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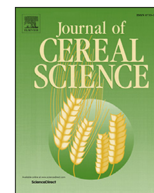
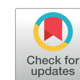


Inflammatory response of gliadin protein isolated from various wheat varieties on human intestinal cell line



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ABSTRACT

Wheat protein contributes a significant part in human diet, apart from its well-known nutritional values, wheat gluten/gliadin proteins are also responsible for the many allergic/inflammatory diseases and chronic inflammation in the small intestine may cause diarrhea and malabsorption, in a specific population of individuals. In the present study, the antigenic characteristics of twelve wheat varieties of diverse origin namely C273, C281, C286, C306, C518, C591, Agra Local, 9D, 8A, Raj4229, HD3027, NP824 released during 1920–2012 were evaluated. Gliadin proteins from these varieties were tested on human colon cancer cell line HCT116 to assess their effect on inflammation, oxidative and nitrosative stress, pro-inflammatory cytokines. The results show that these wheat varieties induced high levels of ROS/RNS and MPO activity which was further supported by the increase in the mRNA levels of a cytokine such as IL-1β and IL-15. It can be concluded that gliadin from these wheat varieties is suggested to act as a potential antigen by enhancing the level of inflammation irrespective of their year of release and origin which if not controlled may lead to the initiation of celiac disease in genetically susceptible individuals or may be responsible for other wheat protein intolerance associated diseases.

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1. Introduction

Wheat provides about 20% of the total food calories consumed worldwide and is staple food in many countries. Besides being an essential source of proteins and carbohydrates, it is also one of the leading causes of many diet-induced health issues, especially wheat protein induced allergies, celiac disease (CD), Non-celiac gluten sensitivity (NCGS) and much more. These are collectively known as wheat intolerance or wheat related diseases, and it is identified all over the world. Its prevalence in general population is very high e.g. occurrence of CD is more than 1%, (Sollid and Khosla, 2005), and incidence of NCGS is about 0.5–13% (Molina-Infante et al., 2015). Out of these CD is an autoimmune inflammatory disorder with complex genetics. The detailed immunopathogenesis of CD has not been fully elucidated (Gujral et al., 2012). Gluten/gliadin protein of wheat is the key player of CD which finally generates inflammatory responses and ultimately leads to a restructuring of the intestinal epithelial architecture i.e. destruction of villi and eventually leads to malabsorption of nutrients. The chronic inflammation during CD subsequently may initiate intestinal cancer (Freeman, 2009).

In the present study, the inflammatory responses of gliadin protein from different wheat varieties available to the population in the north Indian subcontinent are analyzed using various biochemical parameters and standard markers of inflammation along with the gene expression of inflammatory cytokines. The prime objective of this study is to analyze whether the gliadin isolated from wheat varieties based on their origin has some role to play in wheat protein related intolerance and associated inflammatory responses.

Abbreviations: MPO, Myeloperoxidase; AOPP, Advance Oxygen Protein Product; CD, Celiac Disease; NCGS, Non-Celiac Gluten Sensitivity; MTT, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; NO, Nitric Oxide; VC, Vehicular Control; NBT, Nitroblue Tetrazolium; NEDD, N-(1-naphthyl) ethylenediamine dihydrochloride; ILCs, Innate lymphoid cells; FBS, Fetal bovine serum; DMSO, Dimethyl sulfoxide; DAF-FM, 4-Amino-5-Methylamino-2',7'-Difluorofluorescein Diacetate; EtBr, Ethidium bromide; ROS, Reactive oxygen species; RNS, Reactive Nitrogen species; IBD, Inflammatory bowel disease; NO, Nitric oxide.

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2. Material and methods

2.1. Wheat germplasm

Twelve varieties of wheat (*Triticum aestivum)* used in the present study were procured from Punjab Agricultural University, Ludhiana, Punjab, India, and details are listed in Table 1.

2.2. Isolation of gliadin protein

Gliadin protein was isolated by using a modification of the protocol of Lookhart and Bean (1995). Briefly wheat grains was ground to fine powder, and 50 mg powder weighed, 70% ethanol was added and incubated at room temperature for 30–60 min with intermediate vortexing and finally centrifuged to get the supernatant containing gliadin (Lookhart and Bean, 1995). The supernatant was concentrated in vacuum concentrator to remove maximum ethanol. The protein content of this concentrated protein was evaluated using Bradford assay. The aliquots of gliadin protein was stored in −80 °C till further use.

2.3. Cell culture

HCT116 human colon cancer cell line was purchased from National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM) supplemented with 10% heat inactivated (30 min at 56 °C) FBS, 1X penicillin and streptomycin in a tissue culture flask at 37 °C in a humidified atmosphere of 5% CO2 incubator. At 70–80% confluence the cells were trypsinized using 1X trypsin-EDTA and sub-cultured in a new sterile tissue culture flask for further experiments.

2.4. Cytotoxicity assay

The cytotoxicity of isolated gliadin protein on HCT116 cells were analyzed by MTT assay. 1×10^4 cells were seeded in 96 wells plate were treated with different concentration of gliadin protein (0, 5, 10, 20 μg) and incubated for 24 h in FBS-free media. MTT solution (0.5 mg/ml) was added followed by incubation for 4 h at 37 °C. After 4 h; the purple product was solubilized using acidified DMSO (with 0.6% acetic acid) and absorbance was measured at both 570 nm and 620 nm. The cell viability of each group was calculated with their respective control (Dhiman et al., 2012).

2.5. Cell treatments and preparation of total cell lysates

1×10^6 HCT116 cells were seeded in Petri plates containing culture media supplemented with 10% FBS. During the treatment, the media was changed to serum-free media and cells were treated with 20 μg of isolated gliadin protein from different wheat variety for 24 h. After that, treated and untreated cells were washed with PBS and centrifuged at 1500 rpm for 5 min at 4 °C. The pellet was homogenized in lysis buffer [50 mM Tris-HCl (pH-8), 15 mM NaCl, 0.05 mM EDTA, 1% TritonX 100, 2 mM DTT]. After incubation for 15 min in ice, homogenates were centrifuged at 12,000 g at 4 °C for 10 min, protein content of the supernatant was measured using Bradford assay and aliquots were stored at −80 °C till further use.

Table 1
List of wheat germplasm used in present study.

Groups	Varieties	Year of release/cultivation
Group 1	C 273, C 281, C 286, C 306, C 518, C 591	1940–60
Group 2	Raj-4229	2012
Group 3	HD-3027	2012
Group 4	NP(New Pusa) 824	1950–60
Group 5	8 A, 9 D	1920–40
Group 6	Agra local	1920–40

2.6. Respiratory burst assays

2.6.1. NBT assay

The cells (1×10^6) cultured and treated with gliadin protein in a 24-well plate were incubated with 100 μl of NBT solution (0.04% NBT) for 3 h. The cells were washed twice with PBS, then once with methanol after incubation and air-dried. The NBT deposited inside the cells were then dissolved, first by adding 2 M KOH followed by addition of DMSO to dissolve blue formazan with gentle shaking for 10 min at room temperature. The absorbance of dissolved NBT solution was read at 570 and 620 nm using microplate reader (Synergy H1) (Choi et al., 2006).

2.6.2. Nitrite/nitrate assay

The nitrite levels were measured using Griess assay. Briefly, equal volumes of culture supernatant and Griess reagent (1% sulfanilamide and 0.1% of NEDD) were mixed. The absorbance of chromophore formed during nitrate diazotization with sulfanilamide and subsequent coupling with NEDD was read at 543 nm in a microplate reader (Synergy H1). The nitrite content of each sample evaluated from a standard curve obtained after near regression made with sodium nitrate and expressed in μM (Dhiman et al., 2013b).

The intracellular nitrite levels were also measured using the fluorescent probe based DAF-FM dye. Briefly, the cells (1×10^6) cultured and treated with gliadin protein in a 24 wells were incubated with 20 μM DAF-FM prepared in DMSO at 37 °C for 30 min. The dye was removed, and cells were washed with PBS. Fresh PBS was added and fluorescence reading was taken at excitation wavelength at 478 nm and emission wavelength at 515 nm in a microplate reader (Synergy H1). The plate was incubated at 37 °C for 30 min, and another fluorescence reading was measured after 30 min (Dhiman and Garg, 2014).

2.6.3. Assay for Myeloperoxidase (MPO)

The cell lysate (100 μg protein) was mixed with 0.53 mM O-dianisidine dihydrochloride and 0.15 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 6.0) and incubated for 5 min at room temperature. The change in absorbance was measured at 460 nm using microplate reader (Synergy H1) and results expressed as units of MPO/mg protein using 11.3 $M^{-1}cm^{-1}$ extinction coefficient. One unit of MPO was defined as the amount of enzyme degrading 1 nM H_2O_2 per min at 25 °C (Dhiman et al., 2009).

2.6.4. Assay for Advanced Oxygen Protein Products (AOPP)

100 μg of total cell lysate was mixed with 1.16 M potassium iodide followed by addition of glacial acetic acid (20%). The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing all reagents except the samples. Chloramine-T was used as a standard with a linear range of 0–100 μmol/L. An AOPP concentration was expressed in μmol/liter of chloramine-T equivalents (Baskol et al., 2006).

2.7. Semi-quantitative RT-PCR

Total RNA was isolated from the treated and non-treated HCT116 cells by using TRIzol® reagent (Invitrogen) and followed the manufacturer instructions, finally RNA pellet was resuspended in nuclease-free water. The quality of isolated RNA was checked on NanoDrop 2000 (Thermo Scientific) and on denaturing agarose gel. RNA was then treated with DNase1 enzyme for complete removal

of DNA contamination followed by phenol–chloroform extraction. Again the quality of DNase1 treated RNA was checked, followed by synthesis of the first strand of cDNA using the SuperScript® First-Strand Synthesis System (Invitrogen) as per the instructions by the manufacturer. cDNA was used as a template for the PCR reaction with pro-inflammatory cytokines (IL-1β, IL-15, IL-18, TNF-α) gene-specific primer pairs (Supplement data Table 2) along with a housekeeping gene (β-actin) as a loading control. The PCR product was separated on 1.2% agarose gel containing ethidium bromide (EtBr) along with 100bp DNA ladder of Invitrogen (TrackIt™ 100 bp DNA ladder). The gel image was taken by using Bio-Rad Gel Doc™ XR system and densitometric analysis was done using Image Lab™ software of Bio-Rad version 5.2 (Thakur et al., 2018).

2.8. Statistical analysis

The sample analysis was done in triplicates and assays were repeated at least three times. Data presented as mean ± S.D for control as well as experimental samples. For all statistical comparison, Student's t-test was performed with p-value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of gliadin protein on cell viability

To examine the cytotoxicity of the isolated gliadin protein of different wheat varieties on colon cancer cell line HCT116 and to determine the optimum concentration of gliadin protein for further experiment MTT assay was performed with broad concentration range of isolated gliadin protein. The HCT116 cells were treated with different concentrations (5, 10, 20 μg) of isolated gliadin protein from different wheat varieties for 24 h. Untreated cells served as control and cells treated with solvent served as vehicle control (VC). When cells were treated with 5 μg of gliadin, except C 273, NP 824 and 8 A, all other wheat varieties show significant increase in the cell viability (Fig. 1a). Where as when cells were treated with 10 μg of gliadin, except C 273, C 591 and NP 824, all other wheat varieties show significant increase in the cell viability as compared to VC (Fig. 1b). Cells when treated with 20 μg of gliadin protein, other then NP 824 and 9 D, gliadin from all other wheat show a significant increase in cell viability as compared to VC (Fig. 1c). Based on our results and available literature 20 μg of isolated gliadin protein was chosen for further experiments.

3.2. Gliadin induced total intracellular ROS level in cells

ROS level produced in HCT116 cells was determined by NBT assay. Intracellular reactive oxygen species (ROS) are responsible for the reduction of NBT inside the cells. A highly significant increase of percent ROS level was observed in all the HCT116 cells treated with 20 μg of Gliadin protein for 24 h when compared with the VC cells. About 1.5–4 fold increase

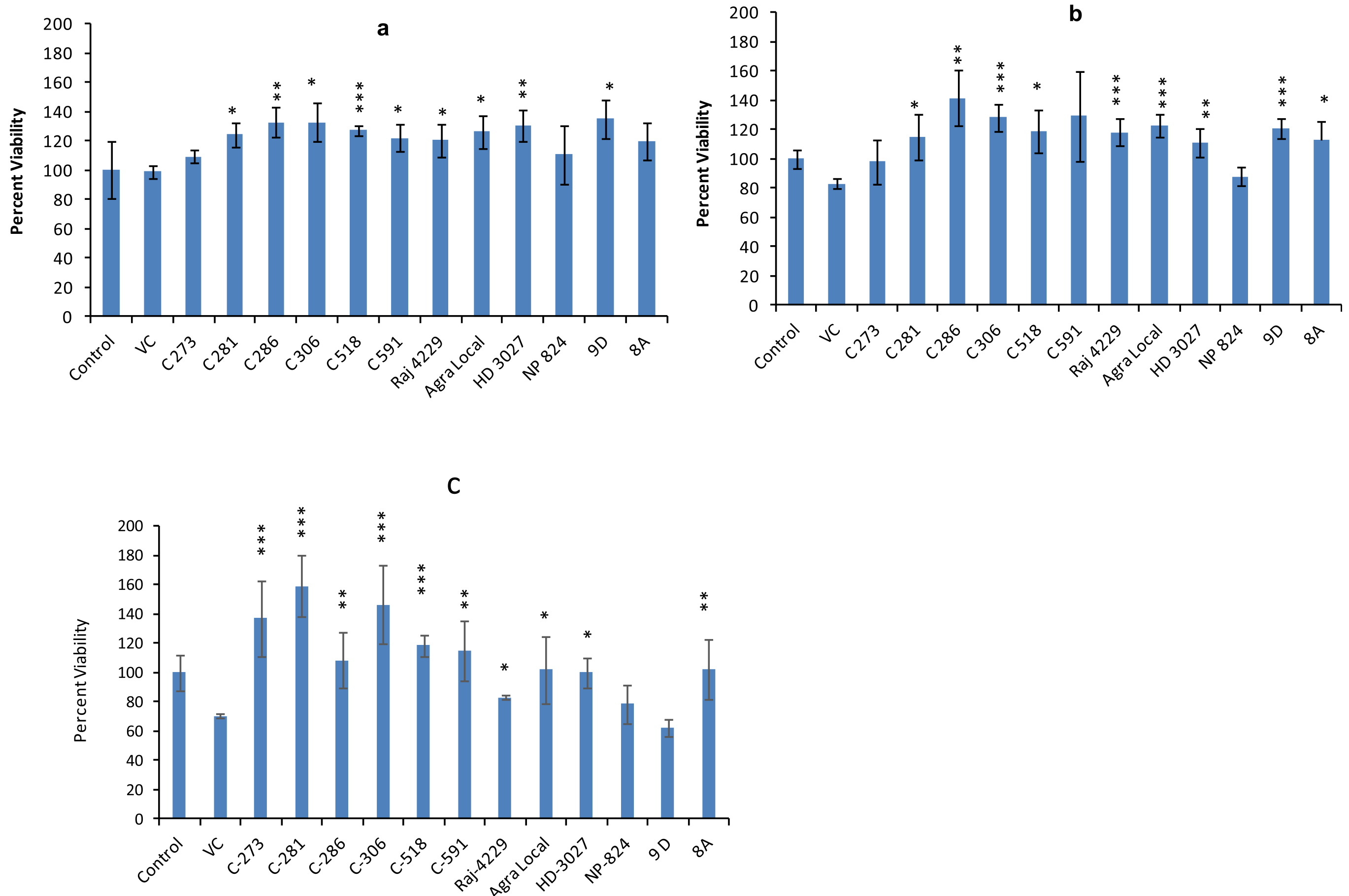


Fig. 1. a) The percent viability of HCT116 cells after treatment with 5 μg of gliadin protein for 24 h **b)** The percent viability of HCT116 cells after treatment with 10 μg of gliadin protein for 24 h **c)** The percent viability of HCT116 cells after treatment with 20 μg of gliadin protein for 24 h. The results shown are the representatives from three different experiments presented as mean ± standard deviation (n = 3) statistically significant at ***p < 0.005, **p < 0.01 & *p < 0.05.

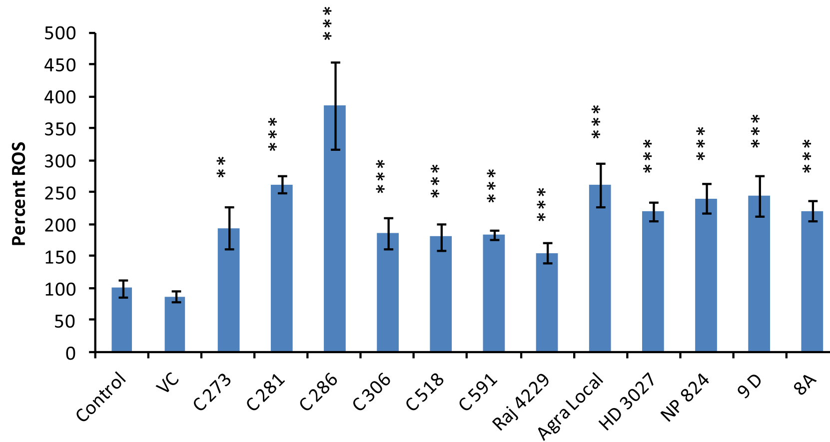


Fig. 2. Levels of Reactive Oxygen Species (ROS): Percent ROS level in HCT116 cells after treatment with gliadin protein for 24 h using NBT assay. The results shown are the representatives from three different experiments presented as mean ± standard deviation (n = 3) and statistically significant at ***p < 0.005, **p < 0.01.

was observed in the group-1 (C series) wheat varieties treated cells, about 1.5 fold increase in the group-2 (Raj 4229) wheat treated cells and more than 2–2.5 fold increase in all other 4 groups wheat varieties treated cells (Fig. 2) when compared with the VC cells.

3.3. Nitric oxide (NO) level in gliadin treated cells

The concentration of extracellular NO was determined by using Griess assay. This assay is a chemical analysis test that detects the presence of organic nitrite compounds, uses sulfanilamide and N-1-

Fig. 3. Nitric oxide level in HCT116 cells treated cells: a) Extracellular NO estimation (μM) in culture supernatant of HCT116 cells after treatment with 20 μg of gliadin protein of different wheat varieties. **b)** Extracellular NO estimation (μM) in total cell lysates of HCT116 cells after treatment with 20 μg of gliadin protein of different wheat varieties. **c)** Intracellular NO levels estimated using DAF-FM in HCT116 cells after treatment with 20 μg of gliadin protein of different wheat varieties. The results shown are the representatives from three different experiments presented as mean ± standard deviation (n = 3) and statistically significant at *p < 0.05.

napthylethylenediamine dihydrochloride (NEDD) under acidic conditions. The amount of extracellular NO was determined by standard curve plotted by using Sodium Nitrite ($NaNO_2$). The mean concentration of NO in culture supernatant of all the treated samples did not show any significant change except varieties C 273 and C 286 when compared with VC (Fig. 3a). About 1.4 fold increase in the concentration of NO was observed in C 273 C 286 and Raj 4229 treated cells when compared with VC cells after 24 h.

The mean concentration of NO in total cell lysates of all the treated samples was significantly high except C 306, C 518, Raj 4229 and Agra local varieties when compared with VC (Fig. 3b). About 1.2 folds increase of NO level in total cell lysates was observed in all the group-1 (C series) varieties treated cells except C 306 and C 518. Similarly, group-3 (HD 3027) showed about 1.15 fold increase, group-4 (NP 824) showed about 1.11 fold increase and group-5 (9 D, 8 A) showed about 1.14 fold and 1.28 fold increase in NO level, respectively as compare with VC after 24 h of treatment. Group-2 (Raj 4229) wheat protein treated cells show an increase in NO level, but it was not statistically significant. Group-6 (Agra local) treated cells show no change in NO level.

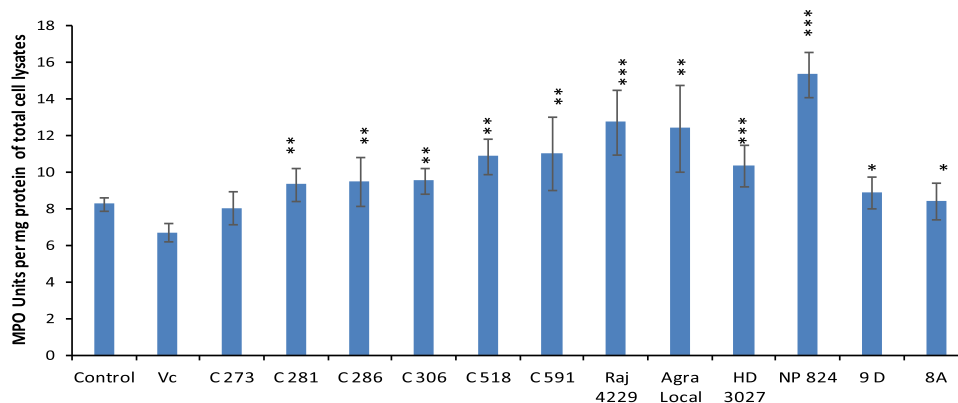
The concentration of intracellular NO was determined by using DAF-FMassay. The mean concentration of intracellular NO level was not statistically significant in HCT116 cells after 24 h of treatment with 20 μg of gliadin protein of different wheat varieties (Fig. 3c) except in Raj 4229 and Agar local.

3.4. Myeloperoxidase (MPO) activity in gliadin treated cells

A significant increase in MPO activity was observed in all the HCT116 cells (Fig. 4a) when treated with 20 μg of gliadin protein from different wheat varieties. An increase of 1.40 fold in MPO level was observed in the cells treated with group-1 (C-series) wheat varieties except for C 273 treated cells, which showed an increase in MPO level but it was not statistically significant. Group-2 (Raj 4229) gliadin treated cells showed about 1.90 fold increase, group-6 wheat (Agra local) treated cells showed about 1.85 folds increase, group-3 (HD 3027) wheat treated cells showed about 1.55 folds increase, group-4 wheat (NP 824) treated cells showed highest about 2.28 fold increase whereas group-5 (9 D, 8 A) showed 1.32 fold and 1.26 fold increase in MPO level as compared with VC.

3.5. Advanced Oxygen Protein Products (AOPP) in gliadin treated cells

A significant increase in AOPP level was observed in the



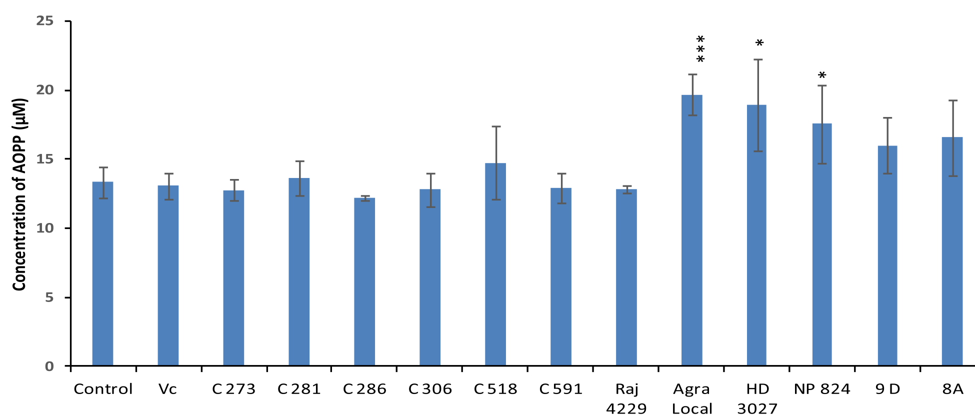


Fig. 4. Level of inflammatory markers: a). Concentration of MPO unit per mg of protein observed in HCT116 cells when treated with 20 μg of gliadin protein of different wheat varieties. The results shown are levels of MPO activity in Units/mg protein from three different experiments presented as mean ± standard deviation (n = 3) and statistically significant at $^{***}p < 0.005$, $^{**}p < 0.01$, $^{*}p < 0.05$. **b).** Concentration of AOPP (μM) in HCT116 cells after treatment with 20 μg of gliadin protein of different wheat varieties. The results shown are AOPP is expressed as μM/mg protein, from three different experiments presented as mean ± standard deviation (n = 3) and statistically significant at $^{*}p < 0.05$ and $^{**}p < 0.01$.

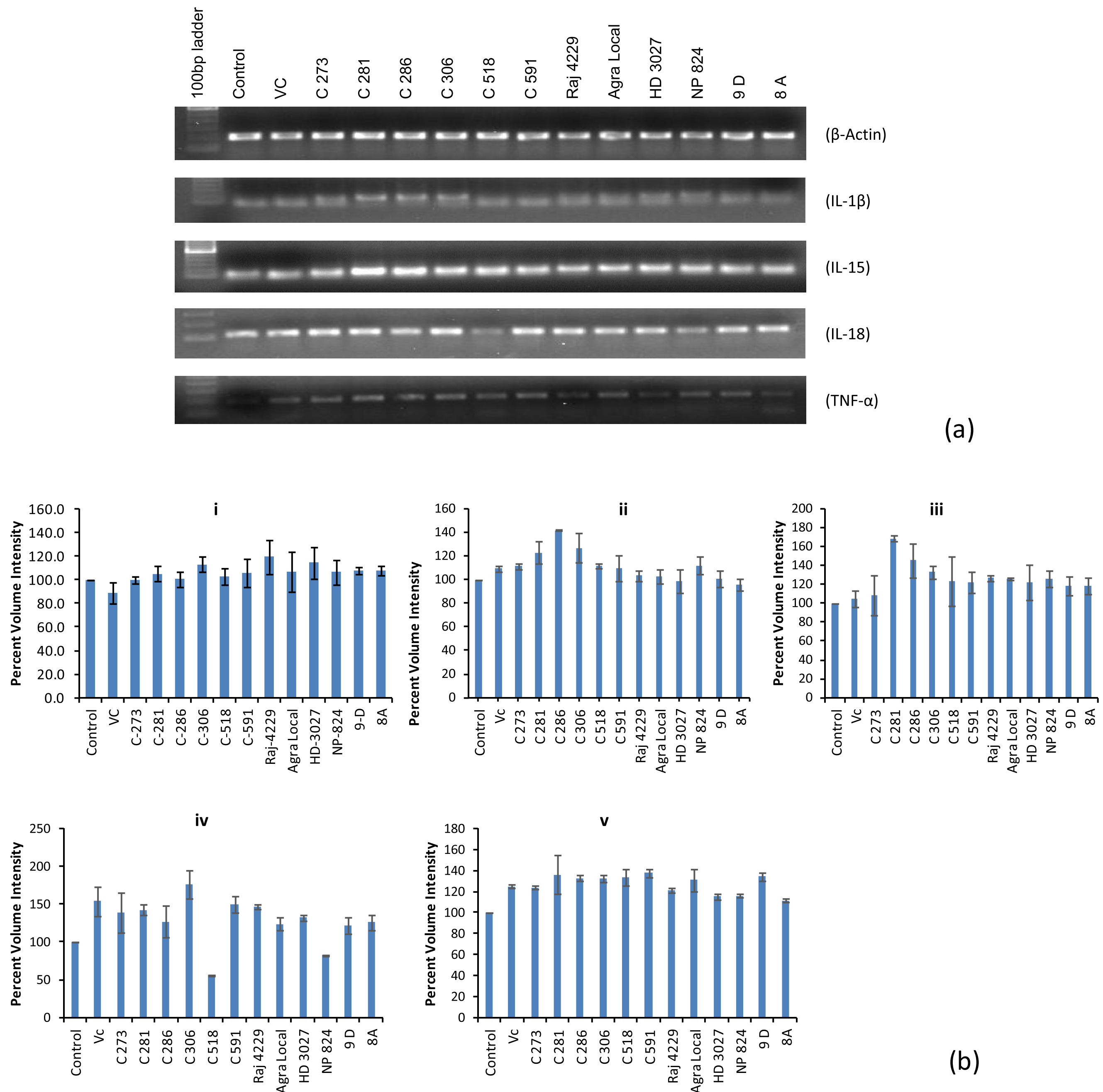


Fig. 5. a) mRNA expression of various pro-inflammatory cytokines: mRNA expression of various pro-inflammatory cytokines when HCT116 cells were treated with gliadin protein from different wheat varieties. The amplified product separated on 1% agarose gel. Control: Without treated cells, VC: Cells treated with solvent (Vehicular Control). **b). Densitometric analysis of mRNA expression of various pro-inflammatory cytokines. i).** Densitometric analysis of mRNA expression profile of β-Actin in HCT116 cells after treatment with gliadin protein from different wheat varieties. **ii)** Densitometric analysis of mRNA expression profile of IL-1β in HCT116 cells after treatment with gliadin protein from different wheat varieties after normalisation with β-Actin. **iii)** Densitometric analysis of mRNA expression profile of IL-15 in HCT116 cells after treatment with gliadin protein from different wheat varieties after normalisation with β-Actin. **iv)** Densitometric analysis of mRNA expression profile of IL-18 in HCT116 cells after treatment with gliadin protein from different wheat varieties after normalisation with β-Actin. **v)** Densitometric analysis of mRNA expression profile of TNF-α in HCT116 cells after treatment with gliadin protein from different wheat varieties after normalisation with β-Actin. The results shown are the representatives from three different experiments presented as mean ± standard deviation (n = 3).

HCT116 cells (Fig. 4b) when treated with 20 μg of gliadin protein from varieties Agra local, HD 3027 and NP 824 but in cells treated with other varieties, no any significant changes were observed. About 1.48 fold increase in AOPP level was observed in group-6 wheat (Agra local) treated cells, about 1.42 fold increase in AOPP level was seen in group-3 (HD 3027) whereas about 1.32 fold increase in AOPP level was observed in group-4 (NP 824) treated cells.

3.6. Gene expression analysis of pro-inflammatory cytokines in gliadin treated cells

Pro-inflammatory cytokines like IL-1β, IL-15, 1L-18, TNF-α, are used as a marker of inflammation. Semi-quantitative RT-PCR was done by using gene specific primers of these genes along with a housekeeping gene (β-Actin). mRNA expression profile for the β-

Actin gene in all the samples including control and VC were nearly the same (Fig. 5a–b). In cells treated with gliadin protein of wheat variety C 281, C 286 and C 306 showed an increased level of expression of the IL-1β mRNA (Fig. 5a–b ii). Similarly, all the wheat varieties except C 273, 8 A and 9 D treated cells showed an increased level of expression of the IL-15 mRNA (Fig. 5a–b iii). Similarly the expression of IL-18 mRNA is nearly same in all the treated cells except C 306 showed increase but C 518 and NP 824 showed decrease in expression (Fig. 5a–b iv). The expression of TNF-α increased significantly in C 281, C 286, C 306, C 518, C591 and 9 D wheat treated samples (Fig. 5a–b v).

4. Discussion

Gluten protein of wheat which is a combination of glutenin and gliadin fractions is the contributing element of cellular inflammatory responses. α and γ fractions of gliadin were able to generate a robust antigenic response that was postulated to play a central role in wheat induced autoimmunity (Fasano, 2012). Inflammation-mediated by gliadin is mainly due to the production of ROS/RNS (Reactive Oxygen Species/Reactive Nitrogen Species) in the intestine (Ferretti et al., 2012). Chronic inflammation in the small intestine could create a flat mucosa and leads to avillus condition which is the rooting cause of diarrhea and malabsorption.

The present study is a comparative analysis of inflammatory responses induced by the gliadin protein extracted from different wheat varieties on the human cell line. Biochemical parameters and standard markers of inflammation were examined to determine the effect of gliadin on mammalian cells under *in vitro* condition. A preliminary cytotoxicity assay presented a significant change in the proliferation of HCT116 cells as compared to VC in the presence of 20 μg gliadin and was further chosen for all experiments in present study.

The antioxidant capacity during wheat intolerance disease such as CD has been reported to be significantly reduced mainly due to depletion of glutathione (Stojiljkovic et al., 2009). In this context, an adjunct of natural antioxidants and appropriate dietary supplements is often recommended as a standard therapy for a CD patient. Human colon cancer cell lines- T84 and Caco2, when exposed to gliadin, revealed an intracellular oxidative imbalance. It was accompanied by an increase in the levels of lipid peroxidation products [4-hydroxy-2(E)-nominal 4-HNE], an increase in the oxidized to reduced glutathione ratio (GSSG: GSH) and a decrease of protein-bound sulfhydryl groups (Ferretti et al., 2012).

In present work, we determined the superoxide anion (O_2^-), an indicator of intracellular ROS levels, by using NBT assay. ROS level in all the gliadin treated cells was significantly higher when compared to VC as observed in our study. Similar results were also testified in earlier reports involving other cell lines (T84 and Caco2) (Drago et al., 2006; Luciani et al., 2010).

Nitric oxide (NO) is an inflammatory molecule with several pathophysiological roles and is designated as a contributor to the RNS-level upsurge. Increased NO production has been correlated with inflammatory gastrointestinal disorders as well as with wheat intolerance (Everts et al., 1999) The exact role NO is still unclear for both defensive and detrimental roles have been suggested (Rachmilewitz et al., 1995). Total NO concentration was calculated in both culture supernatant and total cell lysate using Griess assay. NO level in whole cell lysate was elevated in nearly all the wheat varieties treated cell. On the other hand, culture supernatant showed no change except C 273 and C 286 treated samples. A higher urinary nitrite/nitrate level was reported in children with CD, which declined significantly when the patients were on a gluten-free diet of oats (Hollen et al., 2006). On the contrary, no significant change in the intracellular RNS level after treatment with gliadin protein was observed which may be due to the short half-life of RNS and their reactivity.

Myeloperoxidase (MPO) and Advanced Oxygen Protein Products (AOPP) are the primary inflammatory mediator markers (Dhiman et al., 2013a; Witko-Sarsat et al., 2003). AOPP accumulation has been linked with several pathological conditions; their levels tend to increase during oxidative stress as a result of reactions between plasma proteins and chlorinated oxidants produced by MPO (Bochi et al., 2014). Gliadin treatment was found to alter the cellular MPO content to significantly higher levels. Our results are supported by previous studies on IBD (Kristjansson et al., 2004; Peterson et al., 2002). The AOPP level was increased significantly in Agra local, HD 3027, and NP 824 treated samples whereas no significant change was observed with 8 A, and 9 D-treated cells.

Previous studies have correlated T cells with the induction of intestinal damage during CD, yet little is known about the role of gliadin (the toxic component of gluten) in inducing inflammation. Pro-inflammatory cytokines such as IL-1β, IL-15, IL-18 and TNF-α rise simultaneously during CD and other inflammatory diseases (Garrote et al., 2008; Mention et al., 2003; Nilsen et al., 1998). Assessment of innate lymphoid cells (ILCs) isolated from the duodenal biopsies of CD patients revealed that CD-related inflammation is due to the accumulation of ILCs producing TNF-α and IFN-γ in the mucosa which also expresses toll-like receptor 3 (TLR3) and thus, contributes to small intestinal atrophy (Marafini et al., 2015). Herein, our study supplements the current understanding of a mechanism by which an undesirable immune response is initiated by gliadin protein and the consequences after that. Expression of IL-1β remain unaltered in all the cells treated with gliadin protein, except for the C 281, C 286 and C 306 treated cells where increased expression level was observed. Also, the IL-15 expression increased in almost all the treated cells but highly significant increase in expression occurred in gliadin from C 281, C 286 and C 306 wheat varieties treated cells. Our results are consistent with previous report which conveyed that the rise in IL-15 levels could also stimulate autoimmunity in CD patients (Meresse et al., 2015). The IL-18 mRNA expression was nearly the same, except a decrease was observed in C 518, group-4 (NP-824), group-6 (Agra Local), and group-5 (9 D, 8 A) wheat varieties treated cells. At last, the expression of TNF-α was nearly constant in all the cells treated with gliadin protein of all the wheat varieties except a decrease in group-2 (Raj 4229), group-3 (HD 3027), group-4 (NP 824) and 8 A when compared with VC.

The present study showed an enhanced expression of ROS, NO, MPO and inflammatory cytokines in the HCT116 cells confirms the probable correlation between inflammation and the protein component of wheat that leads to CD in genetically compromised individuals. This study has indicated that gliadin obtained from group-1 (C-series) wheat varieties might be responsible significantly for the wheat intolerance disease. However, the gliadin protein isolated from group-5 and 6 (9-D, 8-A and Agra local) varieties showed either slight or no change in ROS/RNS, inflammatory markers, and pro-inflammatory cytokines suggesting that the old wheat varieties are less immunogenic and with due course of time the germplasm of wheat has undergone changes with respect to their gliadin protein which might be responsible for various wheat related allergies and celiac disease.

5. Conclusion

The results clearly established that gliadin protein from all the wheat varieties used in present study irrespective of their origin and time of release showed potential antigenic properties by increasing the levels of various oxidative stress marker, inflammatory markers and pro-inflammatory cytokines. Although the cell

line model used in present study has some limitation and the results cannot be compared directly with the epithelial lining but the present study has provided preliminary but necessary information for further study on gliadin induced toxicity and suggest that the inflammatory responses due to gliadin may lead to the initiation of celiac disease or may be responsible for wheat protein intolerance. The precise mechanism of this action remains to be elucidated and whether pro/anti-inflammatory cytokines can further alter some signaling pathways related to these inflammation markers will be done in the near future.

Conflicts of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jcs.2018.03.012.

References

Baskol, G., Demir, H., Baskol, M., Kilic, E., Ates, F., Karakukcu, C., Ustdal, M., 2006. Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis. Cell Biochem. Funct. 24 (4), 307–311. https://doi.org/10.1002/cbf.12572.

Bochi, G.V., Torbitz, V.D., Cargnin, L.P., de Carvalho, J.A., Gomes, P., Moresco, R.N., 2014. An alternative pathway through the Fenton reaction for the formation of advanced oxidation protein products, a new class of inflammatory mediators. Inflammation 37 (2), 512–521. https://doi.org/10.1007/s10753-013-9765-13.

Choi, H.S., Kim, J.W., Cha, Y.N., Kim, C., 2006. A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. J. Immunoassay Immunochem. 27 (1), 31–44. https://doi.org/10.1080/15321810500403722.4.

Dhiman, M., Coronado, Y.A., Vallejo, C.K., Petersen, J.R., Ejilemele, A., Nunez, S., et al., 2013a. Innate immune responses and antioxidant/oxidant imbalance are major determinants of human Chagas disease. PLoS Neglected Trop. Dis. 7 (8) https://doi.org/10.1371/journal.pntd.00023645 e2364.

Dhiman, M., Estrada-Franco, J.G., Pando, J.M., Ramirez-Aguilar, F.J., Spratt, H., Vazquez-Corzo, S., et al., 2009. Increased myeloperoxidase activity and protein nitration are indicators of inflammation in patients with Chagas' disease. Clin. Vaccine Immunol. 16 (5), 660–666. https://doi.org/10.1128/cvi.00019-096.

Dhiman, M., Garg, N.J., 2014. P47phox-/- mice are compromised in expansion and activation of CD8+ T cells and susceptible to Trypanosoma cruzi infection. PLoS Pathog. 10 (12) https://doi.org/10.1371/journal.ppat.1004516 e1004516.

Dhiman, M., Wan, X., Popov, V.L., Vargas, G., Garg, N.J., 2013b. MnSODtg mice control myocardial inflammatory and oxidative stress and remodeling responses elicited in chronic Chagas disease. J. Am. Heart Assoc. 2 (5) https://doi.org/10.1161/jaha.113.000302 e000302.

Dhiman, M., Zago, M.P., Nunez, S., Amoroso, A., Rementeria, H., Dousset, P., et al., 2012. Cardiac-oxidized antigens are targets of immune recognition by antibodies and potential molecular determinants in Chagas disease pathogenesis. PLoS One 7 (1), e28449. https://doi.org/10.1371/journal.pone.0028449.

Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente, M., Tripathi, A., Sapone, A., et al., 2006. Gliadin, zonulin and gut permeability: effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand. J. Gastroenterol. 41 (4), 408–419. https://doi.org/10.1080/00365520500235334.

Everts, B., Stotzer, P., Olsson, M., Kilander, A., Pettersson, A., 1999. Increased luminal nitric oxide concentrations in the small intestine of patients with coeliac disease. Eur. J. Clin. Invest. 29 (8), 692–696. https://doi.org/10.1046/j.1365-2362.1999.00519.x.

Fasano, A., 2012. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann. N. Y. Acad. Sci. 1258 (1), 25–33. https://doi.org/10.1111/j.1749-6632.2012.06538.x.

Ferretti, G., Bacchetti, T., Masciangelo, S., Saturni, L., 2012. Celiac disease, inflammation and oxidative damage: a nutrigenetic approach. Nutrients 4 (4), 243–257. https://doi.org/10.3390/nu4040243.

Freeman, H.J., 2009. Malignancy in adult celiac disease. World Gastroenterol. 15 (13), 1581–1583. https://doi.org/10.3748/wjg.15.1581.

Garrote, J.A., Gomez-Gonzalez, E., Bernardo, D., Arranz, E., Chirdo, F., 2008. Celiac disease pathogenesis: the proinflammatory cytokine network. J. Pediatr. Gastroenterol. Nutr. 47 (Suppl. 1), S27–S32. https://doi.org/10.1097/MPG.0b013e3181818fb9.

Gujral, N., Freeman, H.J., Thomson, A., 2012. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J. Gastroenterol. 18 (42), 6036–6059. https://doi.org/10.3748/wjg.v18.i42.6036.

Hollen, E., Forslund, T., Hogberg, L., Laurin, P., Stenhammar, L., Falth-Magnusson, K., et al., 2006. Urinary nitric oxide during one year of gluten-free diet with or without oats in children with coeliac disease. Scand. J. Gastroenterol. 41 (11), 1272–1278. https://doi.org/10.1080/00365520600684563.

Kristjansson, G., Venge, P., Wanders, A., Loof, L., Hallgren, R., 2004. Clinical and subclinical intestinal inflammation assessed by the mucosal patch technique: studies of mucosal neutrophil and eosinophil activation in inflammatory bowel diseases and irritable bowel syndrome. Gut 53 (12), 1806–1812. https://doi.org/10.1136/gut.2003.036418.

Lookhart, G., Bean, S., 1995. Separation and characterization of wheat protein fractions by high-performance capillary electrophoresis. Cereal Chem. 72 (6), 527–532.

Luciani, A., Villella, V.R., Vasaturo, A., Giardino, I., Pettoello-Mantovani, M., Guido, S., et al., 2010. Lysosomal accumulation of gliadin p31-43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa. Gut 59 (3), 311–319. https://doi.org/10.1136/gut.2009.183608.

Marafini, I., Monteleone, I., Di Fusco, D., Cupi, M.L., Paoluzi, O.A., Colantoni, A., et al., 2015. TNF-alpha producing innate lymphoid cells (ILCs) are increased in active celiac disease and contribute to promote intestinal atrophy in mice. PLoS One 10 (5). https://doi.org/10.1371/journal.pone.0126291 e0126291.

Mention, J.J., Ben Ahmed, M., Begue, B., Barbe, U., Verkarre, V., Asnafi, V., et al., 2003. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology 125 (3), 730–745. https://doi.org/10.1016/S0016-5085(03)01047-3.

Meresse, B., Korneychuk, N., Malamut, G., Cerf-Bensussan, N., 2015. Interleukin-15, a master piece in the immunological jigsaw of celiac disease. Dig. Dis. 33 (2), 122–130. https://doi.org/10.1159/000369521.

Molina-Infante, J., Santolaria, S., Sanders, D., Fernández-Bañares, F., 2015. Systematic review: noncoeliac gluten sensitivity. Aliment. Pharmacol. Ther. 41 (9), 807–820. https://doi.org/10.1111/apt.13155.

Nilsen, E.M., Jahnsen, F.L., Lundin, K.E., Johansen, F.E., Fausa, O., Sollid, L.M., et al., 1998. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology 115 (3), 551–563. https://doi.org/10.1016/S0016-5085(98)70134-9.

Peterson, C.G., Eklund, E., Taha, Y., Raab, Y., Carlson, M., 2002. A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease. Am. J. Gastroenterol. 97 (7), 1755–1762. https://doi.org/10.1111/j.1572-0241.2002.05837.x.

Rachmilewitz, D., Karmeli, F., Okon, E., Bursztyn, M., 1995. Experimental colitis is ameliorated by inhibition of nitric oxide synthase activity. Gut 37 (2), 247–255. https://doi.org/10.1136/gut.37.2.247.

Sollid, L.M., Khosla, C., 2005. Future therapeutic options for celiac disease. Nat. Clin. Pract. Gastroenterol. Hepatol. 2 (3), 140–147. https://doi.org/10.1038/ncpgasthep0111.

Stojiljkovic, V., Todorovic, A., Pejic, S., Kasapovic, J., Saicic, Z.S., Radlovic, N., Pajovic, S.B., 2009. Antioxidant status and lipid peroxidation in small intestinal mucosa of children with celiac disease. Clin. Biochem. 42 (13–14), 1431–1437. https://doi.org/10.1016/j.clinbiochem.2009.06.009.

Thakur, S., Dhiman, M., Mantha, A.K., 2018. APE1 modulates cellular responses to organophosphate pesticide-induced oxidative damage in non-small cell lung carcinoma A549 cells. Mol. Cell. Biochem. 441 (1–2), 201–216.

Witko-Sarsat, V., Gausson, V., Descamps-Latscha, B., 2003. Are advanced oxidation protein products potential uremic toxins? Kidney Int. Suppl. 84, S11–S14. https://doi.org/10.1046/j.1523-1755.63.s84.47.