Saccharomyces cerevisiae modulates Aflatoxin-induced toxicity in male Albino mice

[a] Darwish, H.R.[a]; Omara, E.A.[d] ; Abdel-Aziz, K.B.[a]; Farag, I.M. [a]; Nada, S.A.[c] and Tawfek, N.S.[b]

[a] Cell Biology Dept., National Research Centre, El- Tahrir Street, Dokki, Cairo 12622, Egypt.; [b]Zoology Dept., Faculty of Science, Al-Minia Uni., Egypt; [c] Pharmacology Dept., National Research Center, Egypt.; [d] Pathology Dept., National Research Centre, El-Tahrir Street, Dokki, Cairo 12622, Egypt.

Hr_darwish@yahoo.com

Abstract: Aflatoxins are unavoidable food contaminant and reducing their toxicity *in vivo* is of major interest. The potential of *Saccharomyces cerevisiae* (Sc) was evaluated for reducing the AFs-induced toxicity in mice. Four experimental groups were used, each comprising 30 mice; control group, Sc-treated group (4×10^8 CFU), AFs-treated group (0.7 mg/kg b.w.), and a group given Sc two hours before AFs intoxication. Alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), uric acid and creatinine were measured in mice serum. The levels of malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) activity were measured in homogenates of liver and kidney; as well as histopathological investigations of liver and kidney. Results revealed the following: control and Sc fed mice had similar constant weight increase; mice fed only AFB1 showed weight decrease; finally, Sc improved weight gain. Also, results showed that AFs significantly elevated the serum ALT, AST, uric acid and creatinine levels. The levels of non-enzymatic antioxidant (GSH) as well as enzymatic antioxidant (SOD) activity were significantly decreased in both liver and kidney of AFs-treated mice in comparison with control. Histopatholigical examinations showed a severe damage in liver and kidney tissues of AFs-treated mice. Mice received Sc before AFs gavage, showed a significant amelioration in serum biochemical parameters and improvement in liver and kidney tissues architecture. In along with, a significant amelioration in LPO in liver and kidney by increasing the contents of GSH and activities of SOD antioxidant have been occurred compared to control. In conclusion, Sc was found to be safe and successful agent counteracting the AF toxicity and protected against the toxicity induced by AFs.

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Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *A. parasiticus*, and they have carcinogenic, mutagenic, hepatotoxic and teratogenic effects (Massey et al., 1995). Several diseases are associated with the human consumption of these toxins, including toxic hepatitis and even primary hepatocellular carcinomas (Pitt, 2000). Furthermore, a wide spectrum of toxic responses is related to the exposure of animals to aflatoxins, with most of them causing economical losses resulting from decreased production. AFB1-mediated toxicity was also found to be related to its pro-oxidant potential. This is because reactive oxygen species (ROS) including superoxide anion (O-), hydrogen peroxide (H2O2), and hydroxyl radical (-OH) are generated during the metabolic processing of AFB1 by liver enzymes (Preston and Williams, 2005; Towner, et al. 2003). ROS cause oxidative stress by damaging cellular membranes and components. Therefore, it can be assumed that natural components having antioxidant potential are capable of inhibiting ABF1-induced oxidative damage either by scavenging ROS or stimulating antioxidant defense systems (Yener et al., 2009). *Saccharomyces cerevisiae* (Sc) has proven to benefit health in several ways including stimulation of the growth of intestinal microflore in mammals, pH modulation in ruminants, improvement of reproductive parameters in milk cows and fowls, as well as reduction in the number of pathogenic microorganisms in monogastric animals (Dawson, 1993; Wallace, 1994). In recent years, yeasts have also been reported to have high adsorption ability against mycotoxins in aqueous solution; *Sc* had the potential to bind AFB_1 (Santin et al., 2003; Baptista et al., 2008). The yeast Sc was reported to be the most efficient microorganism for aflatoxin B_1 quenching (Bueno et al., 2006). 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 present study was designed to investigate the effect of Sc on weight performance, biochemical analyses and liver and kidney pathology; and to evaluate the protective role of this yeast against aflatoxin B1- in mice.

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 B_1, B_2, G_1 and G_2 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.

Yeast strain and culture preparation:

Yeast (*Saccharomyces cerevisiae*) was purchased from market, as lyophilized powder (PAK GIDA, Turkey) and stored at 4C°; SC was dosed at (4 x 10^8 CFU) in 0.4 ml dist water and given once-a-day daily treatment.

Experimental animals:

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

Experimental design

Mice were randomly divided into four groups each consisting of 30 mice, each group was subdivided into three subgroups (10 mice for each). Animals were treated orally for successive 7 days as follows: (1) untreated control given 0.4ml $NaHCO_3$ daily, (2) treated with AFs (0.7 mg/kg b.w.) in 0.4ml $NaHCO_3$, , (3) treated with Sc (4×10^8) in H_2O and (4) treated with the Sc (4×10^8) 2 hours before Afs gavage (0.7 mg/kg b.w.). On the 8th day of the study, blood was collected directly from the retro-orbital venus plexus and serum samples were prepared. Then, animals were sacrificed by cervical decapitation and liver and kidney samples were dissected out and washed immediately with ice-cold saline and stored immediately at -80º C until analysis.

Body weight

Mice were weighed at the beginning of the study, at the 8th day, 15th day and the 35th day from the beginning of the study. The percentage of weight gain or loss is then calculated.

Determination of the serum biochemical parameters

Liver and kidney function:

At the end of the experimental period, animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected directly from the retro-orbital venus plexus and serum samples were prepared by centrifugation at 3,000 rpm for 10 min. The above collected serum was used for the assay of marker enzymes of liver function serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST). As well as the markers of kidney function: uric acid and creatinine were carried out using the commercially available standard kits and according to manufacturer's instructions.

Measurement of lipid peroxidation:

Liver and kidney tissues were homogenized individually in 20 mm Tris – HCl (pH 7.4). Homogenates were centrifuged at 6000 g for 30 min. MDA levels in the supernatants were determined using a spectrophotometric assay kit according to the manufacturer's instructions. Briefly, thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm (Ohkawa et al., 1979). The lipid peroxidation values are expressed as nm MDA/mg tissue.

Reduced Glutathione (GSH) content

GSH levels were measured using a spectrophotometric assay kit according to the manufacturer's instructions. 5,5' dithiobis-2-nitrobenzoic acid (DTNB) is reduced by glutathione (GSH) to produce a yellow compound .The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm (Beutler et al., 1963). GSH values are expressed as mmol/g tissue.

Superoxide dismutase (SOD) activity

Liver and kidney homogenates were prepared in cold Tris–HCl (5 mmol/L, containing 2 mmol/L EDTA, pH 7.4) using a homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10,000g for 10 min at 4º C. The

supernatant was used immediately for the assays for SOD. 100µl of supernatants were added to 2.8 ml tris HCL buffer containing 25µl pyrogallol and 20µl catalase (Marklund and Marklund, 1974). The activities of all of these enzymes were determined. The SOD activities were expressed as units per mg of tissue.

Histopathological examination:

For histopathological studies, samples of the liver and kidney tissues of each animal were excised and processed for light microscopy. The processing involved fixing the tissue specimens in a 10% neutral buffered formalin solution, preparing the blocks in paraffin, cutting sections 5 µm in thickness, and stained with hematoxylin and eosin (H&E) (Drury et al., 1976).

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).

Results:

The impact on body weight

The current results indicated that no animal death occurred in any of the treatment groups. The effect of different treatments on body weight gain of mice is depicted in Figure (1). Mice treated with AFs showed a high significant loss in weight, at the 8th day of the assay respect to control group (4.84 % at P< 0.001). At the 15th day, body weight loss continued and reached 10.14 % at (P< 0.001); it was highly significant; this weight loss showed partial recovery at the 35th day of the assay and reached to 8.50 % which was statistically highly significant compared to all other groups at P<0.001.In contrast, animals treated with Sc alone showed an insignificant increase in weight gain compared with control group at P>0.05. On the other hand, mice treated with Sc before AFs-intoxication showed a strong recovery in body weight gain which was very high significant (P< 0.001) at all time points although they did not reach the control values. When comparing the Sc plus AFs group with control, an insignificant decrease in the body weight gain at the 8th day at P>0.05, whereas this decrease was significant at the 15th day (P<0.05) and at the 35th day of assay (P<0.01).

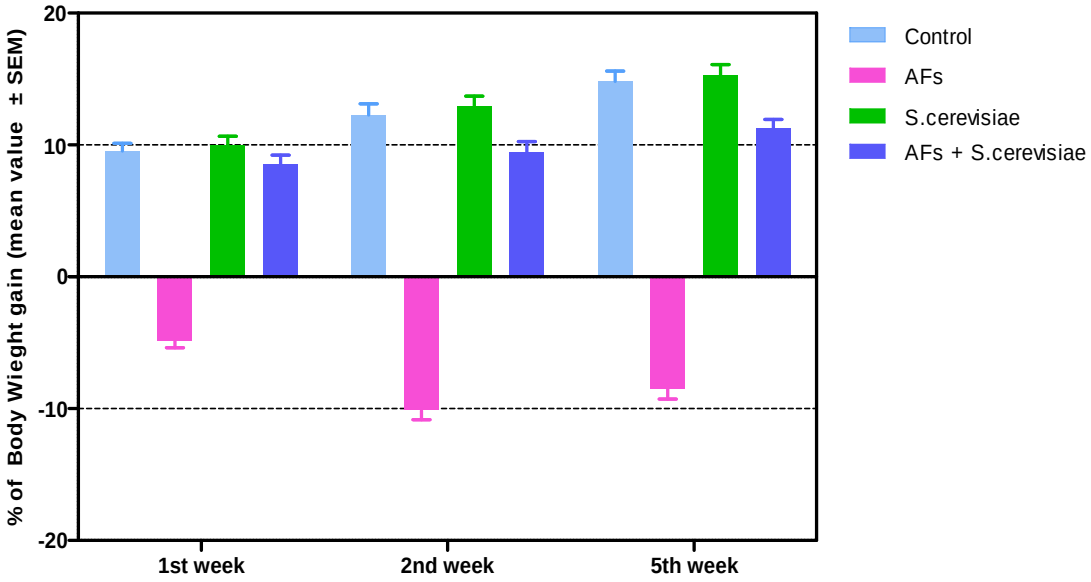

Figure 1: Effects of Sc on AFs-induced mice body weight change in male mice (Data represented the % weight gain/loss at weekly intervals).

Biochemical studies

Serum biochemical parameters

The effect of different treatments on serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), uric acid and creatinine levels in mice is presented in Table (1). Mice given AFs showed an increase in activities of ALT (~180%) and AST (~217%), which were statistically high significant at P<0.01, as compared to control. In addition, On the other hand, oral administration of Sc before AFs-intoxication caused significant amelioration in ALT and AST activities as compared to the AFs alone treated groups at P<0.01. This reduction was still below that in control group, and it was statistically higher than that of control at P<0.05; whereas it was significant at P<0.01 when compared to the Sc group. Mice treated with Sc alone showed a nonsignificant reduction in ALT and AST activities when compared to control at P>0.05.

Data in Table (1) showed that AFs treatment caused significant elevation in serum creatinine by 185% and uric acid by 212%, as indicators of kidney function, in comparing with control group, while animal treated with Sc before AFs-intoxication ameliorated raised creatinine and uric acid value when compared with the AFs group, which was significant at P<0.01. This recovery was not complete and showed significant differences compared to control at P<0.05 in case of serum creatinine; whereas it was significant at P<0.01 for uric acid. On the other hand, treatment with Sc alone showed insignificant reduction in creatinine and uric acid levels on comparing with control at P>0.05.

Table 1: Effect Sc on serum biochemical parameters of liver and kidney functions of AFs-treated male mice.

EXPERIMENTAL GROUPS	*ALT (U/L)*	*AST (U/L)*	*CREATININE (mg/dL)*	*URIC ACID (mg/dL)*
Control (H_2O / corn oil)	28.9 ± 1.20^{AB}	45.0 ± 1.58^{A}	0.42 ± 0.021^{AB}	1.32 ± 0.046^{AB}
AFs (0.7 mg/kg b.w.)	51.8 ± 1.69^{C}	97.8 ± 3.23^{C}	0.78 ± 0.020^{C}	2.80 ± 0.090^{C}
S. cerevisiae (4×10^8)	$24..8 \pm 1.36^{A}$	$35.3 \pm 1.44^{A*}$	0.34 ± 0.020^{A}	1.12 ± 0.079^{A}
Sc plus AFs	$35.0 \pm 1.58^{B*}$	63.0 ± 1.95^{B}	$0.52 \pm 0.022^{B*}$	1.60 ± 0.098^{B}

-Means with different superscript letters (A, B, C) are significantly different (P <0.01).
-Means with a star are significantly different (P <0.05).
-All data are expressed as means ± SEM.

Effect on oxidative status of mice liver and kidney:

A. Effect on lipid peroxidation (MDA content):

AFs-treatment elevated the level of MDA in liver and kidney tissues; this increase was highly significant as compared to control and Sc groups at P<0.01(Table 2). In mice receiving Sc alone, significant reduction was found in the level of MDA in kidney tissues at P<0.05, while it was non-significant in case of liver tissue when compared with control at P>0.05. Oral administration of Sc before AFs-intoxication reduced significantly the MDA content in both liver and kidney tissues compared to AFs-treated group at P<0.01. In this group, the levels of MDA in liver and kidney tissues were significantly higher than that of control and Sc groups (P<0.01).

B. Effect on the reduced glutathione (GSH level):

Results revealed that mice treated with AFs exhibited sharp depletion in level of reduced glutathione in both liver and kidney tissues, this reduction reached to 2.7 folds in liver tissues and 1.7 folds in case of kidney tissues, it was statistically significant at P<0.01 as compared to other groups. Mice given Sc alone showed an increase in GSH content as compared to control, which was insignificant for liver tissue at P>0.05 and significant in case of kidney tissue at P< 0.05. On the other hand, mice received Sc before

AFs gavage showed a significant recovery in GSH level when compared with AFs-intoxicated group at P<0.01.This enhancement was significantly below the GSH level of control and Sc groups at P<0.01.

C. Effect on Superoxide dismutase (SOD) activity

SOD activity in liver and kidney tissues was significantly decreased in AFs administered group, as compared to all groups at P<0.01 (Table 2). By contrast, mice received Sc alone showed an enhancement in SOD activity which was insignificant, in liver tissue and significant in kidney tissue when compared with the control group at P<0.01. On the other hand, the activity of SOD in group received Sc before AFs-treatment was significantly elevated as compared to the AFs-treated group. This amelioration was still significantly below that of control groups in kidney tissues at P<0.01; while it showed no significance in liver tissues at P>0.05.

Table 4.12: Effect of Sc on AFs-induced lipid peroxidation and antioxidative defense mechanisms in liver and kidney of male mice

Experimental groups	*Parameters*					
	MDA (nmoles /gm protein)		*GSH (mmol/gm protein)*		*SOD (units/mg protein)*	
	LIVER	*KIDNEY*	*LIVER*	*LIVER*	*KIDNEY*	*LIVER*
Control (H_2O / corn oil)	*12.0 ± 0.46* ^{A}	*17.1 ± 0.27* ^{A}	*337 ± 4.7* ^{A}	*260 ± 5.71* ^{A}	*30.0 ± 1.90* ^{AB}	*67.9 ± 1.79* ^{A}
AFs (0.7 mg/kg b.w.)	*4.4 ± 0.30* ^{C}	*10.1 ± 0.39* ^{C}	*781 ± 15.6* ^{C}	*663 ± 11.9* ^{C}	*15.0 ± 1.30* ^{C}	*32.6 ± 2.04* ^{D}
S. cerevisiae (4×10^8)	*13.0 ± 0.46* ^{A}	*18.9 ± 0.50* ^{A*}	*306 ± 3.82* ^{A}	*219 ± 4.72* ^{A*}	*53.0 ± 1.80* ^{A}	*79.4 ± 1.75* ^{B}
Sc plus AFs	*8.3 ± 0.34* ^{B}	*14.1 ± 0.31* ^{B}	*440 ± 13.6* ^{B}	*341 ± 10.8* ^{B}	*26.0 ± 1.40* ^{B}	*57.4 ± 1.27* ^{C}

-Means with different superscript letters (A, B, C, D) are significantly different (P <0.01).
-Means with a star are significantly different (P <0.05).
-All data are expressed as means ± SEM.

Histopathological examinations of liver

In control animals liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and central vein (Plate 1A). No obvious histological changes could be noticed in the liver of mice treated with Sc (Plate 1B). Histological evaluation of the effect of AFs showed distortion of hepatic architecture with dilated and congested in central vein. Ballooning of cytoplasm and darkening of nuclei were also noticed (Plate 1D). On other side, the combined administration with Sc and AFs resulted in restoration of normal hepatic architecture (Plate 1 E and F).

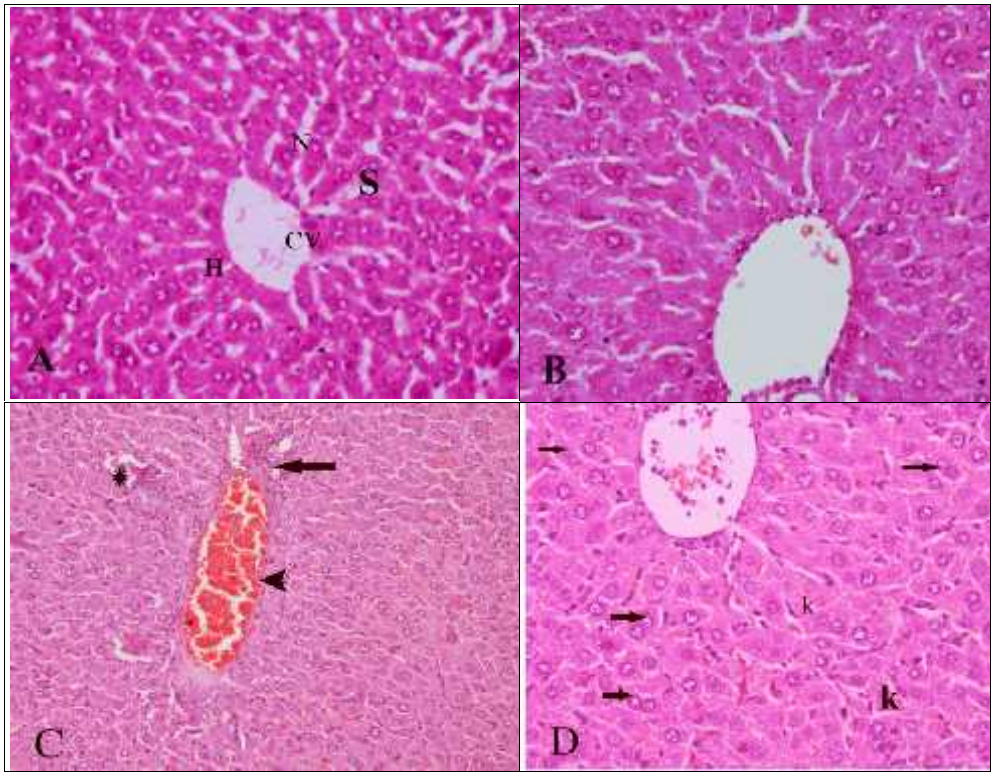

Plate 4.4: (A)-Photomicrograph of section of liver from control mice showing normal structure of liver, central vein (CV) and hepatic cords of hepatocytes (H) with prominent nucleus (N) separated with blood sinusoids (S). (H & E X 400) **(B)** Photomicrograph of section of liver of mice administration of ***S. cerevisiae*** showing normal structure of liver. **(C)** Section of liver of mice treated with **AFs** showing abnormal architecture of liver tissue, dilated with congested central vein (arrow head) ►, inflammatory cells → and necrotic area *. **(D)** Section of liver of mice treated with **AFs** and ***S. cerevisiae*** showing normal architecture with bineucleated cells →, kuppfer cells (K).

Histopathological examinations of kidney

Microscopic examination of kidney sections in control group stained with H&E showed normal appearance of glomeruli, bowman capsule, proximal and distal tubules as demonstrated in Plate (2) Administration of Sc showed normal architecture of rat kidneys (Plate 2B). The most consistent finding in the histological section of mice–renal tissues treated with AFs showed hypercellularity and sever degeneration in glomeruli and tubules (Plate 2D). Examination of kidney tissue revealed no evidence of renal tubule injury after treatment with AFs and Sc, except hypercellularity of glomeruli and interstitial hemorrhage was also noticed (Plate 2 E and F).

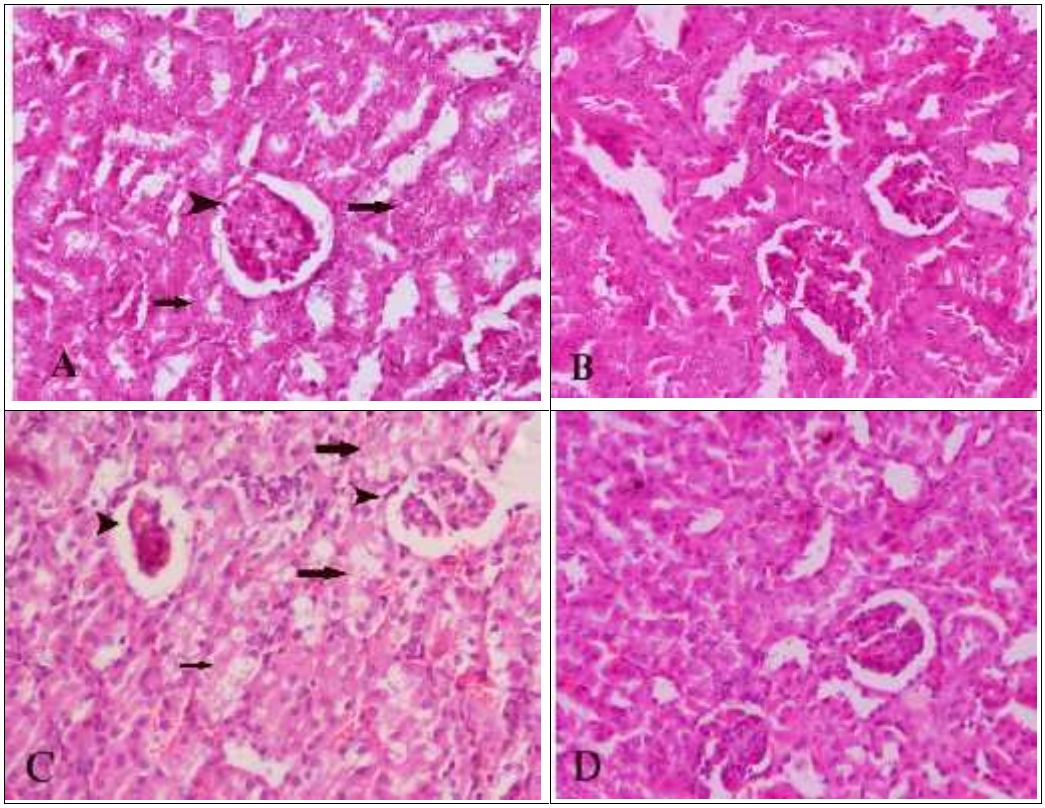

Plate 4.5 (A): Section in the kidney of control mice showing normal glomerulus (►), and renal tubules structures (→). (H & E X 400) **(B):** Section in the kidney of mice administered ***S. cerevisiae*** showing normal structure of glomerulus and renal tubules. **(C):** Sections in the kidney of mice treated with **AFs** showing atrophy and hypercellularity of glomerulus's (arrowhead ►), and degenerated tubules (→) with interstitial hemorrhage. **(D):** Sections in the kidney of mice treated with **AFs** and ***S. cerevisiae*** showing almost normal architecture with little amount of degeneration of some tubules (arrow head).

DISCUSSION

The present results clearly indicated that intoxication with AFs resulted in a significant reduction in body weight. Similar decrease in body weight was reported in rats and mice fed AFB_1-contaminated diet (Madrigal-Santillán et al., 2006, 2007). This reduction in body weight may explained by the ability of AFs to generate free radicals (Abdel-Wahhab et al., 2008), which may lead to DNA breakage, inhibition of protein biosynthesis and gluconeogenesis, lipid peroxidation, disruption of oxidative phosphorylation in mitochondria, inhibition of blood clotting and apoptosis (Parhizkar et al., 2002). Furthermore, the decrease in body weight in the animals treated with the mycotoxin may be due to their effects on the balance between orexigenic and anorexigenic circuits that regulate the homeostatic loop of body weight regulation, leading to cachexia (Rastog et al., 2001). In this regards, Abdel-Wahhab et al. (2006) reported that rats treated with AFB_1 showed a significant decrease in leptin; which and its receptor are the key players in the regulation of energy balance and body weight control which together act to influence the feeding response, causing weight loss (Yuan et al., 2004). By contrast, the current results showed that Sc gavage before AFs administration, also, enhanced growth performance and resulted in a significant recovery in body weight. These findings are in agreement with previous studies; Addition of Sc to diets could improve growth performance (Onifade et al., 1999) and viability of weaned rabbits (Maertens and

De Groote, 1992). Madrigal-Santillán et al. (2006) indicated that animals treated with the Sc plus AFB_1 gained considerable weight in the third and the sixth week of the assay; which was more than double

the usual level reached by AFB_1 treated mice. Improvement in growth performance had been previously observed in adult and juvenile fish and sea bass larvae fed live yeast (Lara-Flores et al., 2003; Tovar-Ramírez et al., 2010). Yeast viability seems critical for such growth-promoting effect, since it was not observed when inactive yeast was incorporated into the feed (Métailler and Huelvan, 1993; Oliva-Teles and Gonçalves, 2001). Moreover, digestion of yeast cells releases active compounds like polyamines, proteases and phosphatases, which could be beneficial for the digestive process (Zanello et al., 2009). Moreover, Dönmez and Keskin (2008) studied the effects of glucomannan supplemented on oxidative stress caused by AF in rabbits; they found that glucomannan enhanced antioxidant status compared with control group.

Data presented herein showed significant biochemical changes in kidney and liver functions in mice treated with AFs. The activity of ALT and AST is a sensitive indicator of acute hepatic necrosis and hepatobiliary disease; and increase in AST and ALT activity indicates initial hepatocellular damage (Abdel-Wahhab et al., 2006). Treatment with AFs was found to alter serum biochemical parameters; in our study, very great elevation in serum ALT and AST activity was observed as a result of AFB_1 treatments. Similar to our results, Aravind et al. (2003) reported an increase in ALT and AST in broiler chickens fed AFs-contaminated diet. AFB_1 (3 mg/kg feed) caused increase in ALT and AST serum activity by 5.2-fold and 3.8-fold, respectively (Abdel-Wahhab and Aly, 2003). AFB_1 reported to significantly increase the activities of all serum ALT and AST (Preetha et al., 2006; Yener et al., 2009), Such increase can be attributed to cell necrosis, changes in cell membrane permeability or impairment of biliary excretion (Pozzi et al., 2000). On the other hand, AFs resulted in a significant increase in serum uric acid and creatinine concentrations. This findings are in agree with previous studies reported the elevation of creatinine in serum and urine of rabbits receiving AF-contaminated feed (Verma and Raval, 1997) and in mice treated with the AF (Ghaly et al., 2010). Verma and Kolhe (1998) showed time dependent rises in creatine and creatinine concentrations in the serum and urine of AF-fed rabbits. They suggested that AF causes adverse changes in skeletal muscle and kidney at a very early stage. It is well established that any changes of creatinine and uric acid levels in serum are indicative of an impairment of kidney function. The results showed that the pretreatment with Sc reduced the ALT, AST, creatinine and uric acid and protected against hepatic and renal injuries caused by the mycotoxin. These data are in agreement with previous studies; adding glucomannan or Mannan Oligosaccharides to diets of aflatoxin-treated rabbits and broilers lowered AST and ALT levels (Dönmez and Keskin, 2008; Yalcinkaya et al., 2008). Baptista et al. (2008) stated that the yeast strains Y1026 and Y904 were able to reduce ALT and AST activity and reduced liver damage caused by aflatoxins in Wistar rats. Recently, mice treated with the yeast Strains *of Rhodotorula glutinis* significantly decreased ALT, AST, creatinine uric acid levels of AFB_1-treated animals (Ghaly et al., 2010). Oxidative stress arises when the generation of ROS, by-products of the oxidative metabolism primarily produced in the mitochondria, exceeds the cellular ability to eliminate them and to repair cellular damage, thus leading to oxidation of biomolecules including DNA, lipids and proteins (Hwang and Kim, 2007). Lipid peroxidation (LPO) is one of the main manifestations of oxidative damage and it has been found to play an important role in the toxicity and carcinogenicity. The data obtained in this study show that both AFs and OTA induce a significant increase in LPO in liver and kidney tissues as increasing in malondialdehyde (MDA) production. MDA is an end product of lipid peroxidation, and it is considered a late biomarker of oxidative stress and cellular damage (Carampin et al., 2003). These results confirm and extend previous data which have demonstrated that these mycotoxins induce a significant increase in LPO under in *vitro* and in *vivo* conditions (Petrik et al., 2003; Abdel-Wahhab et al., 2008; Verma and Chakraborty, 2008).

On the other hand, peroxidative damages are encountered by elaborate defense mechanisms, including enzymatic and non-enzymatic antioxidants (Janssen et al., 1993). To assess the balance of reactive oxygen species (ROS) production in liver and kidney, levels of non-enzymatic antioxidants GSH and enzymatic antioxidant (SOD) activity were measured. The current results show a dramatic decrease in GSH contents and SOD activity in liver and kidney homogenates of mice treated with the mycotoxins. Our findings of decrease in GSH contents and the activities of SOD corroborate with that of previous studies (Abdel-Wahhab et al., 2008). Thus significant decrease in GSH level will further aggravate the toxic effects of these mycotoxins. GSH plays a critical role in the protection of tissues from AFB_1 exposure by directly interacting with ROS or as a cofactor for enzymatic detoxification and the liver necrosis begins when the glutathione stores are almost exhausted (Larsson et al., 1994; Janssen et al., 1993; Abdel-Wahhab and Aly, 2003). GSH depletion

might be a consequence of mycotoxins conjugation with GSH or/and continuous attack of free radicals which known to generate reactive intermediates (such as α, β-unsaturated aldehydes) that covalently bind to GSH (Duke et al., 1996; Glaab et al., 2001).

Furthermore, Ilic et al. (2010) stated that the A3 subunit of GST subunit has a crucial role of in protection of normal mice against AFB_1 toxicity. On the other side, SOD converts superoxide radicals to H_2O_2, which induces hydroxyl radicals by Fenton and/or Haber–Weiss reactions if the agent is not removed by CAT and/or GSH (Johnson and Giulivi, 2005). So, the decrease in SOD activity will increase the level of superoxide radicals, leading to an increase in oxidative stress enhancing early cell death, probably by apoptotic mechanisms (Ozçelik et al., 2004). Consequently, concomitant depletion in GSH contents and decrease in SOD activity lead to an increase in oxidative stress enhancing liver and kidney damage. The pretreatment with Sc before intoxication with the mycotoxin ameliorated the GSH levels and SOD activity compared to control, where MDA level decreased and SOD activity increased, along with an increase in GSH contents. Recent studies showed that dietary yeast *Debaryomyces hansenii* stimulates both immune and antioxidant responses in juveniles of gilthead sea bream and in leopard grouper *Mycteroperca rosacea* after exposure to pathogens (Reyes-Becerril et al., 2008a, b). In addition, feeding rabbits with glucomannan caused an increase in SOD activity and GSH contents (Dönmez and Keskin, 2008). More recently, Tovar-Ramírez et al. (2010) reported that live marine yeast *D. hansenii* CBS 8339 positively enhanced 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. Moreover, oral administrations of LGG cultures or Sc before mycotoxins gavage decrease their bioavailability by their ability to bind the mycotoxins in gastrointestinal tract (Celyk et al., 2003; Santin et al., 2003; Gratz et al., 2006).

Histopathological study of both liver and kidney may reflect and explain the above biochemical changes. Microscopic examination of the liver sections of the AFs -treated groups showed severe histological changes typical to those reported in the literature. In this concern, treatment with AFB_1 resulted in cirrhotic livers with numerous regenerative and dysplastic nodules encircled extensively by ballooning and fatty degeneration cells. Moreover, increased numbers of both apoptotic cells and mitoses were predominantly found in the periportal regions; the nuclei of these cells were enlarged; hyperchromatic and pleomorphic with a coarse chromatin pattern and their cytoplasms were distincly large (megalocytosis) (Naaz et al., 2007; Balaji et al., 2009; Yener et al., 2009; El-Agamy, 2010). In contrast, the microscopic examination of the rat liver in the groups pretreated with Sc before AFs-intoxication resulted in a mild improvement in liver architecture, and livers exhibited an almost normal architecture with little amount of inflammatory cell and congestion of central vein.

Histological sections of mice renal tissues treated with AFs showed hypercellularity and severe degeneration in glomeruli and tubules. The obtained data are agreement with the previous literature, where AF administration induced cloudy swelling of the epithelial lining of renal tubules with mild interstitial fibrosis and congestion and frequent vacuolar degeneration (Mollenhauer et al., 1989; Ezz El-Arab et al., 2006; Orsi et al., 2007). The adverse effects mentioned above were protected by the pretreatment with Sc before mycotoxins-administration; kidney tissue revealed no evidence of renal tubule injury after treatment with AFs and probiotics, except hypercellularity of glomeruli and interstitial hemorrhage, which were also noticed.

In conclusion, our data support the hypothesis of oxidative stress mediated toxicity induced by AFs; where the hepatotoxicity, nephrotoxicity and genotoxicity were accompanied by an elevation in LPO along with a reduction in GSH contents and SOD activity. By contrast, the present study indicates that oral administration of live yeast *S. cerevisiae* to mice positively enhances growth performance and significantly mitigates AFs-induced toxicity by means of preventing oxidative stress, and by maintaining glutathione content, as well as a stable activity of SOD, however, further studies are needed to better understand the *in vivo* possible mechanism(s) by which Sc may reduce the toxicity induced by AFs. The results of the histopathological investigations of the kidneys supported these findings confirming the protective effects of the test compounds albeit with some differences in antioxidant potency. Taken together, our data may suggest that Sc could alleviate an AFs-reduced toxicity in mice.

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