integrity of intestinal cells and modulating cytokine production in a co-culture system involving Caco-2 cells and macrophages [29]. *S. platensis* showed protective effects against ulcerative colitis by reducing inflammation markers and improving disease parameters in a rat model. The hydroalcoholic extract of *S. platensis* had dose-dependent effects, while the chloroform extract only lowered certain inflammatory markers [30]. In another study conducted by Mazloomi et al. [31], the effects of *S. platensis* on nonalcoholic fatty liver disease (NAFLD) patients were evaluated. After an eight-week intervention, *S. platensis* showed potential in improving fatty liver grade and modifying liver enzymes, oxidative stress, and certain lipid profiles. Overall, *S. platensis* supplementation holds promise for managing NAFLD-related factors. However, the regulation of intestinal barrier function as well as the improvement in CD-related inflammation and oxidative stress by *S. platensis* has not yet been studied. Our findings suggest that *S. platensis* has the potential to serve as a potent remedy for mitigating oxidative damage and inflammation in intestinal tissue caused by CD. Nevertheless, we recommend conducting a comprehensive evaluation of its toxicity to determine any possible side effects when administering it as a treatment for CD patients.

5. Conclusion

In summary, our findings suggest that *S. platensis* can be considered as a treatment option for addressing inflammation, oxidative stress, and tight junction disassembly caused by CD. Further research should prioritize exploring the impact of *S. platensis*/gliadin interactions on the overall CD response to gliadin, including the activation of immune cells sensitive to gluten, by utilizing more intricate in vitro or ex vivo models.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.RIGLD.REC.1399.036), and did not include any research involving human or animal participants.

Funding

The study was supported by Shahid Beheshti University of Medical Sciences.

Authors' contributions

Conceptualization: Mohammad Rostami-Nejad; Methodology: Somayeh Jahani-Sherafat, Samira Alipour, Mahtab Jahdkaran and Ensiyeh Olamafar; Validation: Mohammad Rostami-Nejad, Mahmood Khodadoost and Mona Zamanian Azodi; Formal analysis: Somayeh Jahani-Sherafat and Mohammad Rostami-Nejad; Original draft preparation: Somayeh Jahani-Sherafat; Review and critical editing: Mohammad Rostami-Nejad, Mona Zamanian Azodii and Mahmood Khodadoost; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The Research Institute for Gastroenterology and Liver Diseases (RIGLD), Shahid Beheshti University of Medical Sciences is gratefully acknowledged.

References

[1] Taraghikhah N, Ashtari S, Asri N, Shahbazkhani B, Al-Dulaimi D, Rostami-Nejad M, et al. An updated overview of spectrum of gluten-related disorders: Clinical and diagnostic aspects. BMC Gastroenterology. 2020; 20(1):258. [DOI:10.1186/s12876-020-01390-0] [PMID]

[2] Asri N, Rostami-Nejad M. The facts of celiac disease; a comprehensive review. International Journal of Celiac Disease. 2019; 7(2):48-52. [Link]

[3] Shewry P. What is gluten-why is it special? Frontiers in Nutrition. 2019; 6:101. [DOI:10.3389/fnut.2019.00101] [PMID]

[4] Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Annals of the New York Academy of Sciences. 2012; 1258(1):25-33. [DOI:10.1111/j.1749-6632.2012.06538.x] [PMID]

[5] Sturgeon C, Fasano A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. Tissue Barriers. 2016; 4(4):e1251384. [DOI:10.1080/21688370.2016.1251384] [PMID]

[6] Ferretti G, Bacchetti T, Masciangelo S, Saturni L. Celiac disease, inflammation and oxidative damage: A nutrigenetic approach. Nutrients. 2012; 4(4):243-57. [DOI:10.3390/nu4040243] [PMID]

[7] Domsa EM, Filip GA, Olteanu D, Baldea I, Clichici S, Muresan A, et al. Gold nanoparticles phytoreduced with Cornus mas extract mitigate some of gliadin effects on Caco-2 cells. Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society. 2020; 71(2). [DOI:10.26402/jpp.2020.2.04] [PMID]

[8] Aspasia S, Emmanuela-Kalliopi K, Nikolaos T, Eirini S, Ioannis S, Anastasia M. The gluten-free diet challenge in adults with coeliac disease: The hellenic survey. PEC Innovation. 2022; 1:100037. [DOI:10.1016/j.pecinn.2022.100037] [PMID]

[9] Asri N, Rostami-Nejad M, Rezaei-Tavirani M, Razzaghi M, Asadzadeh-Aghdaei H, Zali MR. Novel therapeutic strategies for celiac disease. Middle East Journal of Digestive Diseases. 2020; 12(4):229-37. [PMID] [PMCID]

[10] Liu W, Ruan R. Chapter 14 - Microalgae-based biomaterials for environmental remediation and functional use. In: Ngo H, Guo W, Pandey A, Chang JS, Lee DJ, editors. Biomass, biofuels, and biochemicals. Amsterdam: Elsevier; 2022. [DOI:10.1016/B978-0-323-96142-4.00015-4]

[11] Ho JN, Watson RR, Lee J. Chapter 11 - dietary supplements, immune modulation, and diabetes control. In: Watson RR, Preedy VR, editors. Bioactive food as dietary interventions for diabetes. Amsterdam: Elsevier; 2013. [DOI:10.1016/B978-0-12-397153-1.00011-1]

[12] Abdel-Daim MM, Farouk SM, Madkour FF, Azab SS. Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to dunaliella salina in acetic acid-induced rat experimental colitis. Immunopharmacology and Immunotoxicology. 2015; 37(2):126-39. [DOI:10.3109/08923973.2014.998368] [PMID]

[13] Al-Qahtani WH, Binobead MA. Anti-inflammatory, antioxidant and antihepatotoxic effects of spirulina platensis against d-galactosamine induced hepatotoxicity in rats. Saudi Journal of Biological Sciences. 2019; 26(4):647-52. [DOI:10.1016/j.sjbs.2018.01.003] [PMID]

[14] Abdel-Moneim AE, El-Saadony MT, Shehata AM, Saad AM, Aldhumri SA, Ouda SM, et al. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences. 2022; 29(2):1197-209. [DOI:10.1016/j.sjbs.2021.09.046] [PMID]

[15] Manai F, Azzalin A, Gabriele F, Martinelli C, Morandi M, Biggiogera M, et al. The in vitro effects of enzymatic digested gliadin on the functionality of the autophagy process. International Journal of Molecular Sciences. 2018; 19(2):635. [DOI:10.3390/ijms19020635] [PMID]

[16] Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010; 140(6):918-34. [DOI:10.1016/j.cell.2010.02.016] [PMID]

[17] Sharma N, Bhatia S, Chunduri V, Kaur S, Sharma S, Kapoor P, et al. Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. Frontiers in Nutrition. 2020; 7:6. [DOI:10.3389/fnut.2020.00006] [PMID]

[18] Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scandinavian Journal of Gastroenterology. 2006; 41(4):408-19. [DOI:10.1080/00365520500235334] [PMID]

[19] Dolfini E, Elli L, Dasdia T, Bufardeci B, Colleoni MP, Costa B, et al. In vitro cytotoxic effect of bread wheat gliadin on the LoVo human adenocarcinoma cell line. Toxicology in vitro: An International Journal Published in Association With BIBRA. 2002; 16(4):331-7. [DOI:10.1016/S0887-2333(02)00017-6] [PMID]

[20] Maiuri MC, De Stefano D, Mele G, Iovine B, Bevilacqua MA, Greco L, et al. Gliadin increases iNOS gene expression in interferon-gamma-stimulated RAW 264.7 cells through a mechanism involving NF-kappa B. Naunyn-Schmiedeberg's Archives of Pharmacology. 2003; 368(1):63-71. [DOI:10.1007/s00210-003-0771-y] [PMID]

[21] Rivabene R, Mancini E, De Vincenzi M. In vitro cytotoxic effect of wheat gliadin-derived peptides on the Caco-2 intestinal cell line is associated with intracellular oxidative imbalance: Implications for coeliac disease. Biochimica et Biophysica Acta. 1999; 1453(1):152-60. [DOI:10.1016/S0925-4439(98)00095-7] [PMID]

[22] Tucková L, Novotná J, Novák P, Flegelová Z, Kveton T, Jelínková L, et al. Activation of macrophages by gliadin fragments: Isolation and characterization of active peptide. Journal of Leukocyte Biology. 2002; 71(4):625-31. [DOI:10.1189/jlb.71.4.625] [PMID]

[23] Ramedani N, Seidita A, Asri N, Azimirad M, Yadegar A, Jahani-Sherafat S, et al. The gliadin hydrolysis capacity of B. longum, L. acidophilus, and L. plantarum and their protective effects on Caco-2 cells against gliadin-induced inflammatory responses. Nutrients. 2023; 15(12):2769. [DOI:10.3390/nu15122769] [PMID]

[24] Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, et al. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. American Journal of Physiology Gastrointestinal and Liver Physiology. 2004; 286(3):G367-76. [DOI:10.1152/ajpgi.00173.2003] [PMID]

[25] Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. International Journal of Cell Biology. 2010; 2010:215158. [DOI:10.1155/2010/215158] [PMID]

[26] Desai SJ, Prickril B, Rasooly A. Mechanisms of phytonutrient modulation of Cyclooxygenase-2 (COX-2) and inflammation related to cancer. Nutrition and Cancer. 2018; 70(3):350-75. [DOI:10.1080/01635581.2018.1446091] [PMID]

[27] Varasteh S, Fink-Gremmels J, Garssen J, Braber S. α-Lipoic acid prevents the intestinal epithelial monolayer damage under heat stress conditions: Model experiments in Caco-2 cells. European Journal of Nutrition. 2018; 57(4):1577-89. [DOI:10.1007/s00394-017-1442-y] [PMID]

[28] Yu T, Wang Y, Chen X, Xiong W, Tang Y, Lin L. Spirulina platensis alleviates chronic inflammation with modulation of gut microbiota and intestinal permeability in rats fed a high-fat diet. Journal of Cellular and Molecular Medicine. 2020; 24(15):8603-13. [DOI:10.1111/jcmm.15489] [PMID]

[29] Yoshimoto S, Okada K, Hayashi O. Immuno-regulatory and anti-inflammatory actions of phycocyanin on Caco-2/U937 cells co-culture as a model of the intestinal barrier. Functional Foods in Health and Disease. 2019; 9(7):466-83. [DOI:10.31989/ffhd.v9i7.611]

[30] Morsy MA, Gupta S, Nair AB, Venugopala KN, Greish K, El-Daly M. Protective effect of spirulina platensis extract against dextran-sulfate-sodium-induced ulcerative colitis in rats. Nutrients. 2019; 11(10):2309. [DOI:10.3390/nu11102309] [PMID]

[31] Mazloomi SM, Samadi M, Davarpanah H, Babajafari S, Clark CCT, Ghaemfar Z, et al. The effect of spirulina sauce, as a functional food, on cardiometabolic risk factors, oxidative stress biomarkers, glycemic profile, and liver enzymes in nonalcoholic fatty liver disease patients: A randomized double-blinded clinical trial. Food Science & Nutrition. 2022; 10(2):317-28. [DOI:10.1002/fsn3.2368] [PMID]

[32] Molaaghaee-Rouzbahani S, Asri N, Sapone A, Baghaei K, Yadegar A, Amani D, et al. Akkermansia muciniphila exerts immunomodulatory and anti-inflammatory effects on gliadin-stimulated THP-1 derived macrophages. Scientific Reports. 2023; 13(1):3237. [DOI:10.1038/s41598-023-30266-y] [PMID]

[33] Hajinabi Y, Khamene EA, Forouzesh F, Gholam-Mostafaei FS, Ahangari SAH, Lamouki RM, et al. The genes expression of NF-kB inflammation pathway in treated celiac disease patients. New Zealand Journal of Medical Laboratory Science. 2022; 76(2):55-9. [Link]