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EVALUATION OF GENOTOXIC AND CYTOTOXIC EFFECTS OF GLUTEN IN MALE ALBINO MICE

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Abstract

Gluten is a protein commonly found in daily diets in the form of wheat, barley, rye, and other grains. It serves as the structural component in flour, providing the binding qualities that maintain the shape and texture of food items. This study aimed to investigate the genotoxic and cytotoxic effects of gluten on bone marrow chromosomes and DNA of male albino mice. The animals were divided into four groups: a control group, a negative control group that received an oral dose of 0.02M glacial acetic acid, and two groups that were treated with gluten dissolved in 0.02M glacial acetic acid at doses of 1.5 g/kg and 3.0 g/kg body weight. The treated animals received oral doses with non-consecutively three times a week for a period of four weeks. The study evaluated chromosomal aberrations in the bone marrow, micronucleus test, and DNA damage using the comet assay. The results of the study showed that treatment with 1.5 and 3.0g/kg body weight of gluten induced chromosomal aberrations and damage in DNA content, with an increase in the severity of effects at a higher dose of gluten. The chromosomal aberrations seen included deletion, fragment, centromeric attenuation, centric fusion, ring formation, end to end association, chromosomal gap, beaded chromosomes, and polyploidy. The micronucleus test revealed toxicity in the bone marrow, as shown by appearance of micronuclei in polychromatic erythrocytes and a reduction in the ratio of polychromatic erythrocytes. The comet assay showed a significant increase of DNA damage in the tail length of the comet cells. This study concluded that the treatment with gluten has detrimental effects on the bone marrow chromosomes and DNA of mice, as demonstrated by the increased chromosomal aberrations, micronuclei, and DNA damage observed in the treated mice. So, the use of gluten should be within an acceptable and safe range.

Keywords: Chromosomes. Comet. DNA. Gluten. Mice. Micronucleus.

1. Introduction

Gluten, the main storage protein found in wheat grains, is a complex mixture of proteins, primarily composed of gliadin and glutenin (Biesiekierski 2017). In the bakery industry, gluten's proteins act as the "skeleton" of kneaded dough, giving it structure, and determining its physical and chemical properties (Filip 2019). Gluten forms a network structure in dough that provides strength, elasticity, and stability, which contributes to the final texture, volume, and appearance of baked goods. The viscoelastic properties of dough are influenced by several factors including the type and amount of proteins, water content, temperature, and the addition of other ingredients. An optimal balance of these factors leads to a well-structured dough that can be easily processed, shaped and baked into high-quality baked goods (Gabler

and Scherf 2020). Gliadin, a component of gluten, contains high levels of glutamine and proline which protect it from being broken down by proteases in the gastrointestinal system, resulting in lower digestibility (Barbaro et al. 2020).

Celiac disease (CD) is a common enteropathy with a strong genetic predisposition, characterized by a lifelong intolerance to gluten, a protein found in wheat, rye, and barley (Wolters 2008). Gluten sensitivity, also referred to as non-celiac gluten sensitivity (NCGS), is a condition characterized by both intestinal and extraintestinal symptoms that are triggered by consuming gluten-containing foods in individuals who do not have CD or wheat allergy (Atlasy et al. 2022). Patients with NCGS experience symptoms affecting both their gastrointestinal and extra-intestinal systems (Barbaro et al. 2020). Research has suggested that gluten consumption may be linked to hair loss and infertility. Gluten's effects on the reproductive system may play a role in these conditions (Filip 2019). CD patients have much greater rates of alterations in bone and mineral metabolism, which increases their risk of fractures (Di Stefano et al. 2023).

The consumption of gluten by individuals with a genetic predisposition can lead to CD, which is a chronic autoimmune disorder affecting the small intestine. It damages the mucosa of the small intestine and results in the atrophy of the villi (Valvano et al. 2020). There is evidence that celiac disease can affect extraintestinal organs, including the skin, pancreas, heart, and liver (Kerasioti et al. 2017). The autoimmune enteropathy classified as CD, which affects about 1% of the population, is linked to an elevated risk of enteropathy-associated T-cell lymphoma and small bowel adenocarcinoma (Gromny and Neubauer 2023). Larazotide and latiglutenase are now the two medications with the most comprehensive clinical study for reducing gluten contamination in the diet that reduce intestinal permeability by stabilizing enterocyte tight junctions (Machado 2023).

Gluten consumption has been linked to increased secretion of hepatic enzymes, which can lead to inflammation of the liver, primary biliary cirrhosis, colitis, and Crohn's disease. Additionally, gluten has been associated with skin symptoms such as herpetic dermatitis (Filip 2019). The comet assay results showed that celiac patients had higher levels of DNA damage compared to healthy controls, and the frequency of micronuclei and nuclear buds was also elevated in patients (Shan et al. 2002; Moghaddam et al. 2013; Collins 2014). There is substantial evidence connecting the results of the comet assay, which measures DNA damage, to the induction of micronuclei (Ross et al. 1995; Murgia et al. 2008; Monguzzi et al. 2019).

2. Material and Methods

Chemical used

This study used gluten powder (Figure 1) obtained from *Sigma-Aldrich Corporation in Cairo*, with a protein basis assay of ≥75% and a CAS number of 8002-80-0, for the experiment. The stock solution of gluten was prepared by dissolving it in glacial acetic acid (0.02 mM) to improve its solubility. The solution was administered to animals orally through a feeding needle, with doses of 1.5 g/kg and 3.0 g/kg body weight (b.wt.). The gluten challenge was performed 3 non-consecutive days per week for 4 weeks in mice by oral gavage. The control group consisted of mice that were not treated with gluten or acetic acid. A negative control group was also included, which was treated with only 0.02 mM acetic acid (Verdu et al. 2008; Alruwaili 2017).

Experimental animals

The study utilized 40 male albino mice (*Mus musculus*), aged 16-18 weeks, and weighing 25-30 g, purchased from the animal house of *Theodor Bilharz Research Institute in El-Giza, Egypt*. The mice were housed in clean plastic containers filled with wood shavings and were provided with a standard pellet diet for rodents, as well as unrestricted access to water at room temperature (25 ± 2 °C), a 12-hour light-dark cycle, and a relative humidity of 55 ± 5%. The mice were given a week to acclimate before the start of the experiment. The investigation adhered to the international standards for animal laboratory treatment and

was approved by the Institutional Animal Ethics Committee of Ain Shams University, Faculty of Women. Number: ASU/W/R/23-2-50.

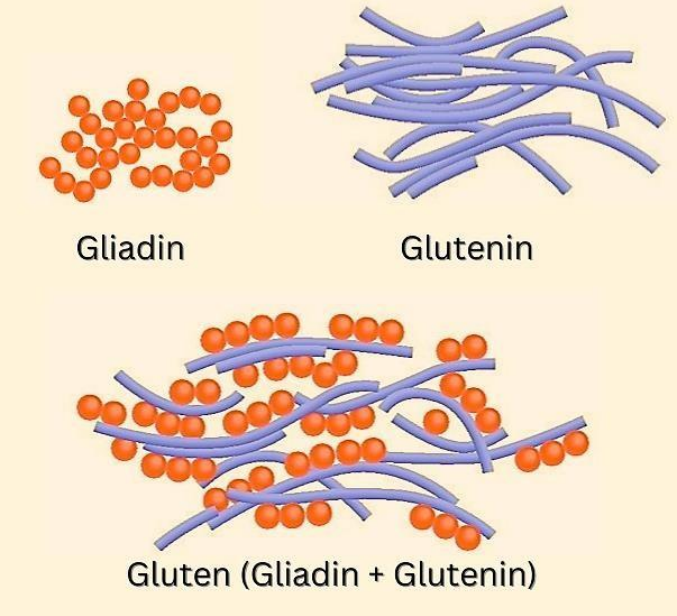

Figure 1. Structure of gluten diagrammatically. Gluten is made up of two main proteins, gliadins and glutenins, which are both toxic to individuals with celiac disease. Glutenin forms a network of fibers that entrap globular gliadins. A diagram demonstrates the interaction between the two types of proteins (Fasano 2011).

Experimental design

This study divided the animals into four groups of ten mice each. The first group served as the control and did not receive any treatment. The second group, serving as the negative control, was given oral doses of glacial acetic acid. The third group received oral doses of gluten (1.5 g/kg b.wt.) dissolved in (0.02Mm) glacial acetic acid. The fourth group received oral doses of gluten (3.0 g/kg b.wt.) dissolved in (0.02Mm) glacial acetic acid, with the doses given non-consecutively three times a week for four weeks.

Chromosomal aberrations assay

The bone marrow chromosomes were prepared for analysis according to a previously established protocol (Preston et al. 1987; El-Alfy et al. 2014; El-Alfy et al. 2016). The mice received an intraperitoneal injection of colchicine solution (0.2 ml/100 g) and were sacrificed after two hours. The bone marrow was collected and used to prepare chromosomes, which were observed under a microscope using bright field. Most of the photographs were taken at 100x magnification with oil immersion. A karyotype was made from the best photograph of a well-spread metaphase stage. Five hundred well-spread metaphase cells (100 cells per mouse) were examined from the treated animals in all groups, including the control, to look for structural and numerical chromosome aberrations and to observe the effects of gluten on the bone marrow chromosomes after four weeks of treatment.

Micronucleus test

The mouse bone marrow was prepared according to a previously established protocol (Kasamoto et al. 2013). The process involved flushing the marrow cavity of the trimmed femurs with 5% bovine albumin and centrifuging the obtained suspension to get a fine one. Then, a smear was prepared by air-drying the slides overnight, fixing them with methanol, and staining them with Geimsa and buffer to differentiate polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs), as well as to stain micronuclei. The slides were washed, dried, and mounted before examination. A minimum of 2,000 PCEs

per animal were examined under a microscope using immersion oil at a magnification of X1,000. The percentage of micronucleated polychromatic erythrocytes (MNPCEs) was calculated, and the PCE/NCE ratio was determined for each animal. The mean and standard deviation for each group were also determined.

Comet assay

The comet assay was performed according to a previously established protocol (Singh et al. 1988). In this study, mouse hepatocytes were used to measure DNA damage. After sacrificing the mice, the liver was isolated, frozen, and homogenized in sterile conditions. The homogenized liver was mixed with a hepatocytes lysis medium and aspirated through centrifugation. The separated hepatocytes were mixed with buffered low-melting-point agarose. The mixture was smeared on a microscopic slide that was coated with buffered normal melting point agarose, solidified, and covered with an outermost layer of normal melting point agarose. The components of the hepatocytes, except for DNA, were digested by carefully dipping the slides in a cold lysis detergent solution. The slides were then subjected to electrophoresis and stained with ethidium bromide under dim lighting to visualize the DNA fragments. One hundred cells per animal were analyzed for DNA migration. The severity of DNA damage was determined by comparing the length of the comet tail (in micrometers) with the diameter of the nucleus of undamaged cells observed in the same field. To determine the effect of gluten on the extent of DNA damage, the mean ± standard deviation of the percentage of DNA in the tail length of DNA migration was evaluated.

Statistical analysis

The data collected from the chromosomal aberrations, micronucleus test, and comet assay were organized and analyzed using statistical methods. The results for each group were expressed as the mean and standard deviation (Std. D.). To determine the significance of differences between the groups, a one-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics for Windows, version 16. The SCHEFFE post-hoc test was also used to further analyze the data. A *p*-value less than 0.05 was considered statistically significant.

3. Results

Chromosomal aberrations

The study examined the mouse bone marrow to assess structural and numerical chromosomal aberrations, and the results are presented in Table 1. The results showed that there were no significant differences ($p \geq 0.05$) in the total number of aberrations between the mice treated with glacial acetic acid and the control mice. However, both the low-dose and high-dose gluten-treated mice showed a significant increase ($p \leq 0.05$) in total aberrations (44.7% and 58.2%) compared to the control group, respectively.

The treatment with gluten caused both structural and numerical aberrations in the chromosomes of the mouse bone marrow cells. These aberrations included structural aberrations like deletion (D), fragment (F), centromeric attenuation (Ca), centric fusion (Cf), ring form (R), end-to-end association (Ee), chromosomal gap (Chg), beaded chromosomes (Bch), chromatid gap (Cg), and insignificant chromosomal aberrations, which are shown in Figures 2B, C and D and numerical aberration such as polyploidy (Po) are depicted in Figure 2E.

As shown in Figure 3, the administration of glacial acetic acid alone did not have a significant impact on the aberrations ($p \geq 0.05$) compared to the control data. However, the low-dose and high-dose gluten-treated mice showed a significant increase ($p \leq 0.05$) in Ca (95% and 99%, respectively), D (40% and 40.86%, respectively), and R (29.8% and 35.6%, respectively) compared to the control group. Additionally, the values of F and Cf in the high dose treated mice were significantly different ($p \leq 0.01$) from the control values (360% and 75.8%, respectively).

The current study showed that the consumption of gluten caused changes in the number of chromosomes in the bone marrow cells of mice. One of these changes was an increase in the number of cells with Po, as seen in Figure 2E. Compared to the control group, the low and high dose treated mice showed a significant increase ($p \leq 0.05$) in the number of cells with Po (3% and 12%, respectively).

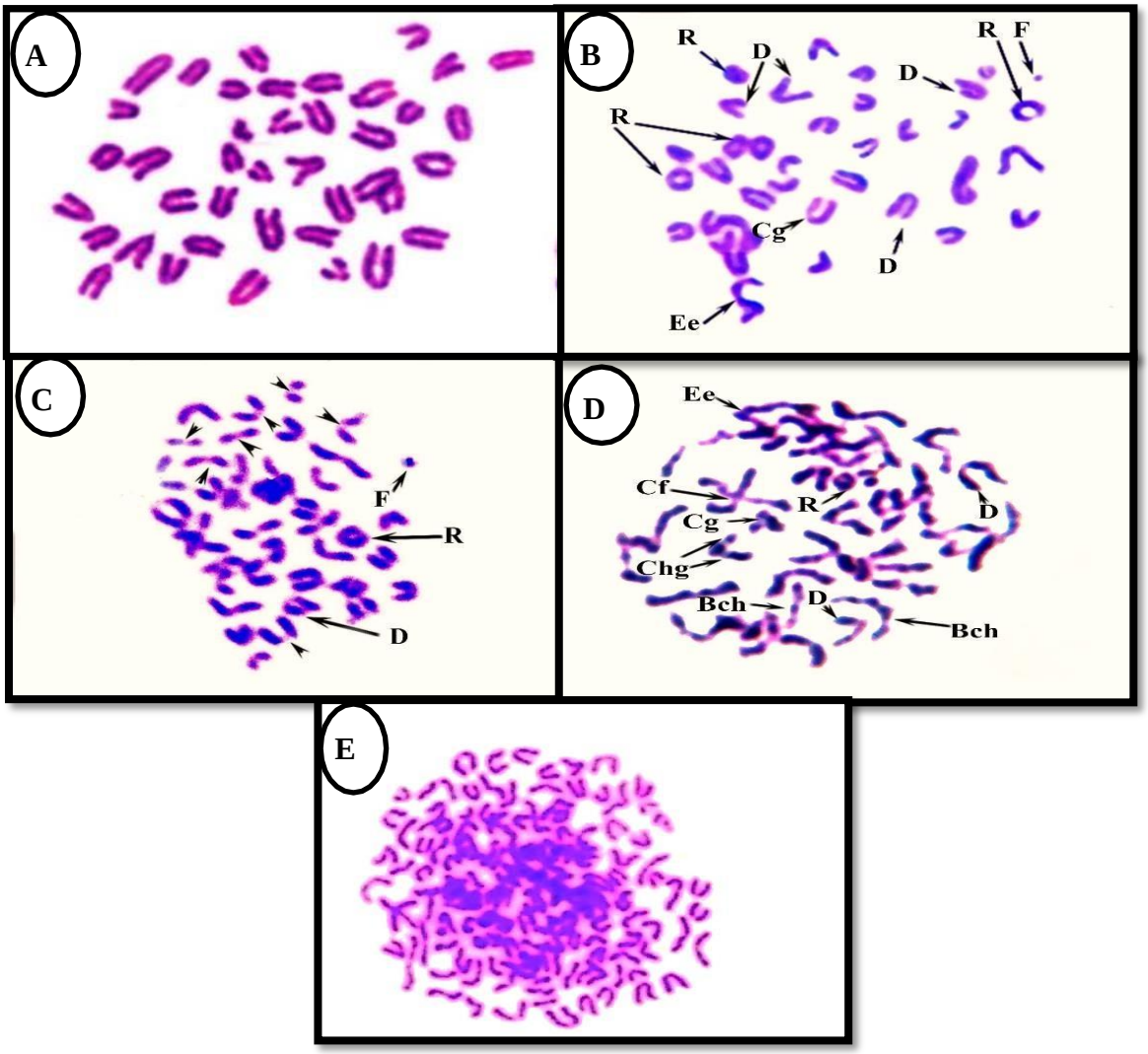

Figure 2. Photomicrographs of metaphase chromosomes of male albino mice (Mus musculus) represented: A - a control group and negative control group chromosomes. Groups received oral dose of gluten 1.5 and 3 g/kg b.wt. for four weeks, three days weekly, showing in B - deletion (D), end to end association (Ee), ring shape chromosome (R), fragment (F) and chromatid gap (Cg). in C - deletion (D), ring shape chromosome (R), fragment (F) and heads arrows showing centromeric attenuation (Ca). Showing in D - beaded chromosomes (Bch), centric fusion (Cf), deletion (D), end to end association (Ee), ring shape chromosome (R), chromatid gap (Cg) and chromosomal gap (Chg), and in E - Polyploidy (Po). X: 2400.

Micronucleus assay and cytotoxicity test

The bone marrow smear of the control mice showed NCE which were small and PCE which were larger than NCE (Figure 4A). The bone marrow smear of the treated mice showed micronuclei, which were bluish in color, in PCE (Figures 4B and C).

In the mice groups treated with either low or high dose of gluten (Table 2 and Figure 5), there was significant increase in the number of MNPCEs compared to the control group (1.667% and 3.33%, respectively). Additionally, the study found that there was a highly significant decrease in the ratio of PCEs to NCEs compared to the control group (-0.322% and -0.662%, respectively).

Table 1. The mean and standard deviation of chromosomal aberrations in metaphases cells of male albino mice (*Mus musculus*) treated with gluten 1.5g/kg b.wt. (group 3), gluten 3.0g/kg b.wt. (group 4), glacial acetic acid 0.02M and control group.

			Control		ve Control		Gluten (1.5 g/kg)		Gluten (3 g/kg)		Sig.
			Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	
Structural aberrations	Chromatid aberrations	D	1.60^{a}	±1.14	2.60^{a}	±1.14	65.60^{b}	±8.50	76.60^{b}	±7.30	0.000
		F	1.00^{a}	±1.00	2.40^{a}	±1.14	65.60^{b}	±8.50	76.80^{c}	±7.29	0.000
	Chromosomal aberrations	Ca	0.80^{a}	±0.837	1.60^{a}	±1.14	76.80^{b}	±11.077	80.0^{b}	±2.45	0.000
		Cf	0.20^{a}	±0.447	1.00^{a}	±1.00	56.80^{b}	±7.120	72.2^{c}	±4.02	0.000
		R	1.20^{a}	±1.30	2.20^{a}	±1.92	37.00^{b}	±7.65	44.00^{b}	±6.28	0.000
		Ee	2.20^{a}	±0.45	3.60^{a}	±1.14	27.80^{b}	±6.099	44.20^{c}	±5.586	0.000
		Chg	0.00^{a}	±0.00	0.60^{a}	±0.89	6.80^{a}	±3.70	27.40^{b}	±6.62	0.000
		Bch	0.00^{a}	±0.00	0.20^{a}	±0.45	2.20^{a}	±1.92	11.40^{b}	±7.33	0.001
Numerical aberrations		Po	0.40^{a}	±0.89	0.40^{a}	±0.548	1.60^{ab}	±2.61	5.20^{b}	±3.35	0.009
Total aberrations			7.40^{a}	±2.30	14.60^{a}	±5.50	338.2^{b}	±21.69	437.8^{c}	±23.64	0.000

Values represent the mean ±Std. D. compared with control group; *p* value > 0.05 was considered significant; Statistically significant means (*p* value > 0.05) are given different letters; a, b and c the groups that showed a non-significant change between each other take the same letter, but the group that showed a significant change compared to the other groups take a different letter.

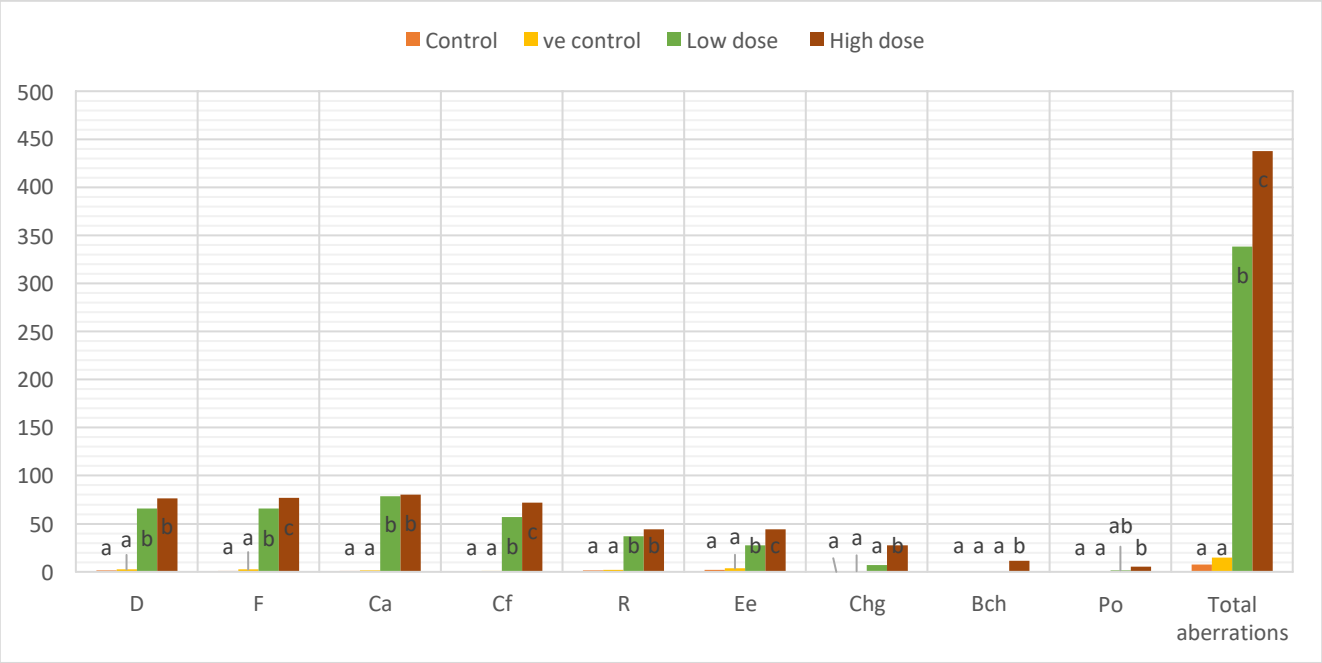

Figure 3. Histogram represents the mean of chromosomal aberrations, deletion (D), fragment (F), centromeric attenuation (Ca), centric fusion (Cf), ring form (R), end to end association (Ee), chromosome gap (Chg), beaded chromosome (Bch), polyploidy (Po) and total aberrations in metaphases cells of male albino mice (*Mus musculus*) treated with gluten 1.5 g/kg b.wt. (group3), gluten 3.0g/kg b.wt. (group 4) after 4 weeks, non-consecutively 3dayse/week. Negative control group treated with 0.02M glacial acetic acid only (group 2), and control group (group 1). The data expressed as mean ±Std. D., Significant p < 0.05 when compared with one-way ANOVA test.

Table 2. The mean and standard deviation of micronucleated polychromatic erythrocytes and ratio of polychromatic erythrocytes to normchromatic erythrocytes of bone marrow smear of male albino mice (*Mus musculus*) treated with gluten 1.5g/kg b.wt. (group 3), gluten 3.0g/kg b.wt. (group 4), glacial acetic acid 0.02M and control group.

	Control		ve Control		Gluten (1.5 g/kg)		Gluten (3 g/kg)		Sig.
	Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	
MNPCEs	1.5^{a}	±0.1	2^{b}	±0.1	4^{c}	±0.1	6.5^{d}	±0.1	0.000
PCE/NCE	0.953^{a}	±0.0462	0.753^{b}	±0.0058	0.667^{c}	±0.0058	0.322^{d}	±0.0189	0.000

Values represent the mean ±Std. D. compared with control group; *p* value > 0.05 was considered significant; Statistically significant means (*p* value > 0.05) are given different letters; a, b, c and d. the groups that showed a non-significant change between each other take the same letter, but the group that showed a significant change compared to the other groups take a different letter.

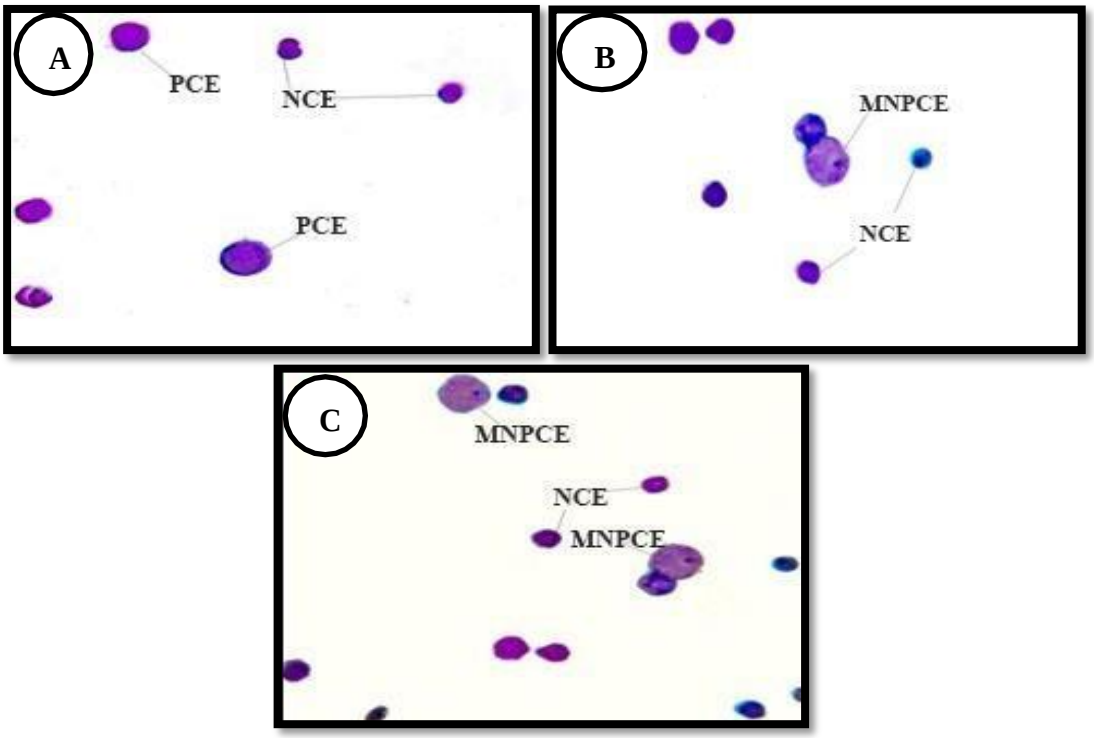

Figure 4. Photomicrographs of bone marrow smear of male albino mice (*Mus musculus*) represented: A - A control group and negative control group, showing polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). B - and C - Groups which received oral dose of gluten 1.5 and 3.0 g/kg b.wt. for four weeks, three days weekly, showing polychromatic erythrocytes with micronuclei (MNPCE) and normochromatic erythrocytes (NCE). X: 1400.

Comet assay

The images of single cell gel electrophoresis revealed that cells from mice treated with gluten displayed a high degree of DNA damage, as indicated by a "comet-like" appearance due to the migration of fragmented DNA through electrophoresis. The tail formation indicates the extent of DNA damage (Figure 6). The analysis of comet classes in the study was conducted using a 515-560 nm filter on a fluorescent microscope. The findings were displayed in Figure 6, which revealed four types of comet shapes: Class 0 comets had no tail, Class 1 comets showed slight damage, Class 2 comets displayed moderate damage, and Class 3 comets had extensive damage. Cells that were damaged beyond recognition, represented by hepatocytes without heads, were not taken into consideration in the analysis.

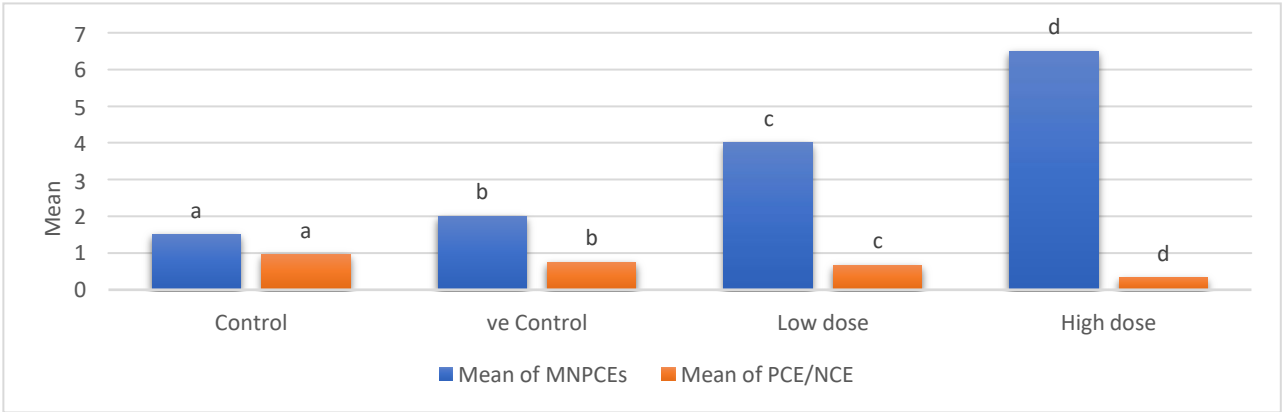

Figure 5. Histogram represents the relationship of mean micronucleated polychromatic erythrocytes (MNPCEs) and mean ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCEs/NCEs) in control group, negative control group, treated groups, 1.5 g/kg b.wt. gluten (group3) and 3.0 g/kg b.wt. (group 4) after 4 weeks (3days/week). The data expressed as mean ±Std. D., Significant $p < 0.05$ when compared with one-way ANOVA test.

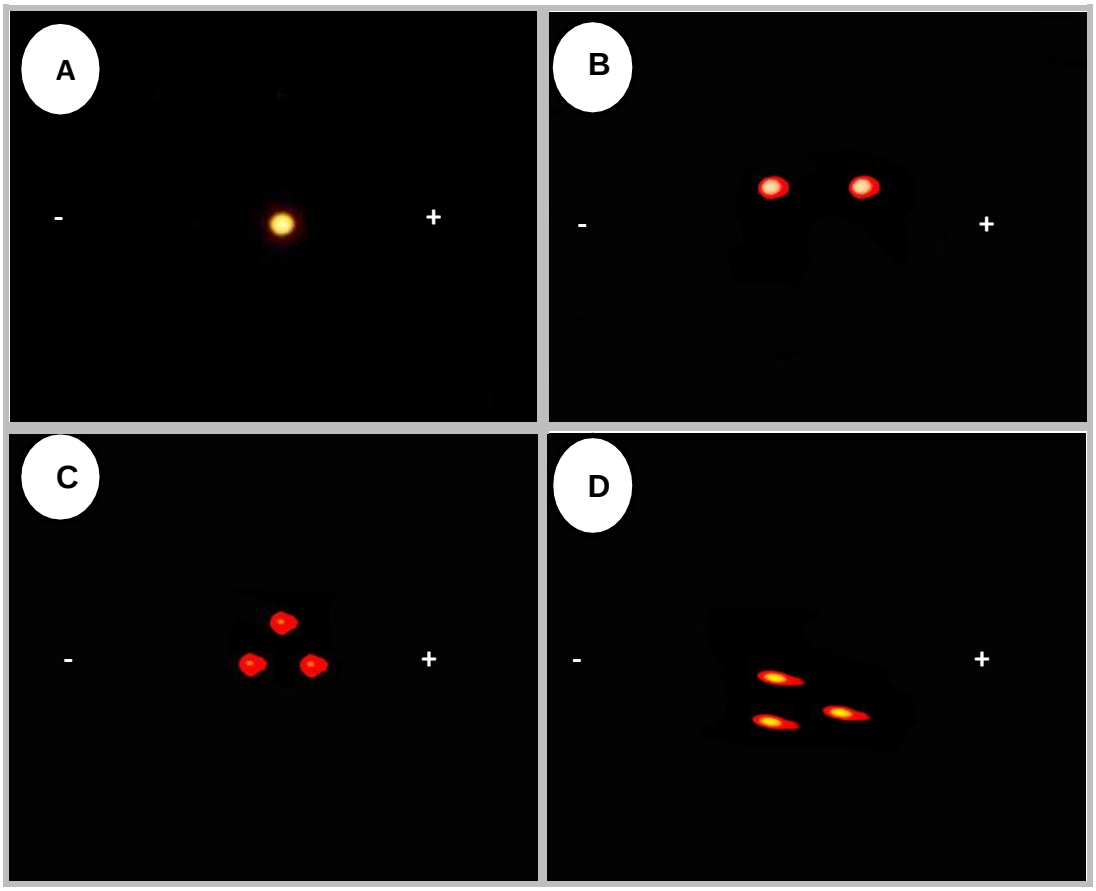

Figure 6. Photomicrographs of bone marrow smear of male albino mice (*Mus musculus*) represented: A - A control group, showing grade 0 in which, no damage occurred with head diameter 37.44 µm and no tail. B - A negative control group which received oral dose of acetic acid showing grade 1 slight damage occurred with head diameter 17.28 µm and tail length 1.44 µm. C - A group which received oral dose of gluten 1.5 g/kg b.wt. showing grade 2 moderate damage occurred with head diameter 37.44 µm and tail length 19.44 µm. D - A group which received oral dose of gluten 3.0 g/kg b.wt. showing grade 3 in which heavy damage occurred with head diameter 16.56 µm and tail length 19.08 µm. X: 1000.

The present study aimed to assess the distribution of DNA damage in liver cells by using comet assay. The damage was evaluated by counting the number of damaged cells out of 100 randomly selected, non-overlapping cells on slides, per each animal, after four weeks of gluten treatment 3 times per week. The severity of DNA damage is illustrated in Table 3 and Figure 7 and is represented as the mean ± standard deviation for all control, negative control, and treated groups (1.5 g/kg and 3.0 g/kg b.wt. of gluten).

Table 3. The mean and standard deviation % DNA in tail and tail length of bone marrow smear of male albino mice (*Mus musculus*) treated with gluten 1.5g/kg b.wt. (group 3), gluten 3.0g/kg b.wt. (group 4), glacial acetic acid 0.02M and control group.

	Control		ve Control		Gluten (1.5 g/kg)		Gluten (3 g/kg)		Sig.
	Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	Mean	±Std. D.	
%DNA in tail	9.103^{a}	±0.86	9.87^{a}	±0.45	12.03^{a}	±1.51	19.01^{b}	±3.71	0.001
Tail Length	1.8^{a}	±0.496	2.38^{ab}	±0.896	2.74^{ab}	±0.238	3.66^{b}	±1.009	0.025

Values represent the mean ±Std. D. compared with control group; *p* value > 0.05 was considered significant; Statistically significant means (*p* value > 0.05) are given different letters; a and b the groups that showed a non-significant change between each other take the same letter, but the group that showed a significant change compared to the other groups take a different letter.

Figure 3 shows a statistically significant increase ($p < 0.01$) in the average percentage of DNA in the tail in the high-dose gluten-treated animals (1.088%) compared to the control animals. There was also a statistically significant increase ($p < 0.05$) in the average tail length, 1.033% compared to negative 0.32% and control animals.

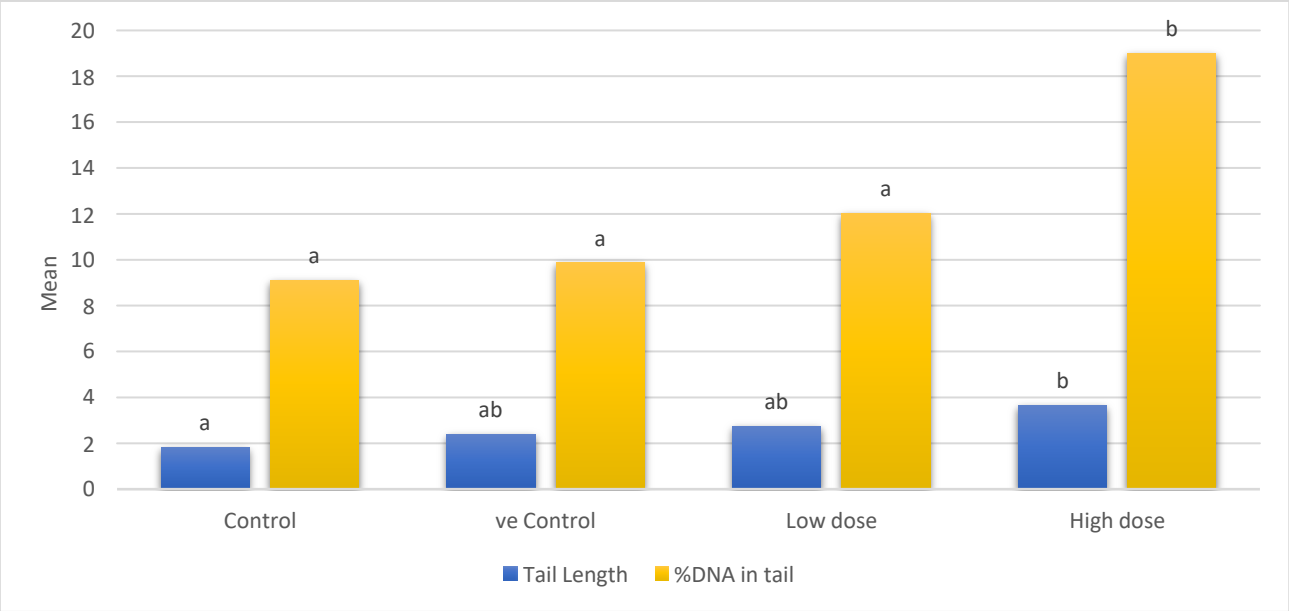

Figure 3. Histogram represents the relationship of mean of tail length and % DNA damage in tail length in control group, negative control group and treated groups, 1.5 g/kg b.wt. gluten (group3), 3.0 g/kg b.wt. gluten (group 4) after 4 weeks (3days/week). The data is expressed as mean ±Std. D., significant $p < 0.05$ when with one-way ANOVA test.

4. Discussion

In this study, the potential genotoxic and cytotoxic effects of gluten were investigated using chromosomal aberrations, micronuclei in the bone marrow cells of mice and comet assay (Maluf et al. 2020). The results showed that the structural chromosomal aberrations were significantly increased ($p < 0.05$) after oral gavage of gluten doses of 1.5 and 3.0g/kg b.wt. for four weeks. The highest number of chromosomal aberrations were observed in mice treated with a high dose of gluten. The aberrations observed in the bone marrow cells were both structural and numerical, including D, F, Ca, Cf, R, Ee, Chg and Bch. Research has shown that gluten consumption can lead to a high frequency of chromosomal abnormalities in peripheral blood lymphocytes, which is a biomarker for cancer risk in humans. This may be an indication of the early biological effects of genotoxic agents or an individual's susceptibility to cancer (Verkarre et al. 2004). The frequency of chromosome abnormalities in the peripheral blood lymphocytes of celiac disease patients decreases significantly after adopting a gluten-free diet (Gunasekarana et al. 2015). Additionally, gluten consumption causes numerical aberrations in the form of Po, with Ca, D and F being the most common aberrations in both doses (Hojsak et al. 2013).

Our results of chromosomal aberrations align with the findings from previous studies indicating that CD causes chromosomal aberrations in the bone marrow cells of adult patients. These aberrations comprise both structural aberrations, including D, F, R, Cf, Chg, Cg, and Ca, and numerical aberrations in the form of Polyploidy (Kolacek et al. 1998). Centromere attenuation is the most prevalent type of aberration and is considered a valid indicator of cancer risk in humans. This reflects the early biological effects of genotoxic carcinogens or the individual's cancer susceptibility, as reported in studies by Verkarre et al. (2004), Mahmoud et al. (2018) and El-Alfy et al. (2020).

Micronucleus test was included in many studies to detect genotoxic effect of many classes of chemicals in mammalian system (Luzhna et al. 2013; Hayashi 2016; El-Alfy et al. 2020). Also, it was reported as the most reliable and widely used bioassay to assess DNA damage in mammalian cells in vivo, because it could detect genomic alterations resulting from chromosomal damage and/or damage to the mitotic apparatus caused by clastogenic agents (El-Alfy et al. 2021). The results of the present study on micronucleus assay showed a highly significant increase ($p < 0.05$) in MNPCEs in the gluten-treated groups (1.5 and 3.0 g/kg b.wt.) compared to the control group, and decrease in the ratio of PCE/NCE, which is considered to be an indicator of toxicity in the bone marrow. These finding is in line with previous studies of Maluf et al. (2020) who reported a higher frequency of micronuclei in CD patient.

The present study suggests that gluten, a protein found in wheat, barley, rye, and other grains, has a genotoxic and cytotoxic effect on the bone marrow cells and DNA of male mice. The component of gluten, gliadin, has been demonstrated to cause oxidative stress and inflammation (Shan et al. 2002; Ciccocioppo et al. 2005). Gliadin peptides can also accumulate in cells, increasing the levels of reactive oxygen species (ROS) (Schumann et al. 2008; Heyman and Menard 2009), leading to further DNA damage (Cooke et al. 2006; El-Alfy et al. 2020). Oxidative stress occurs when oxidizing substances, such as gliadin, surpass the body's antioxidant defenses, due to elevated ROS and RNS or decreased antioxidant levels (Halliwell and Gutteridge 2015; Kerasioti et al. 2017). There is evidence that consuming gluten in CD patients results in an increase in cellular oxidative stress, DNA damage, and pro-apoptotic signals in both cells and duodenal mucosa (Kolacek et al. 2004). The current study found that gluten treatment induced cytotoxicity in the mice, as evidenced by increased levels of chromosomal aberrations, micronuclei, reduction of PCEs, and DNA damage, as measured by the comet assay. These findings are consistent with previous studies reporting similar effects in CD patients (Maluf et al. 2020).

5. Conclusions

In conclusion, the present study showed that treating mice with gluten caused significant structural and numerical aberrations in the chromosomes of the bone marrow cells, increased DNA damage in the liver cells, and a higher percentage of micronucleated erythrocytes. Most likely, these findings indicate that gluten have negative impacts on the genetic material of mice and potentially causes harmful effects on their health. Therefore, the study recommended that the using of gluten should be restricted to a very narrow range border and within an acceptable and safe range.

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References

ALRUWAILI, N.K. Effect of experimentally induced gluten enteropathy in a rat model of inflammation on hepatic transporters and cytochrome P-450 enzymes. Ph.D. thes ed. Massachusetts College of Pharmacy and Health Sciences.

ATLASY, N., et al. Single cell transcriptomic analysis of the immune cell compartment in the human small intestine and in Celiac disease. *Nature Communications*. 2022, **13**, 1–14. https://doi.org/10.1038/s41467-022-32691-5

BARBARO, M.R., et al. Non-celiac gluten sensitivity in the context of functional gastrointestinal disorders. *Nutrients*. 2020, **12**, 1–21. https://doi.org/10.3390/nu12123735

BIESIEKIERSKI, J.R. What is gluten? *Journal of Gastroenterology and Hepatology*. 2017, **32**, 78–81. https://doi.org/10.1111/jgh.13703

CICCOCIOPPO, R., et al. The immune recognition of gluten in coeliac disease. *Clinical & Experimental Immunol*ogy. 2005, **140**, 408–416. https://doi.org/10.1111%2Fj.1365-2249.2005.02783.x

COLLINS, A.R. Measuring oxidative damage to DNA and its repair with the comet assay. *Biochimica et Biophysica Acta* - General Subjects. 2014, **1840**, 794–800. https://doi.org/10.1016/j.bbagen.2013.04.022

COOKE, M.S., et al. Does measurement of oxidative damage to DNA have clinical significance? *Clinica Chimica Acta*. 2006, **365**, 30–49. https://doi.org/10.1016/j.cca.2005.09.009

EL-ALFY, N., et al. Role of propolis against monosodium glutamate genotoxicity by chromosomal aberrations, micronucleus test and comet assay in males. *Der Pharmacia Lettre*. 2020, **12**, 13–22.

EL-ALFY, N., et al. Genotoxic effect of methotrexate on bone marrow chromosomes and DNA of male albino mice (*Mus musculus*). *The Egyptian Journal of Hospital Medicine*. 2016, **64**, 350–363.

EL-ALFY, N., et al. Protective role of the royal jelly against genotoxic effect of endoxan drug on the bone marrow chromosomes of male albino mice. *Academic Journal of Cancer Research*. 2014, **7**, 198–207.

EL-ALFY, N.Z., et al. Ameliorative role of quercetin and/or resveratrol on acrolein-induced clastogenesis in bone marrow cells of male albino mice (*Mus musculus)*. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2021, **10**, 92–114.

FASANO, A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiological Reviews.* 2011, **91**, 151–175. https://doi.org/10.1152/physrev.00003.2008

FILIP, E. Wheat Gluten, Desirable or Dangerous Genes. *American Journal of Biomedical Science & Research*. 2019, **4**, 176–178. https://doi.org/10.34297/ajbsr.2019.04.000794

GABLER, A.M., SCHERF K.E. Comparative characterization of gluten and hydrolyzed wheat proteins. *Biomolecules*. 2020, **10**, 1–15. https://doi.org/10.3390/biom10091227

GROMNY, I., NEUBAUER, K. Pancreatic Cancer in Celiac Disease Patients-A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*. 2023, **20**, 1565. https://doi.org/10.3390%2Fijerph20021565

GUNASEKARANA, V., et al. A comprehensive review on clinical applications of comet assay. *Journal of Clinical & Diagnostic Research*. 2015, **9**, 1–5. https://doi.org/10.7860%2FJCDR%2F2015%2F12062.5622

HALLIWELL, B., GUTTERIDGE, J.M.C. Free Radicals in Biology and Medicine. 5th edition. Oxford. 2015. Online edn, Oxford Academic. 2015 https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

HAYASHI, M. The micronucleus test—most widely used *in vivo* genotoxicity test—. *Genes and Environment*. 2016, **38** (18), 1-6. https://doi.org/10.1186/s41021-016-0044-x

HEYMAN, M., MENARD, S. Pathways of gliadin transport in celiac disease. *Annals of the New York Academy of Sciences*. 2009, **1165**, 274–278. https://doi.org/10.1111/j.1749-6632.2009.04032.x

HOJSAK, I., et al. Chromosomal aberrations in peripheral blood lymphocytes in patients with newly diagnosed celiac and Crohn's disease. *European Journal of Gastroenterology and Hepatology*. 2013, **25**, 22–27. https://doi.org/10.1097/MEG.0b013e328359526c

KASAMOTO, S., et al. In Vivo Micronucleus Assay in Mouse Bone Marrow and Peripheral Blood. *Methods in Molecular Biology*. 2013, **1044**, 179–189. https://doi.org/10.1007/978-1-62703-529-3_9

KERASIOTI, E., et al. Tissue-specific effects of feeds supple-mented with grape pomace or olive oil mill wastewater on de-toxification enzymes in sheep. *Toxicology Reports.* 2017, **4**, 364–372. https://doi.org/10.1016%2Fj.toxrep.2017.06.007

KOLACEK, S., et al. Gluten-free diet has a beneficial effect on chromosome instability in lymphocytes of children with coeliac disease. *Journal of Pediatric Gastroenterology and Nutrition*. 2004, **38**, 177–180. https://doi.org/10.1097/00005176-200402000-00014

KOLACEK, S., et al. Chromosome aberrations in coeliac and non-coeliac enteropathies. *Archives of Disease in Childhood*. 1998, **78**, 466–468. https://doi.org/10.1136%2Fadc.78.5.466

LUZHNA, L., et al. Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. *Frontiers in Genetics*. 2013, **4**, 1-17. https://doi.org/10.3389/fgene.2013.00131

MACHADO, M.V. New Developments in Celiac Disease Treatment. *International Journal of Molecular Sciences.* 2023, **24**, 945. https://doi.org/10.3390%2Fijms24020945

MAHMOUD, E.F., et al. Chitosan nanoparticles suppress the oxidative stress in submandibular salivary glands and prevent the genotoxicity of monosodium glutamate in albino rats: histological, immunohistochemical and chromosomal aberrations analysis study. *Egyptian Dental Journal*. 2018, **64**, 733–746.

MALUF, S.W., et al. DNA damage, oxidative stress, and inflammation in children with celiac disease. *Genetics and Molecular Biology*. 2020, **43**, 1–8. https://doi.org/10.1590%2F1678-4685-GMB-2018-0390

MOGHADDAM, M.A., et al. The Effects of Gluten-Free Diet on Hypertransaminasemia in Patients with Celiac Disease. *International Journal of Preventive Medicine.* 2013, **4**, 700–704.

MONGUZZI, E., et al. Gliadin effect on the oxidative balance and DNA damage: An in-vitro, ex-vivo study. *Digestive and Liver Dis*ease. 2019, **51**, 47–54. https://doi.org/10.1016/j.dld.2018.06.020

MURGIA, E., et al. Validation of micronuclei frequency in peripheral blood lymphocytes as early cancer risk biomarker in a nested case-control study. *Mutation Research*. 2008, **639**, 27–34. https://doi.org/10.1016/j.mrfmmm.2007.10.010

PRESTON, R.J., et al. Mammalian in vivo cytogenetic assays Analysis of chromosome aberrations in bone marrow cells. *Mutation Research*. 1987, **189**, 157–165. https://doi.org/10.1016/0165-1218(87)90021-8

ROSS, G.M., et al. The single cell microgel electrophoresis assay (comet assay): Technical aspects and applications. Report on the 5th LH Gray Trust Workshop, Institute of Cancer Research. *Mutation Research*. 1995, **337**, 57–60. https://doi.org/10.1016/0921-8777(95)00007-7

SCHUMANN, M., et al. Mechanisms of epithelial translocation of the alpha(2)-gliadin-33mer in coeliac sprue. *Gut*. 2008, **57**, 747–754. https://doi.org/10.1136/gut.2007.136366

SHAN, L., et al. Structural basis for gluten intolerance in celiac sprue. *Science*. 2002, **297**, 2275–2279. https://doi.org/10.1126/science.1074129

SINGH, N.P., et al. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*. 1988, **175**, 184–191. https://doi.org/10.1016/0014-4827(88)90265-0

DI STEFANO, M., et al. The Effect of a Gluten-Free Diet on Vitamin D Metabolism in Celiac Disease: The State of the Art. *Metabolites*. 2023, **13**, 74. https://doi.org/10.3390%2Fmetabo13010074

VALVANO, M., et al. Celiac disease, gluten-free diet, and metabolic and liver disorders. *Nutrients*. 2020, **12**, 940. https://doi.org/10.3390/nu12040940

VERDU, E.F., et al. Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice. *American Journal of Physiology Gastrointestinal and Liver Physiol*ogy. 2008, **294**, 217–225. https://doi.org/10.1152/ajpgi.00225.2007

VERKARRE, V., et al. Gluten-Free Diet, Chromosomal Abnormalities, and Cancer Risk in Coeliac Disease. *Journal of pediatric gastroenterology and nutrition*. 2004, **38**, 140–142. https://doi.org/10.1097/00005176-200402000-00006

WOLTERS, V.M. Genetic Background of Celiac Disease and Its Clinical Implications. *The American Journal of Gastroenterology*. 2008, **103**, 190–195. https://doi.org/10.1111/j.1572-0241.2007.01471.x

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