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Ameliorative potential of β-1,3-D-Glucan on acrylamide-induced cytogenetic alterations in mice

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Abstract

Acrylamide (AA) is formed in food during heat preparation (frying and baking), causing DNA toxicity. So, the aim of this study is applied β-1,3-D-Glucan (BDG) as a natural polysaccharide ameliorative to reduce the DNA hepatotoxicity and genotoxicity of bone-marrow chromosomes in male mice by using three parameters: alkaline comet assay, cytochemical DNA and cytogenetical protocols. The AA-oral fed mice are classified into three groups, the first received low dose and the second intake the double fashion of AA in alone or concomitant with BDG for 30 days, besides the fourth group of controls. The study observed that AA induced both numerical and structural chromosomal aberrations in a significant increase (p<0.05 or p<0.0001) in a dose-dependent relationship. The cytochemical study on DNA exhibited that the AA-treated hepatocyte nuclei, showed strong stainability with condensed DNA inclusions and releasing outside their nuclear envelops. Under the comet assay conditions, AA-treated hepatocytes revealed a distinct comet tail electrophoretic migration of DNA fragments that resulted from AA-induced DNA strand breaks. The study also observed similarity configurations of AA-DNA fragmented damage between the findings of cytochemical DNA and comet assays in hepatocytes, and reinforced with the stretching and pulverized chromosome aberrations. But, after enhancing with BDG, the most implications of AA were inclined into mitigation as detected by microscopical and by 3D-comet image analysis, to indicate the potential alleviation role of BDG on AA-induced DNA alterations in hepatocytes and chromosomes of mice.

Keywords: Acrylamide, β-1,3-D-Glucan, Chromosomes, Comet Assay, Cytochemical DNA, Hepatotoxicity, Mice.

1 Introduction

Acrylamide (AA) is a chemical that naturally forms in starchy food products during high-temperature cooking, including frying, baking (biscuits, cakes, rusks, ... etc.), roasting coffee, grilled steak and also industrial processing, at +120°C and low moisture and detected in a wide range of commonly consumed products and detected in a wide range of commonly consumed products (Mottram et al. ,2002; EFSA ,2015; Jakobsen et al., 2016). The same authors reported that AA mainly formed from sugars and amino acids (mainly one called asparagine) that are naturally present in many foods particularly potatoes and cereals. The chemical process that causes this is known as the Maillard reaction; it is a chemical reaction between amino acids and reducing sugars that gives browned food its desirable flavour and taste (Chichester, 1986; Mottram et al., 2002; Everts, 2012). At high temperatures in a range of fried and oven-cooked foods, a potential carcinogen called acrylamide can be formed (Mottram et al., 2002; Tareke et al., 2002). Following ingestion, acrylamide is absorbed from the gastrointestinal tract, distributed to all organs and extensively metabolized, in which, glycidamide is one of the main metabolites resulting from this process (EFSA, 2015). They also added that glycidamide is the most likely cause of these types of adverse effects in animals. Acrylamide exposure can also lead to harmful effects on

the nervous system (including hind-limb paralysis), pre- and post-natal development and adversely affect male reproduction. In more caution, Jakobsen et al. (2016) reported that Acrylamide (AA) is a process-contaminant that increases the risk of developing cancer in humans.

AA is widely studied industrial chemical that is neurotoxic, mutagenic to somatic and germ cells, and carcinogenic in rodents (Kurebayashi and Ohno, 2006). AA could induce DNA damage in the PC Cl3 and FRTL5 rat thyroid cell lines, as well as in human lymphoblastoid TK6 cells in the comet assay (Koyama et al., 2006). ÇEBİ (2016) reported that AA has a toxic potential in tissues including the reproductive and urinary system. It is also a known neurotoxic compound in experimental animals. They stated that it is defined as 2A group carcinogen by International Agency of Research on Cancer.AA decreased glutathione (GSH) levels in Syrian hamster embryo (SHE) cells (Park et al., 2002). It has also been found to be conjugated with GSH (Tong et al., 2004). It is possible that AA itself can be cytotoxic and genotoxic by decreasing the oxidative defense system in the cells (Zamorano-Ponce et al., 2006).From another view to the effects of AA, Kadawathagedara et al. (2016) found the relationship between maternal dietary acrylamide intake during pregnancy and their offspring's anthropometry at birth.

β-1,3-D-Glucan (beta-D-glucan; BDG) is a heterogeneous group of natural polysaccharide of D-glucose monomers linked by (1,3)-β-glycosidic bonds (Stier et al. ,2014). It occurs most commonly as a dietary fiber in cellulose of plants, the bran of cereal grains (especially oat and barley), the cell wall of baker's yeast, certain fungi, mushrooms (such as shiitake and maitake) and bacteria (Teas ,1983; Wasser et al. ,1999; Charalampopoulos et al. ,2002; Holtekjølen et al., 2006; Sikora et al. ,2013; Kusmiati and Dhewantara ,2016). β-1,3-D-Glucan (BDG) is added to foods as supplements on the assumption that this will contribute to health benefits (Holtekjølen et al., 2006, Novak and Vetvicka, 2008). BDG are important structural elements of the cell wall or serve as energy storage in bacteria, fungi including yeast, algae, and plants, while they are absent in vertebrate and invertebrate tissue (Stier et al., 2014).

However, not all β-glucans are able to modulate immune functions. These properties mainly depend on the primary chemical structure of the β-glucans. Cellulose for example, a (1,4)-β-linked glucan, does not exhibit immune-modulatory effects. In contrast, β-glucans derived from fungi and yeast, which consist of a (1,3)-β-linked backbone with small numbers of (1,6)-β-linked side chains, are essentially known for their immune-modulating effects, as reported by Bohn and BeMiller (1995). BDG are known as "biological response" because of their ability to activate the immune system (Miura et al. ,1996).

Due to common dietary application of AA and its presence during food preparation in our life, the present study is aimed to investigate the possibility that BDG may inhibit AA cytotoxicity and genotoxicity in hepatic and bone marrow cells of the male mice.

2 Materials and Methods

Experimental Animals

The present study was carried out on the forty adult male Swiss albino mice of CD-1 *(Mus musculus)* with an average age of 12 weeks and body weight of ~30 g. The animals were obtained from Theodor Bilharz Research Institute, Giza, Egypt. Mice were housed in suitable cages for two weeks for adaptation to laboratory conditions. All animals were fed on standard diet (hay, wheat and milk). Food pellets and water were available *ad libitum*. Mice were kept under suitable laboratory conditions during the whole period of experimentation. As recommended by Ferdowsian and Beck (2011), all experimental procedures were performed taking into account the ethical and scientific considerations regarding animal testing and research.

The Applied Chemicals and Dosages

Acrylamide.

Acrylamide (AA) is 2-propenamide, ethylene carboxamide, acrylic acid amide, vinyl amide or propenoic acid amide (PRI Research Products International, USA), and its molecular formula C_3H_5NO (Lingnert et al., 2002). AA was used at two doses of 0.18 mg/kg b.wt./day and 0.36 mg/kg b.wt./day) (JECFA: FAO/WHO, 2011; EFSA 2015) for successive 30 days.

β-1,3-D-Glucan

According to Bacic et al. (2009) and Chan et al. (2009), the molecular formula of a polysaccharide 'β-1,3-D-Glucan' (Glucan 300® , USA Vial) is $C_{18}H_{30}O_{14}X_2$ (BDG). BGD was used at a dosage of 50 mg/kg b.wt.

The doses of AA and BGD are calculated according to the formula of conversion from human dosage into the mouse dosage (Reagan-Shaw *et al.*, 2008). Both used chemical agents (AA & BDG) are soluble in drinking water, and administered in mice through feeding tube.

Design of Animal Groups

The forty experimental animals are classified into four groups, each 10 mice.

Group1: served as a control group.

Group 2: BDG group, orally fed with 50 mg/kg b.wt./ daily for successive 30 days.

Group 3: AA-oral fed mice. Divided into 2 sub groups: subgroup1 received 0.18 mg/kg b.wt./day and sub group 2 received 0.36 mg/kg b.wt./day for 30 days.

Group 4: the mice treated with 0.36 mg/kg b.wt./day AA+ 50 mg/kg b.wt. a day of BDG fed animals for successive 30 days.

Cytochemical Deoxyribonucleic acid (DNA)

To exhibit DNA inclusions alone, Feulgen nuclear reaction was applied to exhibit the cytochemical DNA in liver specimens fixed in Carnoy's fluid as recommended by Darlington & Cour (1969)and Kasten (2003). Paraffin sections were subjected to mild hydrolysis in N–HCl at 60˚C, then treated with Schiff's reagent. Consequently DNA–containing particles acquired a purple or magenta colouration. Some sections were counter – stained with dilute aqueous solution of 1% light green, followed by a rapid washing in absolute alcohol. DNA inclusions were also showed degrees of purple stainability, as verified by deoxyribonuclease enzyme while the ground cytoplasm took a greenish colouration.

Bone Marrow Chromosomal aberrations technique

The technique as given by Lee and Elder (1980) and modified by Sharma and Sharma (1994) was employed in the present study. Observation was made using bright field and photographs were taken with a 100X oil objective lens. 1000 well spread metaphases of captured chromosomal set from each group were examined at 1000× magnification by use of microscope digital camera 'AmScope' (model number: MU1000) and using Olympus microscope (E330-ADU1X, Japan). A group of eight mice was used for each treatment and 1000 well-spread metaphases were analyzed per group scoring for different kinds of abnormalities including gaps, centric fusion, fragments, deletions, and polyploid metaphases.

Alkaline comet (SCGE) assay

To evaluate baseline frequency of DNA damage in the control, two doses of acrylamide-treated hepatocytes of mice in alone route or accompanied with β-1,3-D-Glucan, the comet assay was carried out under alkaline conditions with single cell gel electrophoresis (SCGE) or comet assay, basically as described by Singh et al. (1988); Sasaki et al. (1997). The liver tissues were surgically removed from each mouse and instantly immersed in liquid nitrogen and grounded using an autoclaved ceramic pestle. Fully frosted slides were covered with 1% normal melting-point (NMP) agarose. After solidification, the gel was scraped off the slide. The slides were then coated with 0.6% NMP agarose. When this layer had solidified, a second layer containing 5 µl of whole hepatocytes sample mixed with 0.5% low melting-point (LMP) agarose was placed on the slides. After 10 min of solidification on ice, the slides were covered with 0.5% LMP agarose. The slides were then immersed overnight in ice-cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris–HCl, 1% sodium sarcosinate, pH 10] with 1% Triton X-100 and 10% dimethyl sulfoxide), which was added immediately prior to use, to lyse the cells and to allow DNA unfolding. The slides were then placed in a horizontal gel-electrophoresis tank, facing the anode. The unit was filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM disodium EDTA, pH 13.0) and the slides were placed in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4 °C under dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis the slides were rinsed gently three times with neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 µg/ml) and covered with a cover-slip.

For comet capture and analysis, fifty randomly captured comets from each slide were examined at 400× magnification using a fluorescence microscope connected *via* a black-and-white camera to an image-analysis system (Comet Assay II; Perceptive Instruments Ltd., Haverhill, Suffolk, UK). To quantify DNA damage, the following comet parameters were evaluated: head intensity (percentage of nuclear head DNA area), and tail length. Tail length (*i.e.*, the length of DNA migration) is related directly to the DNA fragment size. Tail intensity is defined as the percentage of fluorescence migrated in the comet tail. According to Duthie and Collins (1997), the DNA damage was described into four stages (0, I, II, III), whereas no damage (stage 0); mild (stage I) to moderate (stage II) and extensive DNA damage (stage III). The extent of DNA damage within acrylamide-induced hepatotoxicity or improved by β-1,3-D-glucan was expressed as the percentage of cells with the three stages from mild to extensive DNA damage.

According to Helma and Uhl (2000), the 3D-image-analysis of the single-cell gel electrophoresis or comet assay is based on the scientific image-processing program of NIH ImageJ 1.48v, Wayne Rasband, National Institute of Health, USA, as an image-analysis system, which is available at http://rsb.info.nih.gov/nih-image, http://mailbox.univie.ac.at/christoph.helma/comet/.They also illustrated that the comet assay is based on the principle that DNA damage reduces the size of DNA fragments. This effect is detected by applying an electrophoretic field to lysed cells. The stained DNA fragments form a typical comet-shaped migration pattern.

Statistical Analysis

Statistical analysis of the data of chromosomal aberrations was carried out by *t*-test, SPSS statistics 17.0.

Whereas, *p*-value ($p<0.05$) was considered as a statistically significant, whilst *p*-value ($p<0.0001$) was represented as a highly statistically significant

3 Results and Discussion

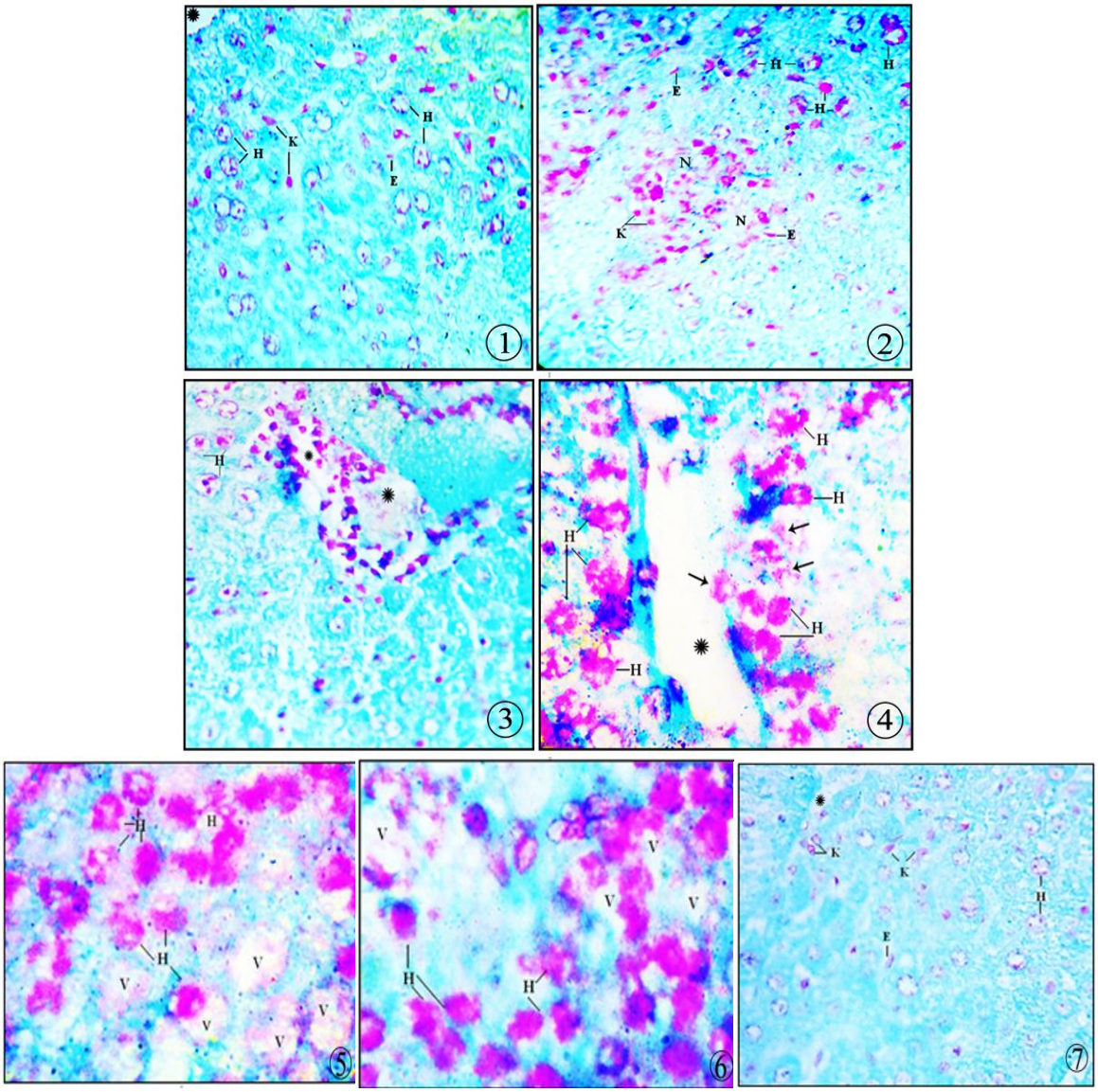
The recent discovery of AA at different levels in a wide variety of commonly consumed foods has energized research efforts worldwide to define toxicity and prevention (Kurebayashi and Ohno, 2006). From this perspective, the idea of the present study was generated in an attempt to find a way to improve and reduce the effects of AA-induced hepatoxicity and genotoxicity in mice by application of potential ameliorative BDG.

The present study observed that the cytochemical DNA in control liver section in mice (Fig. 1) reveals two manners of DNA-containing particles taking intensely magenta colouration of heterochromatin located peripherally and the other is faintly purple stained of loose DNA particles scattered in the nucleoplasm as euchromatin. The Kupffer cell nuclei exhibited a densely DNA stainability, whilst the nuclei of endothelial lining cells showed a relatively moderate staining of Feulgen reaction in counter stained liver sections with light green preparation. Hepatocytes of mice exposed to the effects of successive doses of either

0.18 or 0.36 mg/kg b.wt. of Acrylamide for 3o days have presented a marked increase of DNA inclusions of hepatic parenchymal nuclei, as detected in both peripheral and mid–zonal areas (Figs. 2&3-4, respectively) Hepatocytes of mice exposed to the effects of successive doses of either 0.18 or 0.36 mg/kg b.wt. of Acrylamide for 3o days have presented a marked increase of DNA inclusions of hepatic parenchymal nuclei, as detected in both peripheral and mid–zonal areas (Figs. 2-4, respectively). Such hepatocyte nuclei, showed strong stainability of their condensed DNA inclusions, reflecting a necrotic pattern (Fig. 2). A progressive to this mode of drug application was represented by a highly distinct aggregation of infiltrated inflammatory cells at the portal area (Figs. 3&4). The nuclei of these cells were strongly stained with Feulgen preparation, an indication of condensation of their DNA–containing particles. In this concern, the differentiation between the peripheral condensed DNA inclusions (as heterochromatin) and the loose DNA–tiny elements in the nucleoplasm (as euchromatin) was obviously disappeared from the majority of these nuclei, forming homogeneously packed picture of DNA stainability (Figs. 4-6). The nuclei of this feature may be presumably defined as infiltrated inflamed lymphocytes and plasma cells nuclei, while the larger nuclei probably belong to macrophages and fibroblasts, as described by Foster et al. (1982)and Anderson (1983). In addition, figure (2) have displayed that Kupffer cells nuclei were obviously enlarged taking irregular shapes and sites. Besides, such Kupffer's nuclei were markedly increased packed with rather homogeneously stained DNA–containing masses in a dark magenta colouration. Such Kupffer cells reside within the lumen of the liver sinusoids, and are intimately involved in the liver's response to infection, toxins, ischemia, resection and other stresses (Bilzer *et al*., 2006). Furthermore, The nuclei of the hepatocytes of mice inspected subsequent to the dose of (0.36 mg/kg b.wt.) of Acrylamide for one month, presented more expanding of their fragmented DNA contents as symptomized by their extended stainability patches (like stages II&III in comet assay) in a dark pink colouration (Figs. 5&6). These figures also showed a marked severe deteriorated architecture of their parenchymal cells and their nuclei appeared almost completely devoid of their DNA contents as vacuolated bubbles presumably due to the rupturing and karyolysis of these nuclei and thus the cells visualized suffering from acute degeneration. Examination of liver cells of Z-group (AA-group-2 + BDG), some signs of cure or recovery from the deleterious impacts of AA-induced DNA damage (Fig.7). This figure reflects the nucleoli were manifested more obviously control reddish Feulgen–positive DNA materials, besides, the nuclei of the endothelial–lining of hepatic sinusoids and Kupffer nuclei exhibited a relatively moderate reddish violet colouration. Therefore, the cytochemical DNA in hepatic nuclei after oral fed with BDG indicated its ameliorative role in improving AA-induced DNA toxicity.

The current study observed that AA induced both numerical and structural chromosomal aberrations in a significant increase ($p<0.05$ or $p<0.0001$) under the present test conditions in the bone-marrow cells of male mice (Table 1 & Figs. 9-18) in a dose-dependent manner. Moreover, the study also indicated the ameliorative role of BDG in alleviation the percentage of scored chromosomal aberrations when concomitant with AA. In which, the chromosome aberration test using mammalian cells *in vivo* or *in vitro* is one of the sensitive methods to predict environmental mutagens and or carcinogens, and especially relevant to mutagenic hazard in damage and repair of DNA (Ishidate et al. 1998; Kannan et al., 2014). Moreover, Weisburger et al. (1995) found that the use of grilled meats in diet, where AA formed on the surface of meat may be associated with increased risk of genotoxicity and cancer, which act as direct mutagens. The present work is also in agreement with Zhao *et al*. (2015), who observed that the AA-induced markedly the DNA alteration in lymphocyte and liver cells, as well as micronucleus formation in bone marrow cell. Table (1)

and Figure (19) revealing the magnitudes and types of AA+BDG–induced aberrations (Z-group) are generally decreased in a statistical significance (p<0.05 or p<0.0001). Such scores exhibit the mitigation role of BDG on AA-enhanced chromosome abnormalities in used two doses of AA for 30 days in bone-marrow cells of male mice. These results are in agreement with Zimmermann et al. (2015), who found that the potential cytoprotective and genoprotective effects of β-glucan were evaluated in chicken lymphocytes exposed to increasing concentrations of aflatoxin B_1 inducing DNA damage. On the same manner, Pillai and Uma (2013) recorded the significant reduction in number of aberrant cells and different types of aberrations in beta-glucan on irradiated mushroom cells *in vivo*, reporting that BDG have potential to be a radioprotective.



Figs. (1-7): Photomicrographs of mice liver sections displaying the hepatocyte (H), Kupffer (K) and endothelial (E) nuclei stained with Feulgen reaction for revealing DNAs (as varied densely inclusions of magenta colourations) and counter stained with light green. Fig. 1: represents the liver nuclei of control mouse (C). Fig. 2: showing oral administered ameliorative β-1,3-D-glucan (50 mg/kg) accompanied with the high dose of acrylamide (0.36 mg/kg) for 30 days (Z). Figs. 3-7: revealing the three stages (I, II & III) of different DNA damage levels after feeding with the low dosage of acrylamide (0.18 mg/kg) exhibiting stage-I of DNA alteration (Figs. 3&4) or with the high dosage (0.36 mg/kg) of acrylamide for 30 days, showing a marked DNA deterioration at stage-II (Fig. 5) and stage-III (Figs. 6&7). (Figs. 1-4 X400 & Figs. 5-7 X1000).

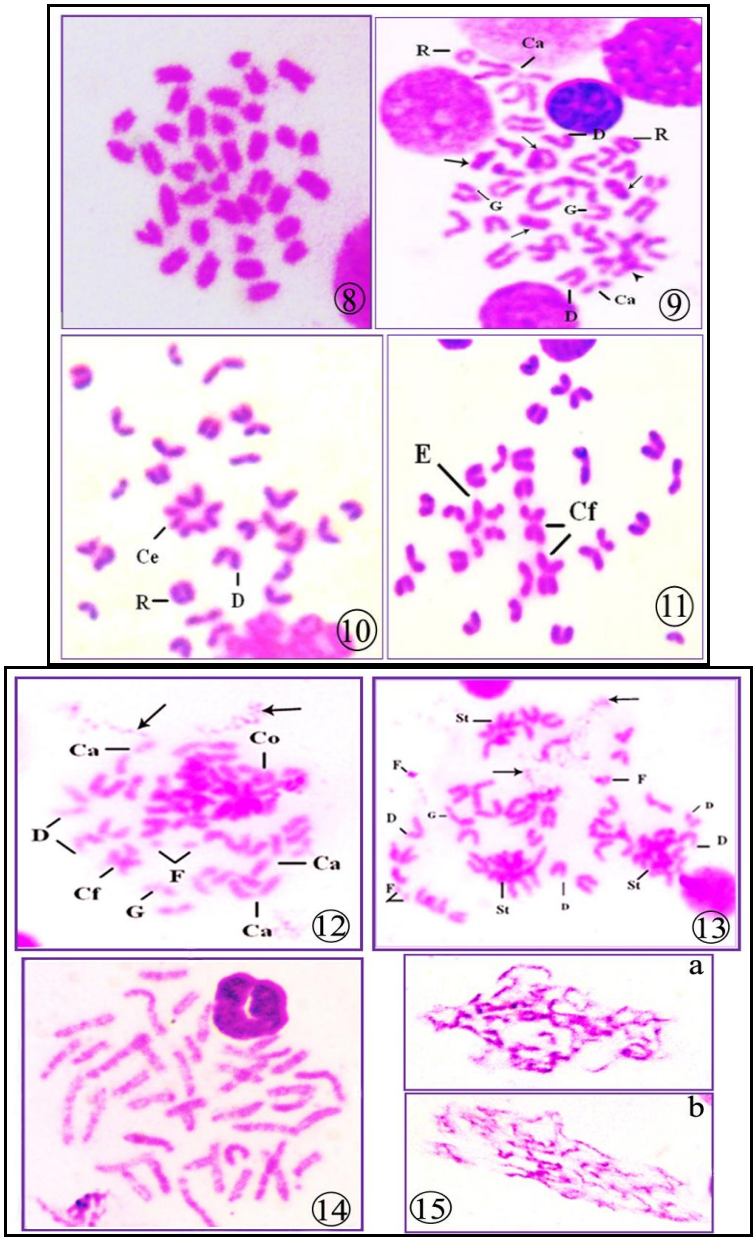
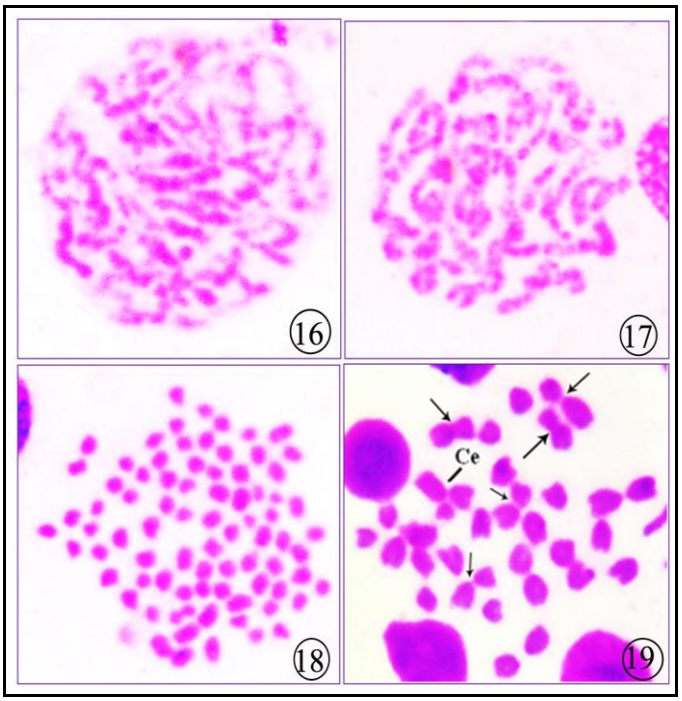


Fig. 8: Normal appearance of metaphase spread obtained from bone marrow of male mouse (*Mus musculus*).

Figs. 9-11: Photomicrographs of metaphases of AA-group-1 (0.18 mg/kg b.wt./daily for 30 days of acrylamide) demonstrating multiple chromosomal aberrations, including, deletions (D), centromeric attenuation (Ca), centric radial of inter-chromatid exchange (Ce), chromatid end-to-end chromatid association (E), centric fusion (Cf), chromatid gap (G), ring chromosomes (R), dicentric chromosome (arrow head) and condensed chromosomes in sticky appearance with clumped chromatin (arrows).

Figs. 12-14: Photomicrographs of metaphases of AA-group-2 (0.36 mg/kg b.wt./daily for 30 days of acrylamide) exhibiting several chromosomal abnormalities involving deletions (D), fragments (F), centric fusion (Cf), chromatid gap (G), ring chromosomes (R), centromeric attenuation (Ca), centric fusion (Cf) and condensed chromosomes in sticky appearance with clumped chromatin (Co/St), beside variety shapes of fragmented-stretching chromosomes (Figs. 12&13: arrows, and all chromosomes of figure 14).

Fig.15. Photomicrographs of metaphase spreads of AA-group-2 (0.36 mg/kg b.wt./daily for 30 days of acrylamide)



Figs. 16-17: Photomicrographs of metaphase spreads of AA-group-2 (0.36 mg/kg b.wt./daily for 30 days of acrylamide) revealing Stretching-fragmented chromosomal set in a large amount of pulverized chromatin and taking different appearances of comet assay stages (I-III).

Fig. 18: Photomicrograph of metaphase spread of AA-group-2 (0.36 mg/kg b.wt./daily for 30 days of acrylamide) showing a numerical chromosomal aberration of tetraploidy**.**

Fig. 19: Photomicrograph of metaphase spread of Z-group (i.e., oral fed mice with the high dose 0.36 mg/kg b.wt./daily of acrylamide concomitant with 50 mg/kg b.wt./daily of ameliorative β-1,3-D-Glucan for 30 days) exhibiting the two chromosomal structural aberrations (centric radial of inter-chromatid exchange, Ce & centric fusion, arrows), which are the most persistent and resistant to improve. (Figs. 8-19: X1000).

Under the comet assay conditions used in this experiment, comet tails reflect the electrophoretic migration of DNA fragments that result from strand breaks. Ethidium bromide–stained nucleoids were examined at 400× magnification and 3D-comet image analysis (Table 2 & Fig. 21). Each cell was visually scored on a 0 to 3 scale using a method described by Duthie & Collins (1997)and Waters et al. (2007), as follows: no damage (stage-0); mild (stage-I) to moderate (stage-II) and extensive DNA damage (stage-III). The extent of DNA damage within acrylamide-induced hepatotoxicity or improved by β-1,3-D-glucan was expressed as the percentage of cells having the three stages from mild to extensive DNA damage in statistical significantce (Table 2 & Fig. 21). The extent of DNA damage in hepatocytes was measured by single cell gel electrophoresis (alkaline comet assay) as described by Singh et al. (1988)and Waters et al. (2007). They also reported that under the assay conditions used in this experiment, comet tails reflect the electrophoretic migration of DNA fragments that result from strand breaks, alkali-labile sites, crosslinks, or base-excision repair sites. DNA damage was carried out using comet assay, a molecular technique that has been increasingly employed to evaluate in vivo and/or in vitro DNA damage in individual cells (Manas et al., 2009). In this concern, the genotoxicity of AA was due to that compound was classified as class 2A carcinogen (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC) and is formed during heating of food from reducing carbohydrates and asparagine by Maillard reaction chemistry (Watzek et al., 2013). In more depth, after dietary uptake, AA is in part metabolically converted into the proximate genotoxic phase I metabolite glycidamide. This metabolite reacts with nucleophilic base positions in DNA, primarily forming N7-(2-carbamoyl-2-hydroxyethyl) guanine (N7-GA-Gua) adducts (Watzek et al., 2013). The ameliorative potential of BDG on hepatotoxicity in AA-induced mice may be due to its tumoricidal activity of polymorphonuclear leukocytes by linear effect as an immune-modulators in murine cells (Morikawa et al. ,1985). The mechanism of ameliorative potential of BDG was illustrated by Xiaolei et al. (2015), whereas they found that the significant protective effect of extracellular polysaccharides from *Grifola frondosa* mycelium on liver injury induced by CCL_4 *in vivo* by decreasing the over-active of CYP2E1 was referred to glucans.

Table 1: Means and Std. Deviation (±SD) of chromosomal aberrations in bone–marrow cells of male mice oral fed with either two doses (low or high dose) of 0.18 or 0.36 mg/kg b.wt./daily for 30 days of acrylamide (AA-group-1 or AA-group-2, respectively), and the high dose of AA (0.36 mg/kg b.wt.) concomitant with β-1,3-D-Glucan (50 mg/kg b.wt./daily) for 30 days (AA+ BDG; Z-group), and their respective control group.

Types of Structural and Numerical Chromosomal Aberrations	Means (±SD) of chromosomal aberrations in 1000 metaphases / 8 mice per each experimental group			
	Control Group	AA-Group-1 Low Dose	AA-Group-2 High Dose	(AA+BDG) Z-Group
I- Structural Aberrations:				
Deletion/ Gap or Fragment (D/G or F)	(2,3,0,0,1,1,1,2) **1.25 ± 1.0350**	(7,9,11,13,8,19,12,10) **11.125 ± 3.7583****	(29,29,33,30,26,40,22,27) **29.5 ± 5.3184****	(5,3,3,4,4,4,3,3) **3.625 ± 0.7440***
Ring chromosome (R)	(0,0,2,2,1,1,1,0) **0.875 ± 0.8345**	(5,6,8,4,6,9,10,5) **6.625 ± 2.1339****	(35,22,21,29,27,27,33,30) **28 ± 4.8697****	(1,2,2,1,2,3,2,1) **1.75 ± 0.7071**
Centric Radial of inter-chromatid exchange (Ce)	(0,0,1,1,1,2,1,0) **0.75 ± 0.7071**	(11,15,16,15,16,11,14,18) **14.5 ± 2.4494****	(44,35,41,47,42,37,39,41) **40.75 ± 3.8078****	(5,5,7,4,1,9,11,8) **6.25 ± 3.1509****
End to-end-association (E)	(1,1,2,0,2,3,0,1) **1.25 ± 1.0350**	(4,3,2,2,1,4,1,2) **2.375 ± 1.1877**	(3,1,5,6,5,3,2,2) **3.375 ± 1.7677***	(2,2,2,3,2,1,1,1) **1.75 ± 0.7071**
Stretching/ or fragmented chromosome	(0,1,1,0,0,1,1,0) **0.5 ± 0.5345**	(23,27,20,29,23,19,33,31) **25.625 ± 5.1530****	(47,59,57,61,66,59,59,62) **58.75 ± 5.4707****	(3,4,1,1,2,2,2,3) **2.25 ± 1.0350***
Condensed / or Sticky chromosomes (Co/St)	(2,2,2,1,1,2,1,1) **1.5 ± 0.5345**	(5,7,7,8,11,13,9,10) **8.75 ± 2.5495****	(34,39,36,29,31,40,38,33) **35 ± 3.9279****	(3,5,5,2,1,1,3,3) **2.875 ± 1.5526**
Centromeric attenuation (Ca)	(1,0,0,2,1,2,1,1) **1 ± 0.7559**	(3,3,4,5,4,2,8,2) **3.875 ± 1.9594***	(7,10,12,14,9,11,18,19) **12.5 ± 4.2426****	(2,1,0,3,5,0,0,1) **1.5 ± 1.7728**
Centric fusion (Cf)	(1,1,0,0,0,2,1,0) **0.625 ± 0.7440**	(11,11,16,19,12,17,21,19) **15.75 ± 3.9551****	(27,21,31,37,34,29,31,29) **29.875 ± 4.7640****	(7,9,11,13,19,11,18,17) **13.125 ± 4.4219****
II- Numerical Aberration:				
Hyperdiploidy/ or Polyploidy	(1,1,0,0,0,0,0,1) **0.375 ± 0.5175**	(7,4,9,2,4,2,6,2) **4.5 ± 2.6186***	(23,21,19,17,19,22,28,20) **21.125 ± 3.3567****	(2,5,1,6,4,2,1,2) **2.875 ± 1.8850***
Total of Means (±SD) of chromosomal aberrations/ 1000 metaphases	(10,7,6,10,4,12,8,5,3) **7.222 ± 3.0322**	(89,53,116,19,205,70, 31,126,36) **82.7777 ± 59.0926***	(236,224,326,27,470, 280,100,239,169) **230.111 ± 128.1107****	(29,14,50,14,18,23, 12,105,23) **32 ± 29.7237**

Level of significance:

***P* > 0.05** **is a statistically non-significant.**

*** *P* < 0.05** **is a statistically significant.**

**** *P* < 0.0001** **is a statistically highly significant.**

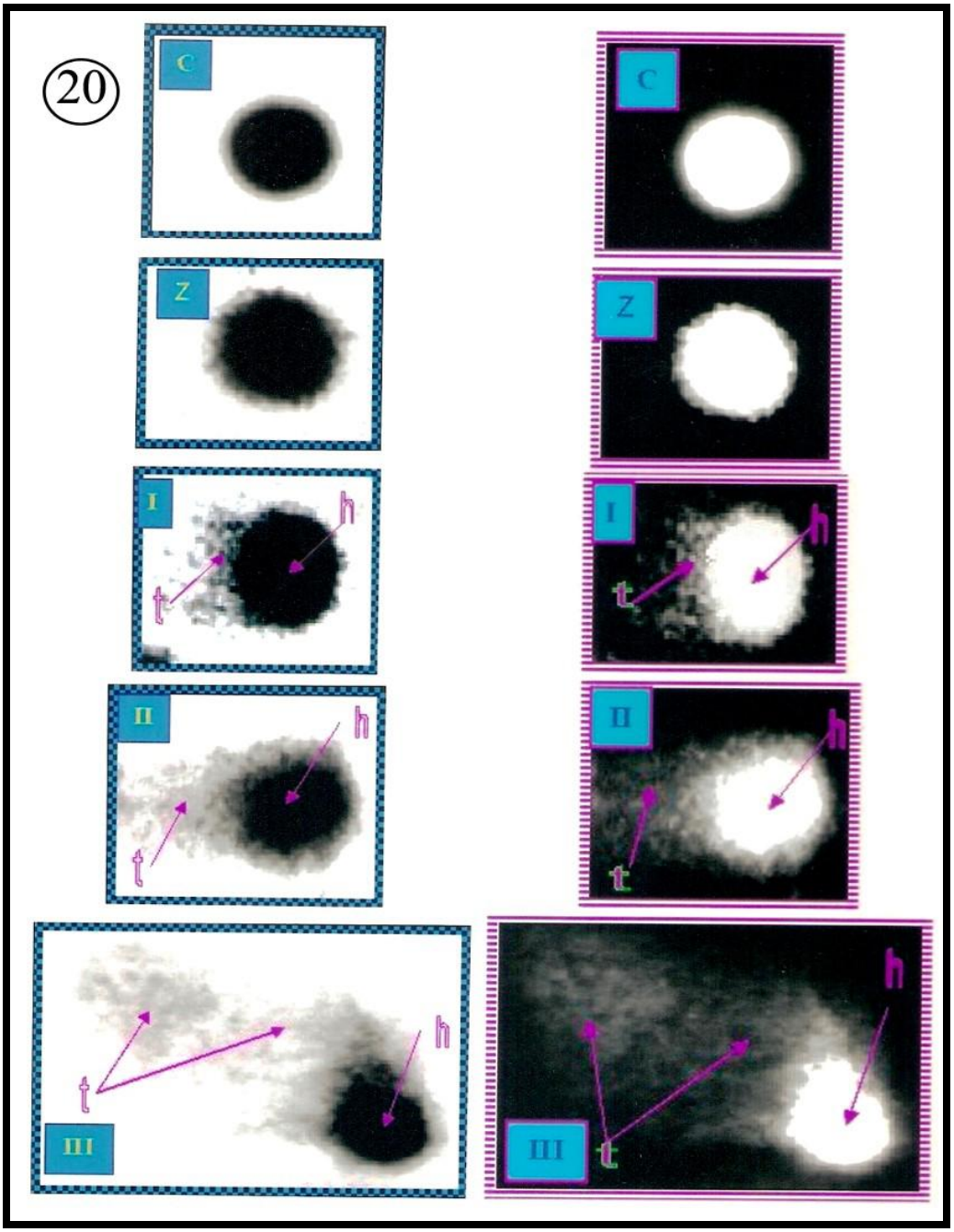


Fig. (20): Inverted and highlighted magnified photomicrographs of DNA comet assay for male mice hepatocytes. C: Control DNA comet assay of hepatic nuclei in the mouse showing normal appearance, reflecting no damage (stage 0), Z: comet assay after feeding both the high dose of acrylamide (0.36 mg/kg b.wt.) accompanied with ameliorative β-1,3-D-glucan treatment (50 mg/kg b.wt.) for 30 days. I, II & III comet stages of DNA damage of mice hepatocytes post oral feeding with the two doses of acrylamide (0.18 or 0.36 mg/kg b.wt./daily) for 30 days. Where: h= Head intensity & t= Tail length, (X400).

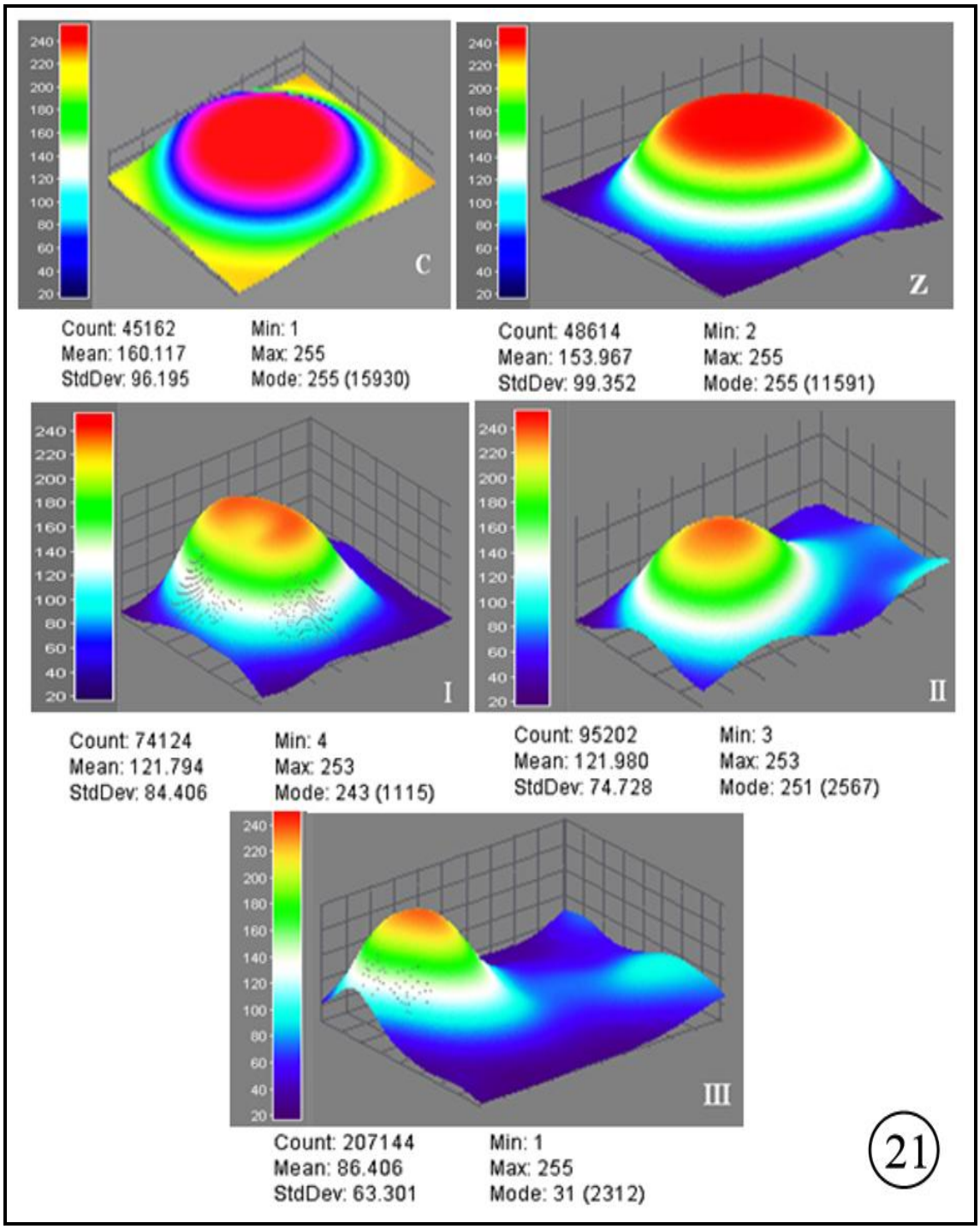


Fig. (21 C): 3D-image analysis of comet assay (0 stage; no damage) and its histogram record for the configuration of the control liver DNA intensity.

Fig. (21 Z): 3D-image analysis of comet assay (pre-stage I) and its histogram record for DNA configurations of mice liver after feeding with the high dose of acrylamide (0.36 mg/kg b.wt./daily) and concomitant with ameliorative β-1,3-D-glucan (50 mg/kg b.wt./daily) for 30 days.

Figs. (21,I-III): 3D-images analysis of comet assay (stages I, II & III) and their histogram records for the intensity damage in DNA configurations for mice hepatocytes after oral treatment with the two doses of acrylamide either (0.18 or 0.36 mg/kg b.wt./daily) for 30 days.

Table 2: Means and Std. Deviation (±SD) of Classes of Comet Assay Stages (0, I, II & III) in liver cells of male mice oral fed with either two doses (low or high dose) of 0.18 or 0.36 mg/kg b.wt./daily for 30 days of acrylamide (AA-group-1 or AA-group-2, respectively), and the high dose of AA (0.36 mg/kg b.wt.) concomitant with β-1,3-D-Glucan (50 mg/kg b.wt./daily) for 30 days (AA+ BDG; Z-group), and their respective control group.

Animal Groups	250 cells/ 5 Mice (50/ mouse)	Comet Assay Stages (Classes)															
		Stage - 0				Stage - I				Stage - II				Stage - III			
		No/ 50 Cells	Mean	*P*-values	±SD	No/ 50 Cells	Mean	*P*-values	±SD	No/ 50 Cells	Mean	*P*-values	±SD	No/ 50 Cells	Mean	*P* -values	±SD
Control Group	*Mouse01*	44	42.8	-	±2.280	5	5.4	-	±1.816	1	1	-	±0.707	0	0.8	-	±0.836
	Mouse02	43				6				1				0			
	Mouse03	45				3				1				1			
	Mouse04	43				5				0				2			
	Mouse05	39				8				2				1			
AA-Group-1 (Low Dose)	*Mouse01*	14	15.2	*p*** ↓	±3.033	23	19.8	*p*** ↑	±2.588	11	11.2	*p*** ↑	±1.923	2	3.8	*p** ↑	±1.303
	Mouse02	17				19				9				5			
	Mouse03	11				21				14				4			
	Mouse04	19				16				10				5			
	Mouse05	15				20				12				3			
AA-Group-2 (High Dose)	*Mouse01*	6	11.2	*p*** ↓	±3.962	21	15.2	*p** ↑	±4.711	8	9	*p** ↑	±4.183	15	14.6	*p*** ↑	±2.073
	Mouse02	11				12				11				16			
	Mouse03	14				17				7				12			
	Mouse04	9				9				15				17			
	Mouse05	16				17				4				13			
(AA+BDG) Z-Group	*Mouse01*	33	30.2	*p** ↓	±4.207	14	14.6	*p** ↑	±3.577	3	2.8	*p*	±3.114	0	2.4	*p*	±2.073
	Mouse02	29				16				1				4			
	Mouse03	36				12				0				2			
	Mouse04	27				20				2				1			
	Mouse05	26				11				8				5			

Level of significance:

***P* > 0.05 is a statistically non-significant.**

*** *P* < 0.05 is a statistically significant.**

*****P* < 0.0001 is a statistically highly significant.**

↑ represents a significant increase.

↓ represents a significant decrease.

Generally, the most characterized detected chromosome aberration in AA-group-1 &-2 is pulverized chromosomes (beaded–shaped chromosomes; Figs: 14-17) in highly significant elevation (p<0.0001), which is distinguished when the chromosomes appear as shattered or segmented structures, having a peculiar fragmented appearance, in which, both chromatic and achromatic segments are weakly attached together at the points of gaps, having a similar appearance of DNA damage (Stages I-III) in both cytochemical DNA (Figs. 4-6) and comet assay (Figs. 20 &21).

In conclusion, the present results demonstrate that BDG could be a suitable ameliorative agent for mitigating AA-induced DNA and chromosome damages in liver and bone marrow cells of male mouse. So, it suggests the use of the cytochemical DNA, cytogenetical and comet assays in closely integrated biomarkers to evaluate AA-induced toxicity in hepatic DNA and genotoxicity and to validate the potential alleviation role of BDG.

4 References

Anderson JR (1983). Inflammation: in Muir's Textbook of Pathology. 11th. edition, Edward Arnold (Publishers) Ltd., London, pp.68-70.

Bacic A, Fincher G, Stone B (2009). Chemistry, Biochemistry and Biology of 1-3 Beta Glucans and Related Polysaccharides, 1st. Edition. Academic Press, eBook ISBN: 9780080920542.

Bilzer M, Roggel F, Gerbes AL (2006). Role of Kupffer cells in host defense and liver disease. Liver Int., 26(10):1175-1186.

Bohn JA, BeMiller JN (1995). 1-3-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr. Polym., 28(1):3–14.

ÇEBİ A (2016). Chapter 4 - Acrylamide Intake, Its Effects on Tissues and Cancer. In: Acrylamide in Food. Pp: 63-91.

Chan GC, Chan WK, Sze MD (2009). The effects of β-glucan on human immune and cancer cells. J. Haematol. & Oncol., 2:25-35.

Charalampopoulos D, Wang R, Pandiella SS, Webb C (2002). Application of cereals and cereal in functional foods: a review. Int. J. Food Microbiol., 79(1-2): 131-41.

Chichester C O ; ed. (1986). Advances in Food Research. Advances in Food and Nutrition Research 30. Boston: Academic Press. p. 79. ISBN 0-12-016430-2.

Darlington C D, Cour L F (1969). The handling of chromosomes. George Allen & Unwin. Ltd., London. pp. 163 – 166.

Duthie SJ, Collins AR (1997). The influence of cell growth, detoxifying enzymes and DNA repair on hydrogen per oxide-mediated DNA damage (measured using the comet assay) in human cells. Free Radic. Biol. Med., 22:717 – 24.

EFSA (European Food Safety Authority), Parma, Italy (2015). EFSA explains risk assessment. Acrylamide in food. www.efsa.europa.eu. doi:10.2805/90424. ISBN: 978-92-9199-685-8.

Everts S (2012). The Maillard Reaction Turns 100. Chemical & Engineering News, 90 (40): 58–60.

Ferdowsian HR, Beck N (2011). Ethical and Scientific Considerations Regarding Animal Testing and Research. PLoS ONE, 6 (9): e24059.

Feulgen R, Rossenbeck H (1924). Z. phsiol. Chem., 135: 303 (Quoted from Kasten, F.H., 2003).

Foster HL, Small JD, Fox JG, editors (1982). Diseases: The Mouse in Biomedical Research. Academic Press, Inc. (London) Ltd., pp.147.

Helma C, Uhl M (2000). A public domain image-analysis program for the single-cell gel-electrophoresis-comet/ assay. Mut. Res., 466 : 9–15.

Holtekjølen AK, Uhlen AK, Bråthen E, Sahlstrøm S, Knutsen SH (2006). Contents of starch and non-starch polysaccharides in barley varieties of different origin. Food Chemistry, 94(3): 348–358.

Ishidate M (1998). In vitro chromosomal aberration test-current status. In: Chromosomal Aberrations: Basic and Applied Aspects. Obe G. and Natarajan A.T. (eds.), Springer-Verlag Pub., pp. 260-271.

Jakobsen LS, Granby K, Knudsen VK, Nauta M, Pires SM, Poulsen M (2016).Burden of disease of dietary exposure to acrylamide in Denmark. Food Chem. Toxicol., 90:151-159.

JECFA (2011). Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on acrylamide. CAS number: 79-06-01. Functional Class: Food Contaminant.

Kadawathagedara M, Tong AC, Heude B, Forhan A, Charles MA, Sirot V, Botton J (2016). Dietary acrylamide intake during pregnancy and at birth in the French EDEN mother-child cohort study. Environ. Res.,149:189-196.

Kannan TP, Lah NA, Ahmad A, Ramli SF, Yusoff NM, Samsudin AR (2014). Genotoxic evaluation of synthetic hydroxyapatite using mammalian bone marrow chromosome aberration test. Arch Orofac. Sci., 9(1): 10-16.

Kasten FH (2003). Robert Feulgen and his histochemical reaction for DNA. Biotechnic & Histochemistry, 78(1): 45-49.

Koyama N, Sakamoto N, Sakuraba M, Koizumi T, et al. (2006). Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells. Mutat. Res., 603 (2): 151–158.

Kurebayashi H, Ohno Y (2006). Metabolism of acrylamide to glycidamide and their cytotoxicity in isolated rat hepatocytes: protective effects of GSH precursors. Arch. Toxicol., 80(12):820-828.

Kusmiati F X , Dhewantara R (2016). Cholesterol-Lowering Effect of Beta Glucan Extracted from Saccharomyces cerevisiae in Rats. Sci. Pharm., 84: 153–165.

Lee MR, Elder FFB (1980). Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenetics and Cell Genetics, 26: 36-40.

Lingnert H, Grivas S, Ja¨gerstad M, Kerstin Skog, Margareta To¨rnqvist M , man P (2002). Acrylamide in food: mechanisms of formation and influencing factors during heating of foods. Scandinavian Journal of Nutrition, 46 (4): 159–172.

Manas F, Paralta L, Raviolo J, Garcia Ovando H, Weyers A, Ugnia L. Gonzalez Cid M, Larripa I, Gorla N (2009). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the comet assay and cytogenetic tests. Ecotoxical. Env. Safety, 72: 834-837.

Miura NN, Ohno N, Aketagawa J, Tamura H, Tanaka S, Yadomae T (1996). Blood clearance of (1-->3)-beta-D-glucan in MRL lpr/lpr mice. FEMS immunology and medical microbiology, 13 (1): 51–57.

Morikawa K, Takeda R, Yamazaki M, Mizuno D (1985). Induction of tumoricidal activity of polymorphonuclear leukocytes by a linear beta-1,3-D-glucan and other immunomodulators in murine cells. Cancer Res., 45(4):1496-1501.

Mottram DS, Wedzicha B L, Dodson A T (2002). Acrylamide is formed in the Maillard reaction. Nature, 419: 448–449.

Novak M, Vetvicka V (2008). Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. J. Immunotoxicol., 5(1): 47-57.

Park J, Kamendulis LM, Friedman MA, Klaunig JE (2002). Acrylamide-induced cellular transformation. Toxicol. Sci. 65, 177–183.

Pillai TG , Uma P (2013). Mushroom beta glucan: Potential candidate for post irradiation protection. Mutat. Res./Genetic Toxicol. and Environmental Mutagenesis, 751 (2): 109-115.

Reagan-Shaw S, Nihal M, Ahmad N (2008). Dose translation from animal to human studies revisited. FASEB J., 22 (3): 659-661.

Sasaki YF, Tsuda S, Izumiyama F, Nishidate E (1997). Detection of chemically induced DNA lesions in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow) using the alkaline single cell gel electrophoresis (Comet) assay. Mutat. Res., 388: 33-44.

Sharma AK, Sharma A (1994). Chromosome techniques. Harwood Academic Publishers. Switzerland.

Sikora P, Tosh SM, Brummer Y, Olsson O (2013). Identification of high β-glucan oat lines and localization and chemical characterization of their seed kernel β-glucans. Food Chem., 137 (1-4):83-91.

Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175: 184–191.

Stier H, Ebbeskotte V, Joerg Gruenwald J (2014). Immune-modulatory effects of dietary Yeast Beta-1,3/1,6-D-glucan. Nutr. J., 13: 38.

Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50(17): 4998–5006.

Teas J (1983). The dietary intake of Laminarin, a brown seaweed, and breast cancer prevention. Nutrition and cancer, 4 (3): 217–222.

Tong GC, Cornwell WK, Means GE (2004).Reactions of acrylamide with glutathione and serum albumin. Toxicol. Lett., 147: 127–131.

Wasser, SP, Weis AL (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. Crit. Rev. Immunol., 19 (1): 65–96.

Waters DJ, Shen S, Xu H, et al. (2007). Noninvasive Prediction of Prostatic DNA Damage by Oxidative Stress Challenge of Peripheral Blood Lymphocytes. Cancer Epidemiol. Biomarkers Prev., 16:1906-1910.

Watzek N, Scherbl D, Schug M, Hengstler JG, Baum M, Habermeyer M, Richling E, Eisenbrand G (2013). Toxicokinetics of acrylamide in primary rat hepatocytes: coupling to glutathione is faster than conversion to glycidamide. Arch Toxicol., 87(8):1545-1556.

Weisburger JH, Rivenson A, Kingston DG, Wilkins TD, Nagao M, et al. (1995).Dietary modulation of the carcinogenicity of the heterocyclic amines, In: Heterocyclic amines in cooked foods: Possible human carcinogens, R. H., Gustafsson J. A., Ito N., Nagao T., Sugimura T., Yamazoe Y. (Eds.) Publishing Co., Inc, Princeton, NJ, p. 240-250.

Xiaolei Ma, Caojiao Li, Wentao Qi, Xiaorui Li, Wang S, Cao X, Chunling Wang C (2015). Protective effect of extracellular polysaccharides from Grifola frondosa mycelium on CCl4-injured liver in vitro. Bioactive Carbohydrates and Dietary Fibre, 6 (1): 7-14.

Zamorano-Ponce E, Morales C, Ramos D, Sepulveda C, Cares S, Rivera P, Fernandez J, Carballo MA (2006). Anti-genotoxiceffect of Aloysia triphylla infusion against acrylamide-induced DNA damage as shown by the comet assay technique. Mutat. Res., 603:145–150.

Zhao M, Wang P, Zhu Y, Liu X, Xiaosong Hu X, Chen F (2015): Blueberry anthocyanins extract inhibits acrylamide-induced diverse toxicity in mice by preventing oxidative stress and cytochrome P450 2E1 activation. Journal of Functional Foods, 14: 95-101.

Zimmermann CEP, Cruz IBM, Cadoná FC, Machado AK, Assmann C, Schlemmer KB, Zanette RA, Leal DBR, Santurio JM (2015). Cytoprotective and genoprotective effects of β-glucans against aflatoxin B1-induced DNA damage in broiler chicken lymphocytes. Toxicology in Vitro, 29 (3): 538-543.