Ameliorative Effect of *Saccharomyces cerevisiae* on Aflatoxin-Induced Genotoxicity and Spermatotoxicity in Male Albino Mice

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Abstract: Aflatoxins (AF) are an unavoidable food contaminant and produce a disease state known as aflatoxicosis. and they have carcinogenic, mutagenic, hepatotoxic and teratogenic effects (Davis and Diener, 1978) and disruption of spermatogenesis is one of its serious consequences. Reducing its toxicity *in vivo* is of major interest. In this study we assessed the potential protective effects of *Saccharomyces cerevisiae* (Sc) against AF in male Albino mice. Four experimental groups were used, each comprising 30 mice; control group, Sc-treated group (4 × 108 CFU), AF-treated group (0.7 mg/kg b.w.), and a group given Sc two hours before AF intoxication. Chromosome aberrations in bone marrow and spermatocytes were recorded; as well as mitotic and meiotic activities. Also, sperm parameters were evaluated. The results revealed that aflatoxin administration increased statistically the frequencies of structural and numerical chromosome aberrations in bone marrow and in spermatocytes. In addition, mitotic and meiotic activities of somatic and germ cells were declined significantly. Also, AF caused a high significant reduction in cauda epididymal sperm count, sperm motility and increased sperm abnormalities, as compared to control. Cytogenetic analyses revealed that Sc administration before AF gavage significantly reduced frequencies of chromosome aberrations in bone marrow and spermatocytes, also recovered mitotic and meiotic activities as well. Moreover, gavage Sc before AF intoxication caused significant recovery in all sperm parameters studied. In conclusion, Sc was found to be safe and successful agent counteracting the genotoxicity induced by AF, in addition to reduction in spermatotoxic alterations.

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1. Introduction:

Aflatoxins are well-known mycotoxins produced by different strains of *Aspergillus flavus* and *Aspergillus parasiticum*. In humans and various animal species it has been reported as a potent hepatotoxic, mutagenic, genotoxic, and hepatocarcinogenic agent (Massey et al., 1995; McLean and Dutton, 1995). Chronic exposure to low levels of aflatoxins is one of the major risk factors in the etiology of human hepatocellular carcinoma in several regions of the world (Preston and Williams, 2005). AFB1 is the most potent of the known AFs, and is a classified within class 1 of human carcinogens (IARC, 1993). Although the liver is clearly the principal target organ for AFB_1, kidney and testis can also be a target following dietary and inhalational exposure. Also, AFs have been detected in boar sperm and the human semen (Picha et al. 1986; Ibeh et al. 2000). AFB_1 is activated by cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB1-8,9-epoxide, which subsequently binds to nucleophilic sites in DNA forming 8,9-dihydro-8-(N7guanyl)-9-hydroxy-AFB_1 adduct, which is regarded as a critical step in the initiation of AFB_1-induced carcinogenesis (Sharma and Farmer, 2004; Preston and Williams, 2005). In addition, the AFB_1-associated mutagenesis was suggested to represent a plausible cause for the higher chromosome instability observed in Chinese Hepatocellular Carcinomas, when compared with European primary liver carcinomas (Pineaua et al., 2008). Several reports suggested that toxicity might ensue through the generation of intracellular reactive oxygen species (ROS), which may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functionality and cytotoxicity (Towner et al., 2003; Berg et al., 2004). Considering the multiple adverse effects of aflatoxin exposure, the reduction or preventing its toxic events is of major interest. Intervention approaches at the individual level to eliminate, inactivate or reduce the bioavailability of this toxin in contaminated products could be promising in preventing the effect of food-related mutagens and a potential measure for reducing cancer risk. Nowadays, there is considerable interest in the potential antigenotoxic and

anticarcinogenic effects associated with probiotics. *Saccharomyces cerevisiae* (Sc), in particular, has proven to benefit health in several ways including stimulation of the growth of intestinal microflore in mammals, pH modulation in ruminants, as well as reduction in the number of pathogenic microorganisms in monogastric animals (Dawson, 1993; Wallace, 1994). Moreover, yeasts have been reported to have high adsorption ability against mycotoxins in aqueous solution (Baptista et al., 2004; Bueno et al., 2006; Emam-Djomeh et al., 2009). In addition, a study in mouse revealed that a component of the Sc cell wall (glucan) reduced the frequency of micronuclei (MN) induced by cyclophosphamide (Chovatovicova and Mavarova, 1992). The aim of the present study was to evaluate the potential *in vivo* antigenotoxic and anti-spermatotoxic effects of Sc against the well-known mycotoxin AF in male Albino mice.

2. **Materials and Methods**

Chemicals, reagents, and reagent kits, used in the present study were purchased from Riedel-de Haën, Germany and Biodiagnostic, Cairo, Egypt. Crude aflatoxins B1, B2, G1 and G2 were obtained as a crude mycotoxin from Food Toxicology and Contaminants Dept., National Research Center, Egypt.

Experimental Animals:

Male Swiss Albino mice (*Mus musculus*) three months old weighing 25-30 grams were obtained from the animal house colony, National Research Center, Giza, Egypt. The animals were maintained on standard casein diet and water *ad libitum* and housed individually in a temperature-controlled and artificially illuminated room free from any source of chemical contamination.

Preparation of *Saccharomyces cerevisiae* cultures

Yeast (*S. cerevisiae*) were prepared by inoculating 100 ml of malt extract (ME) broth medium (Oxoid), in 1.0 liter of distilled water with 1 gm of cells powder. After incubation at 30 ℃ for 24 h, the number of yeast cells was enumerated by serial dilution in peptone-water (0.1 % w/v) and plate counts on MEA, according to the procedure of Petersson and Schnurer (1995). S. cerevisiae suspension was prepared by inoculation of 10 ml of Malt Extract broth (ME) medium with 1gm of fresh comprised yeast and incubated at 25 ℃ for 24 hr, and then stored at 4 ℃ until used.

Experimental design

Mice were randomly divided into four groups each consisting of 30 mice, each group was divided into three subgroups (10 mice for each). Animals were treated orally for successive 7 days as follows: (1) untreated control given corn oil and MRS broth daily, (2) treated with AFs (0.7 mg/kg b.w.) in 0.4ml corn oil, (3) treated with LGG (1×10^{10} CFU) in MRS broth and (4) treated with the LGG (1×10^{10} CFU) 2 hours before AFs gavage (0.7 mg/kg b.w.). On the 8th day of the study, the 1st subgroup was killed and femoral bones were removed, stripped and cleaned from extraneous tissues. Also, liver and kidney samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible, and then stored immediately at -80 ^{o} C until analysis. On the 15th day of the study, the 2nd subgroup was killed and both testes removed and washed in warm citrate saline. At the end of the experiment (35th day), cauda epididymis, of the 3rd subgroup, were quickly isolated, blotted free of blood and utilized for the analysis of various reproductive parameters.

Mitotic Metaphase Chromosome Preparation from Bone Marrow

Metaphases for analysis of chromosome aberration in bone marrow cells were prepared according to the method of Perston et al. (1987). At least 50 Metaphases were scored per animal. For structural aberration analysis, gaps, breaks, fragments, deletions, dicentrics, end to end, complex rearrangements, endoreduplication, and pulverizations were considered. For numerical aberration analysis, aneuploidy, polyploidy and premature centromere division were considered. For Meiotic activity of cells, the number of dividing cells were recorded and the mitotic index was calculated as the following formula: Mitotic index % (M.I.) = the number of dividing cells/Total number of bone marrow cells counted/ per 1000 cells.

Chromosome Preparation from Spermatocytes

Metaphases for analysis of chromosome aberrations in spermatocytes were prepared according to the method of Evans et al. (1964) and recommendations by Russo (2000) were considered. Structural aberrations analysis was studied in metaphase I (MI): MI with only 20 bivalents was scored; the presence of univalents, chromosome breaks, fragments and chain or ring multi-valents, which are classified as reciprocal translocations were considered. For aneuploidy assay, metaphase II (MII) was studied: MII with 18<n>22 chromosomes were recorded and polyploidy was considered as 2n, 3n or 4n. Fifty metaphase spreads were analyzed per animal. For Meiotic activity of cells, meiotic index was calculated as the frequency of MII/MI, normal ratio should be equal 2.

Sperm parameters:

Sperm parameters were prepared and analyzed according to the protocols of Wyrobek and Bruce (1975): Epididymal sperm counts and evaluation of the motility were performed visually using counting chamber. The count was repeated three times for each sample to minimize error, and calculated as 10^6 per sperm dilution. Sperm motility was determined by counting both motile and non-motile sperms in at least 16 separate and randomly selected fields. These results were expressed as percent motility. For evaluation of sperm morphology; a drop of sperm suspension was smeared onto a slide, left to dry; then stained with Eosin A, the slides were washed in water and air dried again. The smears were microscopically analyzed at a magnification of ×1000 for observation of abnormalities.

Statistical analysis

Statistical analyses were performed by one-way ANOVA followed by Tuckey's test or by Two-way ANOVA followed by Bonferroni's test comparing all groups. Analysis was conducted with GraphPad Prism software V.5.0.3 (Inc., San Diego, CA; USA).

3. Results:

Effects of Sc on AFs genotoxicity in bone marrow cells:

Table (1) presents data of different types of chromosomal aberrations induced by AFs and different treatments in bone marrow cells of male mice. Results showed that the oral administration of AFs induced both structural and numerical chromosomal aberrations. Structural chromosomal aberrations recorded were chromatid breaks, chromatid gaps, deletions and fragments. The results showed a high increase in frequencies in structural aberrations, which exhibited a high significance as compared to control group at P<0.001. Chromatid breaks, gaps and deletions showed a high statistical significant increase at P<0.001, whereas, accentric fragments were only statistically significant at P<0.05 when compared with control. Moreover, AFs induced very high incidence in numerical chromosome aberrations, which were statistically significant at P<0.001. Numerical aberrations were recorded as PCD, periploidy and polyploidy. Treatment with Sc before AFs-intoxication significantly decreased the frequencies of structural chromosome aberrations (2.4 folds); this recovery was significant in comparison to the AFs group at P<0.001. Regarding numerical aberration, the frequency of PCD and polyploidy showed highly significant recovery when compared to the AFs group (P<0.001) and total numerical aberrations at P<0.01, but this recovery was still below the values of control and Sc groups. PCD, polyploidy and the total numerical aberrations were significant at P<0.001 and periploidy was significant at P<0.05 when comparing Sc plus AFs group with the control. Treatment with Sc alone showed insignificant differences in chromosome aberrations in bone marrow compared to the control group at P>0.05.

The mitotic index (Table 1) revealed a significant mitotic delay (53.2 %) in mice treated with AFs with respect to control group (P< 0.01). On the other hand, Sc gavage before AFs intoxication recovered the mitotic activity to 79 %, this recovery was statistically significant (P< 0.01) when compared to the AFs intoxicated group from one side and it was statistically below that of control at P<0.01 from the other side. In Sc group, mitotic activity showed insignificant enhancements at P<0.01 when compared with control group at P>0.05.

Effects of Sc on AFs genotoxicity in germ cells (spermatocytes MI, MII)

Table (2) presents data of chromosomal abnormalities induced by different treatments in mice spermatocytes. X-Y and autosomal univalents were recorded as structural chromosome aberration in metaphase I while numerical abnormalities were recorded in metaphase II (MII) as periploidy (n ± 1, 2) and polyploidy. Data clearly showed that AFs-intoxication induced very high significant increase in X-Y, autosomal and total structural abnormalities compared to control at P<0.001. In contrast, *S. cerevisiae*-treated group showed no significant differences when compared with control group at P>0.05. On the other hand, in mice given Sc before AFs-intoxication, XY univalents showed a significant reduction compared to control at P<0.01, while autosomal univalents and total structural aberrations decreased significantly compared to the AFs-treated animals at P<0.001. However, structural aberrations recovery was still above the values of control and Sc groups; autosomal univalents were statistically significant at P<0.01 and the total structural aberrations were significant at P<0.001, whereas no significant differences were found between this group and the control group for XY univalents at P>0.05.

Furthermore, AFs increased the frequencies of periploidy, polyploidy and the total numerical aberrations which were significant (P<0.001) compared to all other groups. Whereas, Sc treated group showed no significant differences in numerical aberrations in respect to the control at P>0.05. Sc gavage before AFs-intoxication caused a significant reduction in frequencies of numerical aberrations as compared to the AFs-treated group (P<0.001). In this group, polyploidy was insignificant compared to control, whereas periploidy was higher significantly

respect to control at P<0.05. Moreover the total numerical aberrations were significantly higher when compared to the control at P<0.001.

The meiotic index (Table 2) revealed a significant meiotic delay in mice treated with AFs with respect to all other groups (P< 0.001). In Sc group, meiotic activity showed a significant enhancement when compared with the control group at P<0.05. Sc gavage before AFs treatment recovered meiotic activity to the baseline of control which was statistically significant at P<0.01 when compared to AFs group; meanwhile it showed no significant differences when compared with control group at P>0.05.

Spermatological examinations:
Sperm count and motility

Table (3) presented the data of sperm concentration, motility and morphology of different treatments. AFs treatment caused a highly significant decrease in sperm concentration (13.9×10^6) at P<0.01. Whereas, Sc gavage elevated the sperm concentration; this increase was statistically insignificant at P>0.05 compared to control. On the other hand administration of Sc before AFs-intoxication caused a significant increase in sperm count respect to the AFs-treated group (P<0.01); this enhancement showed significant differences when compared to the *Sc*and control groups at P<0.01.

Sperm Morphology:

AFs induced a dramatic increase in sperm abnormalities (80.6 %) which was significant in comparing with control at P<0.001 (Table 4.19). Sperm abnormalities observed were head, mid-piece and tail abnormalities as well as detached heads, sticky flagella (agglutination) and retained cytoplasmic droplets were existed. Head abnormalities were head without hook, unusual head shapes and big head (Plate 1 B, G). The mid-piece abnormalities consisted of hair-pin, folded, and disrupted neck (Plate 1 H-J). The tail abnormalities essentially consisted of angular and bi- or coiled tail (Plate 1 K-M). In AFs-treated mice, 20.4 % of sperm head was detached from the flagellum, which was significant compared to control at P<0.001. In addition, AFs caused a fairly high percentage of sperm (18.8 %) that had sticky flagellum (Plate 1M), it was statistically significant at P<0.001.The retention of cytoplasmic droplet (CD) by the cauda epididymal sperm of control as well as AFs-treated mice was observed (Plate1 N). The retention of CD by the cauda epididymal sperm was 8.4% in control mice whereas it was 42.2% in the AFs-treated mice, this difference was statistically highly significant at P<0.001.

In mice receiving Sc before AFs-intoxication, different sperm abnormalities significantly reduced (39.0%) in comparing with AFs-treated group; however, this enhancement showed significant differences with respect with either control or Sc groups at P<0.001. Head abnormalities showed a significant reduction at P<0.001, with respect to AFs group. Also, mid-piece abnormalities, decapicitation and agglutination decreased significantly when compared with AFs group at P<0.001. Meanwhile, the decrease in tail abnormalities showed no significant change when compared with control group at P>0.05. Similarly, the retention of CD, in Sc plus AFs group, showed a significant reduction (19.2%) when compared with AFs-treated group (P<0.001), but it was still higher than that of control and Sc groups and statistical differences were shown at P< 0.001. On the other hand, mice received Sc alone showed no significant changes in all types of sperm morphology (8.40%) with respect to control at P>0.05. Also, CD retention showed insignificant reduction (6.80%) when compared with the control group (P>0.05).

4. Discussion:

The present results showed clearly that AF is genotoxic in bone marrow and spermatocyte cells and had cytotoxic effects in both cell types. Moreover, AF affected the DNA synthesis and chromosome segregation and progression through cell division. AF genotoxicity was revealed by induction of structural chromosome aberrations (total structure abnormalities ~11%) and numerical (total numerical abnormalities ~17%) in somatic cells and (12% for structure and 13% for numerical aberrations) in germ cells. In addition, AFs reduced the meiotic and mitotic activities. These findings coincide with previous reports; El-Arab et al. (2006) reported that AFs (B1, B2, G1 and G2) induced structural and numerical chromosomal aberrations in bone marrow and germ cells of male mice. AFB_1 has induced different chromosomal abnormalities in bone marrow cells and spermatocytes and shown to reduce the meiotic and mitotic activities of male Swiss albino mice (Hassanane et al., 2009a, and b). In ealier study, the effect of oral consumption of 200 ppb of crude AFs showed testicular degeneration and a decrease in the meiotic index (Sahay, 1993). Aneuploidogenic ability of AFB_1 was reported and it appeared to affect assembly of tubulin into microtubules and/or bring about tubulin deplymerization and would result in generation of meiotic micronucleate giant spermatocytes in Swiss mice (Faisal et al., 2008), which may explain the high percentage of premature centromere division and aneuploidy found in this work. AFB_1 genotoxicity might be caused through

Table 1: Effect of Sc on different types of chromosomal aberrations and mitotic activity induced by AFs in bone marrow cells of male mice

Experimental Groups	Structural aberrations					Numerical aberrations				% of Mitotic Activity Change
	Breaks	Gaps	Deletions	Fragments	Total structural	PCD	Peri-ploidy	Poly-ploidy	Total numerical	
Control (H_2O / corn oil)	0.20 ± 0.22^{A}	0.60 ± 0.25^{A}	0.40 ± 0.25^{A}	0.40 ± 0.25^{A}	1.60 ± 0.40^{A}	1.20 ± 0.37^{A}	0.40 ± 0.25^{A}	0.80 ± 0.37^{A}	2.40 ± 0.68^{A}	100.0 ± 0.00^{A}
AFs (0.7 mg/kg b.w.)	3.60 ± 0.25^{B}	3.80 ± 0.37^{B}	2.00 ± 0.32^{CB*}	2.00 ± 0.32^{A*}	11.2 ± 0.60^{C}	7.8 ± 0.37^{C}	4.20 ± 0.37^{B}	4.80 ± 0.25^{C}	16.8 ± 0.68^{C}	53.2 ± 2.82^{C}
S. c. (4×10^8)	0.40 ± 0.25^{A}	0.40 ± 0.25^{A}	0.20 ± 0.20^{A}	0.40 ± 0.25^{A}	1.00 ± 0.45^{A}	1.40 ± 0.25^{A}	0.40 ± 0.25^{A}	1.20 ± 0.37^{AB**}	3.00 ± 0.45^{A}	108.0 ± 2.86^{A}
Sc plus AFs	1.40 ± 0.25^{A}	1.60 ± 0.25^{A}	1.00 ± 0.32^{A}	0.60 ± 0.25^{A}	4.60 ± 0.40^{B}	3.40 ± 0.25^{B}	1.80 ± 0.20^{A*}	3.00 ± 0.32^{B}	8.20 ± 0.37^{BC**}	79.0 ± 2.51^{B}

- Means with different superscript letters (a, b, c) are significantly different ($P < 0.001$). - Means with two stars are significantly different ($P < 0.01$).
- Means with a star are significantly different ($P < 0.05$). - All data are expressed as means ± SEM.

Table 2: Effect of Sc on chromosomal aberrations and meiotic index in spermatocytes induced by AFs in male mice

Experimental Groups	Structural aberrations (MI)			Numerical aberrations (MII)			Meiotic Index (MII/MI)
	X-Y univalents	Autosomal univalents	Total	Periploidy	Polyploidy	Total	
Control (H_2O / corn oil)	1.2 ±0.37^{A}	1.0 ±0.32^{AB}	2.20 ±0.20^{A}	0.60 ±0.25^{A}	1.60 ±0.25^{A}	2.20 ±0.37^{A}	1.92 ±0.03^{AB}
AFs (0.7 mg/kg b.w.)	4.40 ±0.25^{C**}	7.40 ±0.51^{C}	11.8 ±0.37^{C}	5.20 ±0.58^{B}	7.80 ±0.37^{C}	12.8 ±0.80^{C}	1.37 ±0.04^{C}
S. cerevisiae (4×10^8)	1.00 ±0.32^{AB}	0.60 ±0.25^{A}	1.60 ±0.25^{A}	0.60 ±0.25^{A}	1.20 ±0.20^{AB}	1.80 ±0.20^{A}	2.07 ±0.04^{B*}
Sc plus AFs	2.4 ±0.25^{ACB*}	3.0 ±0.32^{B**}	5.40 ±0.40^{B}	2.00 ±0.32^{A*}	2.60 ±0.40^{AB*}	4.60 ±0.40^{B}	1.88 ±0.02^{A}

- Means with different superscript letters (a, b, c) are significantly different ($P < 0.001$). - Means with two stars are significantly different ($P < 0.01$).
- Means with a star are significantly different ($P < 0.05$). - All data are expressed as means ± SEM.

Table 3: Effect of Sc on AFs-induced change in sperm parameters in male mice

Experimental Groups	Sperm Count ($\times 0^6$)	% Sperm Motility	% Sperm Morphology						
			Head Abnormality	Mid-piece Abnormality	Tail Abnormality	Decapitation	Agglutination	Total Abnormality	C.D.
Control (H_2O / corn oil)	22.3 ±0.78^{A}	81.0 ±1.87^{A}	2.40 ±0.51^{A}	2.40 ±0.25^{A}	2.00 ±0.32^{A}	2.60 ±0.25^{A}	0.40 ±0.25^{A}	9.80 ±0.37^{A}	8.40 ±0.51^{A}
AFs (0.7 mg/kg b.w.)	13.9 ±0.72^{C}	36.4 ±2.50^{C}	13.8 ±1.10^{C}	19.2 ±1.02^{C}	8.40 ±0.93^{B}	20.4 ±0.81^{C}	18.8 ±0.58^{C}	80.6 ±1.36^{C}	42.2 ±1.43^{C}
S. cerevisiae (4×10^8)	25.1 ±0.83^{A}	84.8 ±1.66^{A}	1.60 ±0.25^{A}	1.80 ±0.37^{A}	1.60 ±0.25^{A}	2.60 ±0.25^{A}	0.40 ±0.25^{A}	8..40 ±0.51^{A}	6.80 ±0.37^{A}
Sc plus AFs	18.3 ± 0.50^{B}	65.2 ±1.98^{B}	7.40 ±0.51^{B}	11.0 ±0.71^{B}	4.00 ±0.45^{A}	10.8 ±0.74^{B}	6.40 ±0.25^{B}	39.6 ±1.29^{B}	19.20 ±0.58^{B}

- Means with different superscript letters (A, B & C) are significantly different ($P < 0.001$).
- All data are expressed as means ± SEM.

the formation of AFB_1- DNA adducts, which is regarded as a critical step in the initiation of AFB_1-induced hepatocarcinogenesis (Sharma and Farmer, 2004; Preston and Williams, 2005). Moreover, several reports suggest that oxidative stress is considered to be related to cell injury and DNA damage induced by AFB_1 through the generation of intracellular reactive oxygen species (ROS) (Towner et al., 2003; Theumera et al., 2010). The obtained results showed that the pretreatment with Sc reduced significantly the genotoxicity of AF in both cell types, somatic and germ cells by around two folds and restored the mitotic and meiotic activities. These data are consistent with other experimental studies have evidenced the ability of Sc to reduce oxidative damage to DNA as a consequence of scavenging of both *OH radicals and singlet oxygen induced by H2O2 and visible light-excited Methylene Blue in V79 hamster lung cells (Slamenõvá et al., 2003), reducing MNNE produced by AFB1 in mice (Madrigal-Santillán et al., 2006) as well as the capacity of yeast α-Mannan to protect against the DNA damage induced by AFB1 in mouse hepatocytes (Madrigal-Santillán et al., 2009). Regarding the reproductive toxicity, the present study clearly indicated that oral administration of the mycotoxin caused adverse effects on male reproductive parameters in mice (Tables 3). These findings clearly indicate to severe impact of the mycotoxins on spermatogenesis and/ or spermiogenesis; and it is a clear reflection of a direct or indirect toxic manifestation of this mycotoxin treatment in the spermatogenic compartment. Various authors have reported similar kind of observations in different animals emphasizing AFs as reproductive toxicants (Kumari and Sinha, 1994); it induced a decrease in spermatogenic numbers in mice (Bose and Sinha, 1994), decreased motility and longevity of breeding boar semen (Solti et al., 1999). The toxin was found to impair spermatogenesis and cause accumulation of premeiotic germinal cells (Fenske and Fink-Gremmels, 1990). ROS peroxidized fatty acids producing metabolites that could damage phosphatides of cell membrane; consequently, damage the sperm morphology and might impair sperm motility (Alvarez et al., 1987; Saradha et al., 2006; Hsieh et al., 2006). Consequently, the decline in sperm motility might be due to mitochondrial disruption and/or oxidative stress, where a fairly percent of mid-piece disruption was found, in addition to the deformation of the flagellum. These findings confirm the previous data correlating the decrease in human sperm motility to mitochondrial disruption and/ or an increase in lipid peroxidation (Lodish et al., 2003).

Our data substantiated these claims where midpiece and tail of AF-treated mice showed a very high percentage of malformation (hair-pin, disruption, folding, tail angulation, tail coiling), which might caused by peroxidation of cell components and disrupted the cytoskeletal proteins. The sticky flagellum observed in this study might be formed by fusing of two or more spermatozoa, where two or more axonemes are in a common cytoplasm (Agnes and Akbarsha, 2003). These data reflected the aberrant spermatogenesis and/ or spermiogenesis caused by AF treatment. Moreover, the results of the present study showed that AF intoxication rendered a significantly higher percentage of the cauda epididymal sperm to retain cytoplasmic droplets (CD) than in the control mice. These spermatozoa carrying cytoplasmic droplets are thought to be immature and functionally defective (Huszar et al., 1997). On the other hand, pretreatment with Sc significantly mitigates the mycotoxin-induced alterations in reproductive parameters in mice, where a significant improvement in the sperm motility and raise in the sperm number; along with reducing sperm abnormalities were shown. Moreover, these Sc reduced CD retention by more 2 folds with respect to the mycotoxins-treated groups.

The overall data indicate that *S. cerevisiae* had a broad range of biomodulatory properties; protect against AF genotoxicity and mitigate its spermatotoxic effects. This might be, in part, due to the ability of yeasts to adsorb these mycotoxins; where several studies clearly reported the adsorption mechanisms in vitro (Baptista et al., 2004; Bueno et al., 2006; Emam-Djomeh et al., 2009). In an *in vitro* study with the cell wall material, there was a dose dependent binding of as much as 77% (w/w) and modified mannan-oligosaccharides derived from the S. cerevisiae cell resulted in as much as 95% (w/w) binding (Devegowda et al.,1996). Shetty et al. (2007) showed that some strains of Sc bind high amounts of the carcinogen AFB1 and binding was attributed to physical phenomenon, where non-viable and physically altered cells bound significantly higher levels of toxin than their viable counterpart. Rahaie et al. (2010) investigated the binding ability of *S. cerevisiae* to aflatoxin in pistachio nuts. The obtained results showed that S. cerevisiae had an AF surface binding ability of 40% and 70% (with initial AF concentrations of 10 and 20 ppb) in the exponential phase. Moreover, Sc cells or their cell wall showed antioxidant properties; glucomannan supplemented on oxidative stress caused by aflatoxin in rabbits enhanced antioxidant status (Dönmez and Keskin. 2008). Also, *Debaryomyces hansenii* positively enhanced growth performance and antioxidant status of sea bass larvae by means of preventing oxidative

stress, and by maintaining a stable activity and gene expression of SOD and GPX (Tovar-Ramírez et al., 2010).

In conclusion, oral administration of live yeast S. cerevisiae to mice positively enhances could protect against mycotoxins-induced genotoxicity in somatic and germ cells, as well as restoring mitotic and meiotic activities. Also, Sc mitigates the AF-induced toxicity on sperm.

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