Modulatory role of *Saccharomyces cerevisiae* against cadmium-induced genotoxicity in mice

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Background/aim
Cadmium (Cd) was found to be a major heavy metal utilized in different agricultural and industrial processes. However, exposure to this element was found to cause serious environmental pollution and induce deleterious effects on human and animal health. Therefore, this study was conducted to estimate the protective and therapeutic role of *Saccharomyces cerevisiae* against Cd genotoxicity.
Materials and methods
Forty-eight male mice were used in this study by dividing them into six groups (eight animals each): the control group, which was fed a basal diet; a group fed a basal diet contaminated with Cd (0.03 g/kg diet) for 3 weeks; two protection groups fed a basal diet supplemented with *S. cerevisiae* at low (5%) or high (7.5%) levels along with contamination with Cd; and two therapeutic groups fed a basal diet supplemented with *S. cerevisiae* at the same levels for 15 days after cessation of contamination with Cd. At the end of the experimental period, genetic and sperm parameters of the mice were evaluated. Genetic parameters included the DNA comet assay, random amplified polymorphic DNA-PCR analysis, micronucleus test, and chromosomal examinations. Sperm parameters involved sperm-shape abnormalities and sperm count.
Results
The results showed that feeding on a basal diet contaminated with Cd caused significant increases in abnormal genetic parameters and sperm-shape abnormalities as well as significant decrease in sperm count. The utilization of *S. cerevisiae* yeast as a protective or therapeutic agent significantly improved the genetic and sperm parameters as compared with Cd treatment alone. The best findings were revealed when *S. cerevisiae* was used as a protective agent, especially treatment at high levels (7.5%).
Conclusion
This work showed that *S. cerevisiae* yeast is a strong probiotic agent against Cd detoxification in foods, where the addition of this probiotic to animal diets significantly minimized the deleterious effects caused by Cd on the genetic and sperm parameters of mice.

Keywords:
Cadmium, comet assay, cytogenetics, mice, random amplified polymorphic dna-pcr, *saccharomyces cerevisiae*, sperm

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Introduction

Cadmium (Cd) is considered a major heavy metal that is extensively used in agriculture and industry: for example, in the application of pesticides and phosphate fertilizers, in plastic and pigment production, in paints, electroplating, soldering, and in the manufacture of alloys and batteries [1,2]. Cd was found to be an extremely toxic metal causing serious environmental pollution [3–5].

Humans and animals might be exposed daily to Cd through fumes, dust, smoking, and polluted food and water [2,6,7]. This metal was revealed to accumulate mostly in the liver and kidney, although its toxicity can extend to other organs such the lungs, the bladder, the brain, bone, and the reproductive system, causing deleterious effects and a variety of diseases such as cancer, genotoxicity, reproductive disorders and infertility, arteriosclerosis, immunotoxicity, and anemia [2,5].

The toxicity of Cd might be due to its metabolites in the liver leading to the generation of reactive oxygen

species (ROS) that include hydrogen peroxide and superoxide anion [4,8–10]. ROS can attack cellular biomolecules in several organisms resulting in deleterious oxidation of protein, formation of lipid peroxidation, reduction of antioxidant parameters, DNA damage, chromosomal aberrations, alterations of gene expression, and apoptosis [5,11]. Cd ions were found to have the ability to pass through the blood–testes barrier, inducing testis toxicity [4,12]. Testis toxicity includes spermatogenesis aberrations, inhibition of chromatin condensation of sperm, fragmentation of sperm DNA, decrease in sperm motility and viability, and abnormalities in sperm shape [13,14]. Also, ROS generated through exposure to Cd can affect the sperm structure and function by inducing peroxidation of membrane lipids, as well as by decreasing each cell membrane phosphatide and levels of ATP [14]. Thus, contamination with Cd was found to have multiple deleterious effects on human and animal health. Therefore, control and prevention of toxic effects of this metal has become essential. Interventions focusing on probiotics that lead to inactivation or elimination of the bioavailability of Cd ions in contaminated products show promise in controlling the impact of diet-polluted mutagens.

The yeast of *Saccharomyces cerevisiae* is considered one of the major probiotics (microorganisms that are believed to provide health benefits when consumed). This yeast was for many centuries used in food production, and recently it has been utilized in pharmaceutical industries as well as in the production of certain substances that have beneficial properties in humans and animals, such selenium, zinc, glutathione, and cysteine [15–17]. Glutathione, cysteine, zinc, and selenium were reported to have antioxidant substances that could scavenge free radicals and peroxynitrite; they were also found to be strong hepatoprotective agents against many toxicants [17]. Probiotics were found to stimulate the detoxification process by removing or eliminating toxin traces without leaving deleterious residues and without impairing the quality of nutrition of the commodity [18,19]. Moreover, *S. cerevisiae* was found to be a potent protective agent against genotoxicity induced by exposure to aflatoxin (AF) in somatic and germ cells of mice, where the rate of cytogenetic changes and sperm abnormalities was significantly minimized in the animals exposed to AF and treated with *S. cerevisiae* as compared with those exposed only to AF [20].

Further, a previous study by Eshak *et al*. [21] revealed that the addition of *S. cerevisiae* yeast to quail feed that was contaminated with AF led to better growth performance and improvement in the expression levels of neural and gonadal genes as compared with AF diet alone.

Furthermore, the components of *S. cerevisiae*, such as β-glucan and mannan oligosaccharides, were considered to be strong antioxidant, antimutagenic, and anti-carcinogenic agents, wherein treatment with these components led to a reduction in DNA damage caused by cancer development of human leukocytes [22,23] and minimized the proportion of micronuclei induced by cyclophosphamide in mice [24]. Moreover, the β-glucan treatment was found to improve the quality of life of animals exposed to radiation, as β-glucan was observed to have bioaction and antioxidant activity due to its being capable of bioantimutagenic action, scavenging of free radicals, and exerting desmutagenic action [25]. On the other hand, *S. cerevisiae* yeast and its components were observed to have the ability to adsorb and bind with toxicants and consequently protect the live organisms and their genetic constitutions from deleterious effects [26].

The present work aimed to assess the protective and therapeutic role of *S. cerevisiae* yeast against genotoxicity induced by Cd in mice. DNA comet assay, random amplified polymorphic DNA (RAPD)-PCR analysis, cytogenetic tests, and sperm examinations were performed to elucidate the study purpose.

Materials and methods

Chemicals

Cadmium chloride ($CdCl_2$) in powder form was purchased from Sigma–Aldrich. $CdCl_2$ was added and mixed well to the basal diet at 0.03 g/kg diet. This dose of $CdCl_2$ has been considered an appropriate experimental dose that affects mouse organs [27,28]. All experimental procedures involving animals were conducted in accordance to the ethical guidelines of the medical ethical committee of the National Research Centre in Egypt and approved by medical ethical committee of the National Research Centre in Egypt.

Yeast of *Saccharomyces cerevisiae*

The yeast of *S. cerevisiae* was obtained from Microbial Chemistry Department, National Research Centre, Egypt. Two levels, 5 and 7.5%/kg, of *S. cerevisiae* were added to the basal diet. These levels of *S. cerevisiae* were mixed well and fed according to the procedure detailed in the study by Parlat *et al.* [29]. $CdCl_2$ was incorporated into the basal diets before *S. cerevisiae* addition.

Experimental animals

Albino mice of Swiss strain weighing 25–30 g were obtained from the Animal House, National Research Centre, Giza, Egypt. The animals were housed at an ambient temperature of 25±3.2°C under a light/dark cycle of 12/12 h. All mice were kept in clean polypropylene cages and administered food and water *ad libitum*.

Experimental design

The mice were divided into six equal groups of eight animals each. Group 1 was fed a normal basal diet (control group). Group 2 was fed a basal diet containing $CdCl_2$ at 0.03 g/Kg diet for 3 weeks (cadmium group). The third and fourth groups were fed a basal diet containing *S. cerevisiae* at low (5%) (protection 1) or high (7.5%) (protection 2) levels along with treatment with $CdCl_2$. The fifth and sixth groups were fed a basal diet containing *S. cerevisiae* at low (5%) (therapeutic 1) or high (7%) (therapeutic 2) levels for 2 weeks after cessation of $CdCl_2$ treatment. Groups 3 and 4 were used to evaluate the protective role of *S. cerevisiae* against the potential mutagenic effects of $CdCl_2$. Groups 5 and 6 were used to evaluate the therapeutic effect of *S. cerevisiae* against the potential mutagenic effects of $CdCl_2$. At the end of the experiment, the mice were killed by cervical dislocation for studying their molecular genetics (using the DNA comet assay and RAPD-PCR), cytogenetics (micronuclei and chromosome aberrations), and sperm profile (sperm shape and count).

DNA comet assay (single-cell gel electrophoresis)

DNA damage was measured according to the method of Singh *et al.* [30]. The DNA fragment migration patterns of 100 cells of liver tissue for each treatment, including those of controls, were evaluated with a fluorescence microscope [with excitation filter 420–490 nm (issue 510 nm)]. The comet tail lengths were measured from the middle of the nucleus to the end of the tail at ×40 magnification to determine the count and size of the comet. For visualization of DNA damage, EtBr-stained DNA was observed at ×40 magnification using a fluorescence microscope. Comet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK) linked to a charge-coupled device camera was used to assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. The program also calculates tail moment. Generally, 100 randomly selected cells were analyzed per sample.

Random amplified polymorphic DNA-PCR analysis

DNA extraction using the salting-out method

DNA was extracted from liver tissue of mice according to the method described by Miller *et al.* [31] and Sambrook *et al.* [32].

Random amplified polymorphic DNA-PCR amplification

Three commercial primers with size 10 bases and variable nucleotide proportions were used for the amplification process. The primers (supplied by Operon, Almeda, California, USA) were OPC05 (5′GATGACCGCC3′), OPA04 (5′AATCGGGC-TG3′), and OPA06 (5′GGTCCCTGAC3′). The PCR protocol for RAPD analysis was as described by Williams *et al.* [33]. Approximately 3 µl of the amplified DNA product and 2 µl of 1×loading dye were loaded on a 2% agarose gel and then subjected to electrophoresis in 1×tris/borate/EDTA buffer and stained with ethidium bromide (0.5 µl/ml) for verification. A Bio-Rad (USA) XR+molecular imager apparatus was used to visualize the PCR products. The ladder DNA (100 pb) (Fermentas; Thermo fisher scientific, USA) and LabImage were used for determination of the molecular size of the bands.

Random amplified polymorphic DNA profiles and data analysis

Quantitative estimation of DNA samples was done by a double-beam UV-spectrophotometer (UV-2450; Shimadzu, Japan). The DNA concentration was measured at 260 and 280 nm, respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gel using a DNA molecular weight marker (Fermentas). The marked changes in RAPD profile such as disappearance and/or appearance of bands in comparison with untreated control treatments (b/a bands) were evaluated [34].

Cytogenetic analysis

Micronucleus test

Bone marrow slides were prepared and stained according to the method described by Krishna and Hayashi [35].

Chromosome preparation

For chromosomal analysis both treated and control animals were killed, femurs were removed, and bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Preston *et al.* [36].

Sperm analysis

For sperm-shape analysis, epididymal sperms were prepared and stained with Eosin Y (aqueous), as per the methods described by Wyrobek and Bruce [37] and Farag *et al.* [38]. At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to the standard

method described by Narayana [39]. Epididymal sperm count was also determined by means of a hemocytometer as described by Pant and Srivastava [40].

Statistical analysis

Statistical analysis was performed with SPSS software. Data were analyzed using one-way analysis of variance followed by Duncan's post-hoc test for comparison between different treatments. Results were reported as mean±SE. The polymorphism values were determined according to the method of Taşpınar *et al.* [41].

Results

Comet assay

The results of the DNA comet assay are shown in Table 1 and Fig. 1. These findings showed damaged parameters of genomic DNA that included DNA tailed percentage, DNA untailed percentage, DNA tail length, tail DNA percentage, and tail moment. The present results revealed that feeding on a basal diet polluted with Cd had induced significant increases in DNA tailed percentage, DNA tail length, tail DNA percentage, and tail moment, whereas there was significant decrease in DNA untailed percentage as compared with the control group. In contrast, the addition of *S. cerevisiae* yeast to the basal diet along with (as a protective agent) or after cessation (as a therapeutic agent) of Cd treatment caused significant reduction in DNA tailed percentage, DNA tail length, tail DNA percentage, and tail moment, and significant increase in DNA untailed percentage compared with treatment with Cd alone. The results indicated that the use of a high level of *S. cerevisiae* yeast was more pronounced for improvement of DNA structure than the low level of yeast. Moreover, the best improvement in DNA structure was obtained when *S. cerevisiae* yeast was used as a protective agent than when it was used as a therapeutic agent.

Random amplified polymorphic DNA-PCR

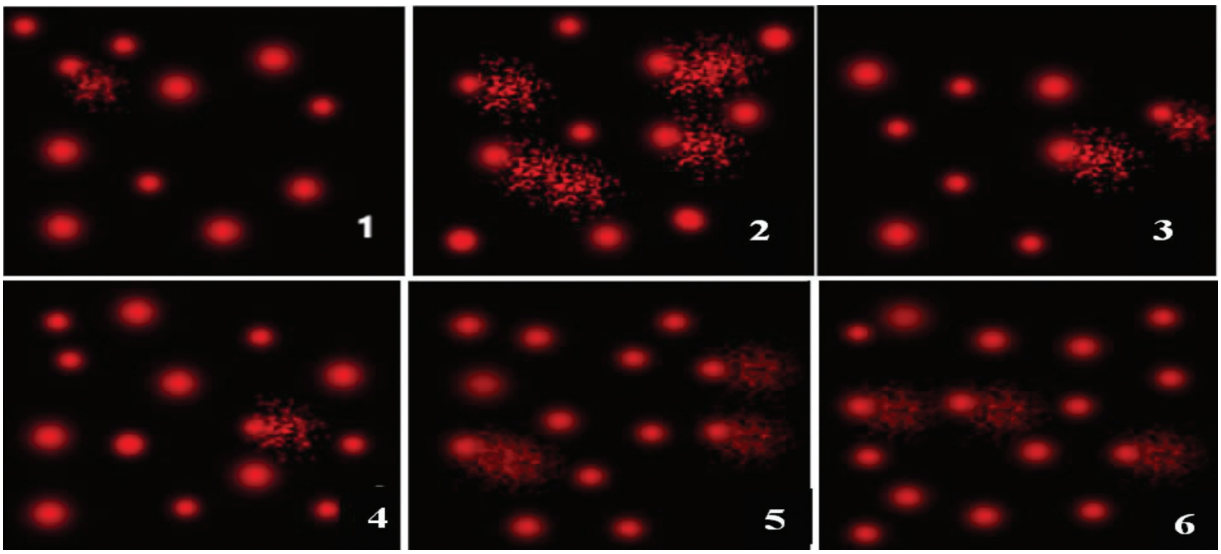
The products of RAPD-PCR are recorded in Table 2 and illustrated in Fig. 2. The obtained results from three primers OPC05, OPA04, and OPA06 displayed a potent PCR amplification with distinct band

Table 1 Parameters of DNA damage in the comet assay in mice treated with *Saccharomyces cerevisiae* yeast as a protective or a therapeutic agent against cadmium toxicity

Treatments	% tailed	Untailed %	Tail length (μm)	Tail DNA %	Tail moment
Control	3.67±0.33^{d}	96.33±0.33^{a}	1.72±0.03^{c}	1.59±0.07^{e}	2.73±0.17^{e}
Cadmium	17.67±0.33^{a}	82.33±0.33^{d}	3.50±0.04^{a}	4.07±0.03^{a}	14.26±0.29^{a}
Protection 1	5.0±0.57^{d}	95.0±0.57^{a}	1.80±0.03^{c}	2.08±0.06^{d}	3.82±0.13^{d}
Protection 2	4.0±0.57^{d}	96.0±0.57^{a}	1.53±0.02^{d}	1.96±0.3^{d}	2.99±0.08^{e}
Therapeutic 1	14.67±0.33^{b}	85.33±0.33^{c}	3.2±0.04^{b}	3.69±0.1^{b}	12.12±0.28^{b}
Therapeutic 2	12.0±0.57^{c}	88.0±0.57^{b}	3.2±0.04^{b}	3.32±0.7^{c}	10.65±0.09^{c}

All data are represented as mean±SE. ^{a,b,c,d,e}Mean with different letters in each column were significantly different using analysis of variance test at $P<0.05$.

Figure 1



Comet assay prepared of mice liver showing varying extent of DNA damage. These mice were treated with *Saccharomyces cerevisiae* yeast as a protective or a therapeutic agent against contamination with cadmium. Figures 1–6 represent control, Cd, protection 1, protection 2, therapeutic 1 and therapeutic 2, respectively.

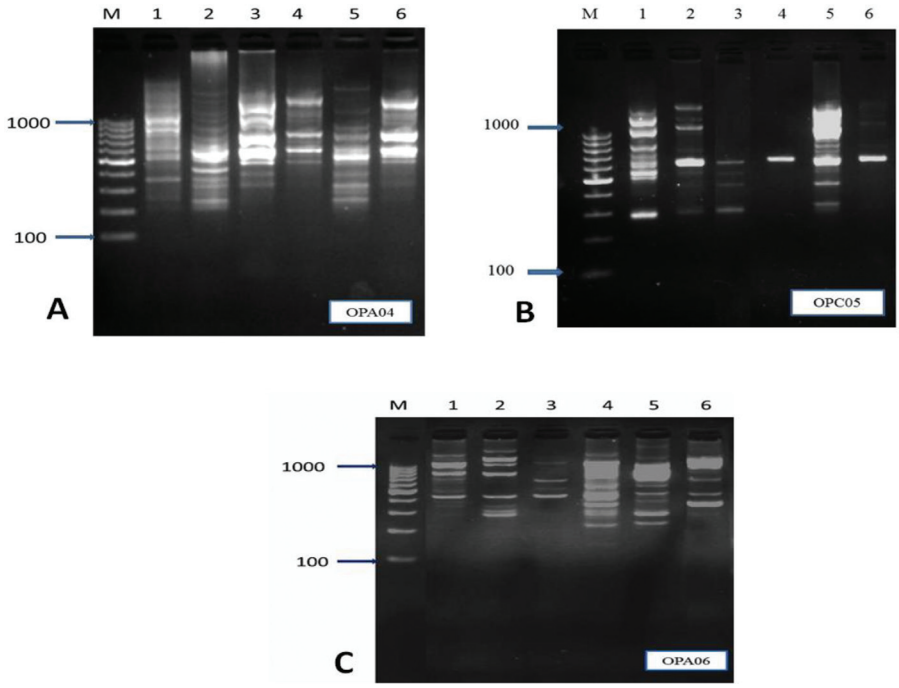
Table 2 Control DNA bands and total polymorphic bands of genomic DNA from random amplified polymorphic DNA-PCR of mice treated with *Saccharomyces cerevisiae* yeast as protective or a therapeutic agent against cadmium toxicity

Primers	Control	Cadmium		Protection 1		Protection 2		Total number of bands	Band size	Polymorphic bands
		A	B	A	B	A	B			
OPC05	9	3	5	1	6	1	6	24	1664–208	22
OPA04	10	4	4	–	3	2	4	45	2091–244	17
OPA06	9	3	4	3	4	3	–	28	1680–298	17
Total	28	10	13	4	13	6	10	97		56 (57.7%)
a+b		23		17		16				
Polymorphism (%)		82.1		60.7		57.1				

Primers	Control	Cadmium		Therapeutic 1		Therapeutic 2		Total number of bands	Band size	Polymorphic bands
		A	B	A	B	A	B			
OPC05	9	3	5	3	2	4	2	37	1664–219	19
OPA04	10	4	4	5	5	3	5	38	2091–244	26
OPA06	9	3	4	3	1	4	1	40	1707–298	16
Total	28	10	13	11	8	11	8	115		61 (53%)
a+b		23		19		19				
Polymorphism (%)		82.1		67.8		67.8				

A, express on gain or new appearance of bands; B, express on loss or disappearance of DNA bands.

Figure 2



Control DNA bands and total polymorphic bands were shown in the random amplified polymorphic DNA-PCR profile. These bands were generated by OPA04 (a), OPC05 (b), and OPA06 (c) of genomic DNA extracted from mouse liver. M, ladder DNA, 100 pb. Lane 1: control; lane 2, cadmium; lane 3, protection 1; lane 4, protection 2; lane 5, therapeutic 1; lane 6, therapeutic 2.

profiles. In protection treatment, the total number of amplified bands was 97. These bands were observed in mice fed a basal diet, a basal diet contaminated with Cd, and a basal diet contaminated with Cd and supplemented with low or high levels of *S. cerevisiae* yeast. The molecular sizes of these bands ranged from 1664 to 208 (for OPC05 primer), from 2091 to 244 (for OPA04 primer), and from 1680 to 298 (for OPC06 primer). The present results showed that 56 bands (57%) out of 97 were polymorphic. The polymorphic bands in the RAPD profile occurred as a result of the appearance (gain) or disappearance (loss) of the amplified bands with respect to those found in the control RAPD profile. The OPC04 primer recorded the highest value (45 bands) of amplified bands including polymorphic ones. The high degree of genetic polymorphism (82.1) was observed in the mouse group fed the basal diet contaminated with Cd.

In contrast, the rate of inducing the genetic polymorphism in genomic DNA was decreased to 60.7 or 57.1% in mice fed the basal diet contaminated with Cd and treated with low or high levels of *S. cerevisiae* yeast, respectively. The supplementation of Cd diet with high level of *S. cerevisiae* yeast caused more reduction in the degree of genetic polymorphism than did treatment with a low level of yeast.

In therapeutic treatment, the total number of amplified bands was 115. These bands were seen in mice fed the basal diet, the basal diet contaminated with Cd, and the basal diet supplemented with low or high levels of *S. cerevisiae* yeast after cessation of Cd treatment. The molecular sizes of amplified bands ranged from 1664 to 219 (for OPC05 primer), from 2091 to 244 (for OPA04 primer), and from 1707 to 298 (for OPA06 primer). The present findings revealed that 53 bands (61%) out of a total of 115 were polymorphic. The OPA06 gave the highest number (40 bands) of amplified bands including amplified polymorphic ones. The degree of genetic polymorphism in the Cd group was 81%; however, this genetic polymorphism decreased to 67.8% in mice fed the basal diet supplemented with low or high levels of *S. cerevisiae* yeast after cessation of Cd treatment. Thus, the present findings showed that using *S. cerevisiae* yeast as a protective agent gave the best results than when using it as a therapeutic agent against Cd toxicity, where the degrees of polymorphisms were lower in protection treatment (Cd+*S. cerevisiae*$_1$ at 5% level and Cd+*S. cerevisiae*$_2$ at 7.5% level) than in therapeutic treatments (Cd followed by *S. cerevisiae*$_1$ at 5% level and Cd followed by *S. cerevisiae*$_2$ at 7.5% level).

Micronucleus

The present results obtained in Table 3 revealed that feeding of a basal diet contaminated with Cd induced highly significant rates of micronuclei as compared with feeding of on a normal basal diet. However, the supplementation of *S. cerevisiae* yeast to the basal diet along (as a protective agent) or after cessation (as a therapeutic agent) of Cd treatment led to significant reduction in micronuclei compared with the Cd diet alone. The use of a high level of *S. cerevisiae* yeast was more effective for decreasing micronuclei than the use of a low level of yeast. Better results were observed by using *S. cerevisiae* yeast as a protective agent than by using *S. cerevisiae* yeast as a therapeutic agent, as the rate of inducing micronuclei in protection treatment was lower than that in therapeutic treatment.

Chromosomal aberrations

The results presented in Table 4 show that mice fed the basal diet contaminated with Cd had highly significant chromosomal aberrations in comparison with mice fed the normal basal diet. In contrast, chromosomal aberrations were significantly decreased in mice fed basal diets that were supplemented with *S. cerevisiae* yeast along with (as a protective agent) or after cessation (as a therapeutic agent) of Cd contamination as compared with those found in mice fed the Cd diet alone. Supplementation with a high level of *S. cerevisiae* yeast in the Cd diet resulted in the lowest rate of chromosomal aberrations compared with addition of a low level of yeast. Further, the frequencies of chromosomal aberrations were more minimized in mice fed a diet supplemented with *S. cerevisiae* yeast along with Cd treatment than those observed in mice fed a diet supplemented with *S. cerevisiae* yeast after cessation of Cd treatment.

Sperm abnormalities and count

The sperm examination in the present study revealed head and tail abnormalities. The sperm head abnormalities included amorphous, no hock, small head, big head, and banana types. The tail aberrations involved coiled and divided ones. The present findings found that sperm abnormalities were more frequent in mice fed the basal diet contaminated with Cd compared with those found in the control. Further, these results revealed that Cd treatment significantly decreased the sperm count as compared with that in control. The addition of *S. cerevisiae* yeast to the Cd diet as a protective or a therapeutic agent significantly decreased the sperm abnormalities and significantly increased the sperm count compared with feeding a basal diet contaminated with Cd alone. The use of a high level of *S. cerevisiae* yeast resulted in minimization of the sperm-shape abnormalities and improvement in the sperm count as compared with low level of yeast. In all cases, the supplementation of *S. cerevisiae* yeast as a protective agent gave much better results by reducing the sperm abnormalities and improving the sperm count

Table 3 The proportions of micronuclei in mice treated with *Saccharomyces cerevisiae* yeast as a protective or therapeutic agent against pollution with cadmium

Treatments	Micronucleus
Control	1.67±0.21^{e}
Cadmium	27.67±0.21^{a}
Protection 1	17.33±0.21^{c}
Protection 2	11.67±0.42^{d}
Therapeutic 1	20.67±0.21^{b}
Therapeutic 2	18.0±0.37^{c}

All data are expressed as mean±SE. ^{a,b,c,d,e}Mean with different letters were significantly different using analysis of variance test at $P<0.05$.

Table 4 Rates of chromosome aberrations in mice treated with *Saccharomyces cerevisiae* yeast as a protective or therapeutic agent against pollution with cadmium

Treatments	Structural chromosomal aberrations						Numerical aberrations		
	Gap	Break	Deletion	Fragment	Centromeric attenuation	Endomitosis	Total	Aneuploidy	Polyploidy	Total
Control	0.50±0.22^{d}	0.33±0.21^{d}	0^{d}	0^{d}	0.67±0.21^{d}	0^{d}	1.50±0.22^{f}	0.67±0.21^{e}	0.33±0.21^{d}	1.0±0.26^{e}
Cadmium	3.67±0.21^{a}	3.17±0.17^{a}	3.67±0.21^{a}	3.67±0.21^{a}	4.67±0.21^{a}	2.83±0.17^{a}	21.6±0.42^{a}	4.67±021^{a}	3.67±0.21^{a}	8.33±0.21^{a}
Protection 1	2.67±0.21^{b}	1.83±0.17^{c}	2.17±0.30^{b}	2.33±0.21^{b}	2.67±0.21^{b}	0.33±0.21^{c,d}	12.0±0.26^{c}	2.67±0.21^{c}	1.17±0.31^{c}	3.83±0.17^{c}
Protection 2	1.67±0.21^{c}	1.50±0.22^{c}	1.33±0.21^{c}	1.17±0.17^{c}	1.83±0.17^{c}	0^{d}	7.50±0.22^{e}	1.67±0.21^{d}	0^{d}	1.67±0.21^{d}
Therapeutic 1	2.83±0.17^{b}	2.50±0.22^{b}	2.33±0.21^{b}	2.17±0.30^{b}	2.83±0.17^{b}	1.67±0.21^{b}	14.33±0.33^{b}	3.33±0.21^{b}	2.33±0.21^{b}	5.67±0.21^{b}
Therapeutic 2	2.33±0.21^{b}	1.67±0.21^{c}	1.33±0.21^{c}	2.0±0^{b}	2.67±0.21^{b}	0.67±0.21^{c}	10.67±0.21^{d}	2.67±0.21^{c}	1.67±0.42^{b,c}	4.33±0.21^{c}

All data are expressed as mean±SE. $^{a,b,c,d,e\,f}$Mean with different letters in each column were significantly different using analysis of variance test at $P<0.05$.

compared with addition of *S. cerevisiae* yeast as a therapeutic agent (Table 5).

Discussion

In the present study, the rates of induced DNA damage significantly increased in mice fed a diet contaminated with Cd as compared with those found in controls. These observations were detected by means of the DNA comet assay and RAPD-PCR analysis. The results of the comet assay showed significant elevation of DNA tailed percentage, DNA tail length, tail DNA percentage, and tail moment and significant decrease in DNA untailed percentage. Our findings were similar to those reported by Karimi *et al.* [10], who, by using the comet assay, observed significant alterations in DNA (including DNA in the head and DNA in the tail) in mouse kidney cells exposed to Cd as compared with control mice. Also, in an in-vitro study, Skipper *et al.* [5] found by using the comet assay significant elevation of tail lengths of DNA comets in liver carcinoma cells of humans treated with Cd compared with those in controls.

The RAPD-PCR analysis revealed that treatment with Cd caused significant elevation of RAPD profile changes, where the rate of induced genetic polymorphisms (the appearance of new bands and absence or loss of existing bands) of DNA amplified bands was higher than that of controls. Also, similar results were observed in fish [42] and plants [41,43]. Mahrous *et al.* [42] reported higher genetic polymorphisms in the genomic DNA of the liver of Tilapia fish treated with Cd compared with those found in the control. Moreover, high modifications of genomic DNA were observed in each of viciafaba [41] and Arabidopsis plantlet shoots [43] that were treated with Cd as compared with the controls.

The obtained results of DNA comet assay showed distinct variation in the size of the nuclear DNA lesion induced by Cd treatment. This damage to DNA reveals the specific genotoxic effect of Cd on the nuclear DNA leading to increase in DNA fragmentation migration (comet tail) from the nucleus (comet head) [5,44,45]. The genotoxicity induced by Cd might be because this metal is metabolized inside liver cells, causing decreased capacity of antioxidant agents and generation of ROS that interact with DNA molecules resulting in oxidative DNA damage [44,46]. Also, the increase in ROS rates due to genotoxic stress leads to many types of DNA damages, such as breaks in single-stranded or double-stranded DNA, insertion or deletion of nitrogenous base pairs, alkylation or oxidation of

Table 5 Sperm shape abnormalities and sperm count in mice treated with *Saccharomyces cerevisiae* yeast as protective or therapeutic agent against pollution with cadmium

Treatments	Head abnormalities						Tail abnormalities			Sperm count
	Amorphous	No hock	Small	Big	Banana	Total	Coiled	Divided	Total	
Control	1.17±0.17^{f}	1.50±0.22^{f}	0^{c}	0^{b}	0.33±0.21^{c}	2.83±0.31^{f}	0^{b}	0^{b}	0^{c}	80.75±0.85^{a}
Cadmium	27.67±0.33^{a}	23.67±0.33^{a}	3.17±0.17^{a}	1.50±0.34^{a}	1.50±0.22^{a}	57.50±0.56^{a}	0.83±0.17^{a}	1.50±0.22^{a}	2.33±0.33^{a}	49.00±1.08^{e}
Protection 1	18.83±0.31^{c}	16.17±0.31^{c}	0.50±0.22^{b,c}	0^{b}	0.50±0.22^{b,c}	36.0±0.63^{c}	0.50±0.22^{a}	0.50±0.22^{b}	1.0±0.26^{b}	69.00±0.41^{b,c}
Protection 2	13.67±0.33^{e}	10.83±0.31^{e}	0^{c}	0^{b}	0^{b,c}	24.50±0.34^{e}	0^{b}	0^{b}	0^{c}	71.75±0.95^{b}
Therapeutic 1	20.83±0.31^{b}	17.50±0.43^{b}	0.67±0.21^{b}	0.50±0.22^{b}	0.67±0.21^{b}	40.17±0.60^{b}	0.67±0.21^{a}	0.50±0.22^{b}	1.17±0.31^{b}	63.25±1.37^{d}
Therapeutic 2	17.83±0.31^{d}	13.83±0.31^{d}	0.50±0.22^{b,c}	0.33±0.21^{b}	0.33±0.21^{b,c}	32.83±0.40^{d}	0^{b}	0^{b}	0^{c}	65.75±1.79^{c,d}

All data are expressed as mean±SE. ^{a,b,c,d,e,f}Mean with different letters in each column were significantly different using analysis of variance test at $P<0.05$.

nitrogenous base pairs, and inhibition of mismatch repair of DNA adduct [5,41,43]. Moreover, the presence of DNA damage in the comet assay might be due to inducing forks of DNA replication during the S-phase under stress of genotoxicity of toxicants leading to high loss of DNA molecules (as comet tails) [47].

The RAPD profile of Cd-treated mice showed characteristic changes in the banding patterns of DNA inducing high rates of genetic polymorphism as compared with control. These genetic DNA polymorphisms might be caused by DNA damage due to Cd genotoxicity especially during DNA replication or during the process of gene expression [48,49]. Thus, the gain (or new appearance) or loss (or disappearance) of the RAPD profile bands of DNA by Cd treatment might be related to changes in sequences, such as inducing point mutation, deletion or addition (insertion) of nitrogenous base sequences, or complex genomic rearrangements that influence the sites of primer binding [43,50,51]. The gain or appearance of new RAPD profile bands might be related to structural changes in the DNA, such as large addition (insertion) of nitrogenous base sequences or transpositions or point mutation that makes available some new sites of nucleotide sequences to bind or interact with primers [43,50]. In contrast, the loss or disappearance of some DNA bands of RAPD profile might be due to DNA polymerase interaction with changes in nucleotide sequences (DNA damage), nucleotide sequence alterations at the sites of primer binding, single-strand and double-strand breaks of DNA, and complex genomic rearrangements [41,43]. Therefore, this study proves that gain or disappearance of DNA bands of the RAPD profile is considered a good biomarker for identification of genotoxicity caused by contamination with Cd.

The obtained results showed that treatment with Cd induced significant increases in the frequencies of cytogenetic changes that included micronuclei and chromosomal aberrations as compared with controls. Similarly, Fahmy and colleagues [52–54] reported significant elevations in induced micronuclei and chromosomal aberrations in murine bone marrow cells exposed to Cd as compared with those found in the control. Also, Mahrous *et al.* [42] observed highly significant frequencies of micronuclei and different types of chromosomal aberrations in *Nile tilapia* fish treated with Cd compared with controls. Moreover, Singh and Sankhla [11] revealed significant reduction in mitotic activity and significant elevation in chromosomal aberrations in bone marrow cells of

mice administered Cd in comparison with controls. El-Refaiy and Eissa [28] found marked chromosomal aberrations with highly significant frequencies in rats that received Cd, when compared with the untreated group. The high frequencies of cytogenetic changes that were observed by Cd treatment in the present study might be due to incorporation of Cd ions into the DNA structure causing random DNA fragmentations or DNA strand breaks that lead to the formation of micronuclei or induce chromosomal aberrations [55]. Several studies reported that Cd ions have strong toxic properties and affect the formation of mitotic spindle fibers, causing anomalies in mitotic division and inducing high rates of micronuclei and different kinds of chromosomal aberrations [11,56].

Moreover, the exposure to various kinds of toxicants, including Cd, causes generation of ROS that interact with different biomolecule cellular components, especially nucleic acids, lipids, and proteins, leading to disturbance of homeostasis, oxidative stress, and consequently cytogenetic changes such as reducted mitotic [28] activity, increased micronuclei formation, and increased chromosomal aberrations. Furthermore, Cd ions were found to have toxic effect on the enzymes responsible for DNA repair, leading to DNA damage and resulting in micronuclei and chromosomal aberration [28,57]. Thus, the Cd genotoxicity in the present work might be related to the incorporation of Cd ions into DNA or due to the toxic effect of such ions on mitotic spindle and enzymes responsible for DNA repair, or due to the formation of ROS that cause DNA lesions leading to increased constitution of micronuclei and chromosomal aberrations.

Concerning the sperm examination in the present study, the results clarified that Cd treatment caused significant increase in sperm-shape abnormalities and significant decrease in viable epididymides sperm count. These findings were in concordance with those of previous studies by Oliveira *et al.* [2], Kaur and Sharma [4], and Metwally and Hashem [58], who observed significant increase in sperm-shape abnormalities and significant decrease in sperm count in mice exposed to Cd as compared with those found in the control groups. Dietrich *et al.* [59] and Annabi *et al.* [60] revealed that treatment with Cd could affect calcium ions (which were found to be a necessary element for sperm activities), leading to sperm abnormalities. Hew *et al.* [61] and Dietrich *et al.* [62] reported that Cd ions can cross the blood–testes barrier, inducing suppression of spermatogenesis; further, these ions can bend with sperm enzymes, affecting sperm cell metabolism and leading to sperm deformation. Moreover, Hew *et al.* [61] detected that Cd ions could alter the adhesion of sertoli cells by disrupting cell junctions and subsequently cause degeneration of seminiferous tubules and lead to a reduction of epididymides sperm count. On the other hand, Acharya *et al.* [63] clarified that ROS generated by genotoxicity of Cd can peroxide the fatty acids in spermatozoa, causing lipid peroxidation and deleterious effects on cell membrane phosphatides as well as inducing sperm DNA oxidation and consequently sperm-shape abnormalities and decrease in viable sperm count.

The present findings identified that the use of *S. cerevisiae* yeast as a protective or a therapeutic agent could suppress the genotoxic and spermatotoxic effects caused by Cd in mouse tissues. The inducing of tail lengths of DNA comets, genetic DNA polymorphism of the RAPD-PCR profile, rate of micronuclei, frequencies of chromosomal aberrations, and proportion of sperm-shape abnormalities were significantly minimized as well as the sperm count significantly ameliorated in *S. cerevisiae* groups (Cd +*S. cerevisiae* or Cd then *S. cerevisiae*) as compared with the Cd group alone. Better results were obtained on using *S. cerevisiae* as a protective agent. Thus, our results revealed that the use of *S. cerevisiae* as a natural product might have protective effects on genetic materials and sperm parameters against Cd genotoxicity. Supporting this, Darwish *et al.* [20] and Abdel-Aziz *et al.* [64] observed that the supplementation of *S. cerevisiae* to mouse diets contaminated with mycotoxin [AF or Ochratoxin (OTA)] significantly decreased the chromosomal aberrations (in somatic and germ cells), micronuclei, and sperm-shape abnormalities as well as significantly increased mitotic and meiotic activities and sperm count in comparison with mycotoxin (AF of OTA) diet alone. Eshak *et al.* [21] found that the addition of *S. cerevisiae* yeast to quail diet contaminated with AFB1 (aflatoxins B1) significantly enhanced growth performances and expression level of neural and gonadal genes compared with AFB1 diet alone. Oliveira *et al.* [65] demonstrated that the utilization of β-glucan (which is a major constituent in the cell wall polysaccharide of *S. cerevisiae* yeast) in the culture of Chinese hamster ovary cell lines exposed to methylmethanesulfonate led to significant decreases in DNA damage (comet assay) and rate of micronuclei formation compared with methylmethanesulfonate culture alone. Also, Oliveira *et al.* [65] found that β-glucan (isolated from *S. cerevisiae* yeast) reduced the DNA damage (comet assay) and micronuclei caused by cyclophosphamide in mice. In a previous

study, Silva *et al*. [25] observed that β-glucan had an anticlastogenic effect on cultured cells that were exposed to ultraviolet radiation.

In human patients with advanced prostate cancer, treatment with β-glucan was found to be a strong protective agent against the development of DNA lesions [66]. Moreover, polysaccharides of *S. cerevisiae* yeast were found to be a potent bioprotective factor in cucumber, tobacco, and bean plants against infection from viruses of necrosis and tobacco mosaic [67,68]. Therefore, *S. cerevisiae* yeast is considered a strong antigenotoxic agent. This property might be related to yeast polysaccharides that include β-d glucans, glucomannans, and mannanoligo – saccharide [69], or some constituents that were found in *S. cerevisiae* cells, such as vitamins, carotenoids, essential amino acids, and minerals [18,29,70,71]. These components were revealed to have the potent antioxidant activities that suppress the reaction of free radicals of the oxidative process or disable and scavenge ROS species, causing reduction of DNA oxidation and consequently minimization or prevention of genotoxicity [65,72]. Furthermore, Tsa1 (CTPX1), which is a major constituent of peroxiredoxins in *S. cerevisiae* yeast, was observed to possess a strong ability to scavenge free radicals, leading to avoidance of several mutation processes and causing stability of genomic DNA [73]. In contrast, Abousadi *et al*. [18] and Parlat *et al*. [29] reported that the cells of *S. cerevisiae* yeast can produce some biological enzymes that have the ability to detoxify by interacting with toxicant molecules and subsequently increasing the efficiency of gut microflora and enhancing the bioavailability of nutrients during the digestion process in the intestine, leading to prevention or minimization of genotoxicity. Eshak *et al*. [21] demonstrated that the action of *S. cerevisiae* yeast (or its components) may be related to its suppression of the activation of cytochrome enzymes, resulting in improvement in liver and kidney functions and leading to mitigation of genetic abnormalities.

Peteri *et al*. [74] observed that *S. cerevisiae* yeast or its components had the ability to biodegrade carcinogens such as OTA by cleaving the amide bond, releasing OTA, and leading to adsorption of such toxicants into viable or nonviable yeast cells. Also, Rahaie *et al*. [26] reported that because of the physical properties of *S. cerevisiae* yeast cells, these cells can bind and adsorb with high levels of carcinogens such as AFB1; the rate of such binding reached 70–95% (w/w) in animal diets.

In conclusion, this work adds evidence that *S. cerevisiae* yeast is a strong probiotic that can attenuate the toxic effects of Cd contamination in food. The addition of this probiotic to animal diets significantly minimized the deleterious effects induced by Cd on the genetic and sperm parameters of mice.

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Conflicts of interest
There are no conflict of interest.

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