Saccharomyces Cerevisiae And Probiotic Bacteria Potentially Inhibit Aflatoxins Production In Vitro And In Vivo Studies

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Abstract
Saccharomyces cerevisiae and Lactic acid bacteria (Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705) potentially inhibited Aspergillus flavus growth and aflatoxins production in YES liquid media. Six groups of rats orally administrated SC (10^{11} CFU / ml) and LGG & LC705 (10^9 CFU/ ml) daily for 15 days alone or with 2 mg / ml aflatoxin B1 (AFB1) in corn oil, significantly reduced serum ALT, AST, GGT, creatinine, and BUN compared with AFB1-teredated group. No deaths occurred in any combined treatment with AFB1, while a 30% mortality rate was recorded in the AFB1-treated group. Blood glutathione (GSH) levels significantly increased in groups treated with single-treatment of S. cerevisiae, LGG & LC705 or concomitant with AFB1; however, AFB1-treatment alone severely depleted GSH level more than other treatments. Histopathological examination of liver and kidney in rats treated with AFB1 showed necrosis, vacuolar degeneration and fatty changes in hepatocytes; cellular swelling and pyknotic nuclei of proximal convoluted tubules in renal tissue. DNA content decreased significantly in liver and kidney tissues with AFB1-administration, these findings were ameliorated by probiotec bacteria and S.cervisiae treatment. Conclusion: These probiotics may have good medical benefits to diminish the aflatoxins production in vitro and in vivo studies.

INTRODUCTION
Aflatoxins are group of closely related difuranocoumarin compounds produced by several fungi, mainly Aspergillus flavus and Aspergillus parasiticus (Steyn,1995). These strains produce aflatoxins B1, B2, G1, G2, and M1 (Wilson and Pyne, 1994), contaminating a number of crops bound to human consumption such as corn, peanut, sorghum, rice, wheat and nut (Cleveland et al., 2003).

Aflatoxin contamination occurs by colonization of the fungus on susceptible crop, or may arise during harvesting, drying, storage, or processing.

Human hepatocellular carcinoma is the fifth most commonly occurring cancer in the world and the third greatest cause of cancer mortality (Anwar et al., 2008). Aflatoxin B1 (AFB1) is the most prevalant and carcinogenic of the aflatoxin, the International Agency for Research on Cancer reported that AFB1 is a carcinogen of group I (Bedard and Massey, 2006).

Filamentous moulds are common spoilage organisms of food products, e.g. fermented milk products, cheese, bread, as well as stored crops and feed such as hay and silage (Filtenborg, et al.,1996). It is estimated that between 5 and 10% of the world’s food production is lost annually due to fungal deterioration (Pitt and Hocking, 1997). In Western Europe, mould spoilage of bread alone is estimated to cause annual economical losses of more than £200 million (Legan, 1993). Penicillium and Aspergillus species are reported as spoilage organisms from a wide range of food and feeds; they are often found on cereal grains, where they might produce numerous mycotoxins (Filtenborg et al., 1996; Samson, et al., 2000). Many techniques are used for the preservation of food and feeds. Drying, freeze drying, cold storage, modified atmosphere storage, and heat treatments are all means of physical methods of food preservation (Farkas, 2001). Several organic acids such as acetic, lactic, propionic, sorbic and benzoic acids are used as food preservatives (Brul and Coote, 1999). Both sorbic and benzoic acid have a broad spectrum of activity (Davidson, 2001). They are used primarily as antifungal agents. The antibiotic natamycin, produced by Streptomyces natalensis, is effective against moulds and a common preservative on
Saccharomyces cerevisiae and probiotic bacteria potentially inhibit aflatoxins production in vitro and in vivo studies

Increasing number of microbial species are becoming resistant to antibiotics. Fungi are no exception and both fungal human pathogens and spoilage moulds in food and feed systems are becoming resistant to currently used antifungals. Furthermore, moulds are not only becoming resistant to antibiotics, but also to preservatives such as sorbic and benzoic acids, as well as, chemical treatment with cleaning compound (Brul and Coote, 1999; Sanglard, 2002).

Probiotics are living microorganisms that when ingested may help to maintain the bacterial balance in the digestive tract of mammals, and may be included in the treatment of pathological conditions, such as diarrhoea, candidiasis, urinary infections, immune disorders, lactose intolerance, hypercholesterolemia, and food allergy (Mombelli and Gismondo, 2000). They also have antigenotoxic effects; for example, species of Lactobacillus, Streptococcus, Lactococcus, and Bifidobacterium, have shown antimutagenicity in the Ames test, and their ability to decrease DNA damage in colon cells treated with N-methyl-N-nitro-N-nitrosoguanidine in vitro study (Pool-Zobel et al., 1996).

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 (which gives rise to an increase in the rate of cellulitic bacteria), improvement of reproductive parameters in milk cows and fowl (fertility and fetal development), as well as reduction in the number of pathogenic microorganisms in monogastric animals (Dawson, 1993). In addition, a study in mice fed AFB1 contaminated corn (0.4 and 0.8 mg/kg) plus Sc (1X10⁸ life cell/g) have been resulted a significant reduction in micronucleated normochromatic erythrocytes rate; authors suggested that Sc had potent adsorbent capacity and there were no structural modification in AFB1 (Madrigal-Santillan et al., 2006).

The aim of our study was to investigate the efficacy of probiotic bacteria (LGG and LC705) and Saccharomyces cerevisiae to inhibit A. flavus growth in vitro and to eliminate aflatoxins from body of mature rat in vivo study.

MATERIALS AND METHODS

MATERIALS

HPLC Apparatus: High performance liquid chromatography (HPLC) was used for aflatoxin determination. A mobile phase consisting of water: acetonitril: methanol (240:120:40) used and the system was equipped with a Waters 600 delivery system, and an. HPLC column (a reverse phase analytical column) packed with C18 material (Spherisorb 5 µm ODS2, 15cmx4.6mm). The detection was performed using the fluorescence detector operated at an excitation wave length of 360 nm and an emission wave length of 440 nm. The separation was performed at 40 °C temperature at a flow rate of 1.0 ml/min. Data were integrated and recorded using a Millennium Chromatography Manger Software 2010 (Waters, Milford MA 01757).

Sep-pak silica cartridge C18 columns Sep-Pak silica cartridge, C18-E (55 µm, 70 Å⁻) 500 mg/6 ml were obtained from Phenomenex Co., Torrance, CA, USA.

Chemical used in the analytical analysis. Chloroform, acetone, trifluroacetic acid (TFA), methanol, acetonitrile, diethylether and acetic acid, of HPLC grade were produced by BDH,Chemicals Ltd., Poole, England.

Freeze-dried powder of Saccharomyces cerevisiae (baker’s yeast strain) and lactic acid bacteria (Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705) were obtained from Valio Ltd. (Helsinki, Finland).

Aflatoxins and Cultures : (a) Potato dextrose agar (PDA), (b) de’Mane- Regosa-Sharp (MRS), (c) Malt Extract Agar (MEA) and (d) Yeast extract-malt extract-sucrose broth medium were obtained from Sigma Chemical Company, St., Louis, MO, USA).

Diagnostic kits were purchased from Boehringer Mannheim GmbH Diagnostica, E.Merck, Postfach 4119, D-6100, and Darmstadt, Germany. All other chemicals were of highest quality available and were obtained from commercial sources.

Animal care and use: The experimental protocols were approved by The National Research Centre, Cairo, Egypt of Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues (Zimmermann 1983).

The experiment was conducted using mature male rats weighing 120-130 g b.wt, purchased from Animal House colony. Animals were divided into equal groups (six rats each) housed under standard environmental conditions (23 ± 1°C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained on a standard laboratory diet ad libitum with free.
access to water.

METHODS

(1) In Vitro Study

(a): Preparation of A. flavus spores and Aflatoxins extraction.

Cultures of toxic molds were grown on potato dextrose agar (PDA) slants for 7 days at 25 °C (Bullerman, 1974). The liberated Aflatoxins were analyzed according to AOAC (2000) and quantified by HPLC technique (Sep-pak silica cartridge C18 columns) according to method’s described by Ferreira et al., (2005).

(b) Spores of Lactic acid Bacilli strains (Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705) were cultured on MRS broth / agar at 37°C until a concentration of 10⁹ bacteria / ml was obtained, the counting of viable bacteria was performed by both traditional plate counting and flow cytometry (FCM) methods. Bacterial counts were expressed as colony-forming units (CFU) per ml media. Viability of bacterial populations was assessed by using SYTOX ® green nucleic acid stain (Molecular probes, S-7020) at 1 µM /10⁶-10⁷ bacteria to detect non viable bacteria. A band pass filter of 525 nm was used to collect the emission for green SYTOX (El-Nezami et al.,1998).

(c ) Preparation of yeast suspensions: A concentration of 10¹⁰ viable cells/ gm was determined for the probiotic yeast Saccharomyces cerevisiae through twelve decimal dilutions made in saline solution. Organisms were seeded in Petri dishes containing Sabourad broth and incubated for 72 h at 25°C and then counted for viability (Tejada de Hernandez, 1985).

(d) Inhibition experiments:

Inhibition of mold growth in the presence of S. cerevisiae, Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705 was performed on PDA plates according to the method of Bjornberg and Schnurer (1993).

(2) In Vivo Study

Eight groups of normal rats (6 rats each) were used in this experiment, the first four groups (1, 2, 3, and 4) were orally administrated Lactobacillus rhamnosus strain GG (LGG) , Lactobacillus rhamnosus strain LC-705(LC 750) and S.cerevisiae (10 ml / Kg b.wt.; 10⁹ cfu /1 ml distilled water), while the control group (group 4) was given distilled water (10 ml / Kg b.wt.). These oral doses were depending on several preliminary studies which had no mortalities among AFB1 co-administration with the three tested probiotics. The second four groups (5, 6, 7 and 8) were orally administrated aflatoxins (2 mg / Kg b.wt. in 10 ml corn oil) AFB1 alone and/or co-administered with LCC, LG 705 10⁹ bacteria /ml and Sc 100 µg cell /ml water (at previously mentioned concentrations). The number of rats in group 5 was increased to 10 animals ( AFB1- treated rats) in order to avoid potentially not being able to attain data because of the potential for an increase in animal mortality rate among this group.

After 15 days of the daily treatment; all animals were sacrificed and blood samples were collected from the retro-orbital venus plexus in- to test tubes : the first was heparinized (imbedded in ice box) for determination of reduced glutathione (GSH) in whole blood by Ellman’s method (1959) ; the second allowed for serum separation (1500 rpm for 15 min) to assess the following biochemical analysis: γ-glutamyl transferase (GGT) according to Rosalki et.al., (1970), aspartate and alanine aminotransferase (AST and ALT) activities and creatinine according to the method of (Thefeld et al., 1974), and BUN (Henri et al.,1974) using BioMerieu kits.

Histopathological examination:

Immediately after sacrifice, a sample of the liver was fixed in 10% formalin. The washed tissue was dehydrated in descending grades (70 % -100 %) of alcohol and finally cleared in zylene. The tissue was embedded in paraffin wax. Sections were cut at 5µm thickness and stained with haematoxylin and eosin (Drury et al., 1980). The sections were then, viewed under a light microscope for Histopathological examination. Histochemical examination of DNA content was performed using the Feulgen technique (Gardikas and Israels, 1948).

Statistical analysis: The obtained results were analyzed by ANOVA (one or two-way) using the Microsoft Excel (Redmond, WA) software package.

RESULTS

S. cerevisiae greatly reduced A.flavus growth in PDA media at concentration of 10⁶ CFU/g from the first week of incubation. Meanwhile, probioteic bacteria did not alter A.flavus-growth during the 1st week of incubation. The presence of LGG in PDA media resulted only in a weak inhibitory effect. A.flavus-growth was not affected by the
presence of LC 705 until two weeks of incubation.

It was clearly demonstrated that the addition of the tested biocontrol microorganisms to YES media containing A. falvus resulted in significant inhibition AFs production and mycelium growth in a variable degrees of inhibition as shown in Table (1). S. cerevisiae and LGG had a similar inhibitory effect on total AFs production (-98.8% & -98.8%). As well as, LC 705 had lesser extend to inhibit AFs production (-85.2%).

Table (1) shows that S. cerevisiae and LGG were nearly similar in their inhibitory effect on AFB1 and AFB2 production (S. cerevisiae: -99.2 % & -98.6% ; LGG : -99.6 & -99.1%) respectively, versus A. flavus –YES media alone. LC 705 also inhibited production of AFB1 & AFB2 to -85% & -84%, respectively.

Moreover, production of AFG1 & AFG2 were inhibited by all studied biocontrol microorganisms. Particularly, LGG at the concentration tested was the most potent inhibitor for AFG1 production when compared to SC or LC 705. The percentage of inhibition of AFG1 and AFG2 production were (S. cerevisiae : -98.4 % & -97.6% , LGG : -99.2 % & -93.3% and LC 705: -85.6 % & -87.0%) respectively, when compared with their respective controls(Table 1).

The dry weight of A. flavus –mycelium is also decreased significantly in YES media containing S. cerevisiae, LGG or LC 705 ( -75.4% , -76.1% and -47%) , respectively.

It was found that S. cerevisiae and LGG were very similar in their effect on AF-production and mycelium growth; in which they possess most effective agents than the effect of LC 705 in A. flavus -YES media.

Figure 1

![Figure 1](image)

Table (1) : Effect of () , GG (LGG) and LC705 (LC 705)on aflatoxins and mycelium production by in YES medium

<table>
<thead>
<tr>
<th>Groups</th>
<th>B1</th>
<th>B2</th>
<th>G1</th>
<th>G2</th>
<th>Total mycelium growth (dry wt./mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.cerv</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGG</td>
<td>B</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 705</td>
<td>C</td>
<td>C</td>
<td>D</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

One-way ANOVA

The different capital letters superscripts are significantly different between treatments at P< 0.05

In vivo results:

Biochemical results (Table 2) showed the effect of different treatments on liver enzymes (ALT, AST and GGT), kidney function tests (BUN and creatinine) and on GSH level in the blood of all groups.

Single treatment with probiotic bacteria (LGG and LC705) showed no-significant changes in ALT, AST and GGT activities comparing with the control group. Liver enzymes were significantly inhibited by S.cerevisiae treatment alone. BUN values did not changed than the control values in groups treated with S. cerevisiae or with the two strains of probiotic bacteria. Whereas, there was a significant decrease in creatinine and significant increase in GSH levels in these single treated groups when compared with the controls or the other treated groups (Table 2).

Rats treated with AFB1 alone, showed significant elevation in amino-transferases activities. This elevation was normalized by co-administration with LGG, while the combined treatment with S. cerevisiae and LC 705 ameliorated transaminases alterations toward the normal levels.

AFB1-administration also affected the Kidney functions; BUN and creatinine values elevated significantly than other treatments (Table 2). Combined treatments with S.
Saccharomyces Cerevisiae And Probiotic Bacteria Potentially Inhibit Aflatoxins Production In Vitro And In Vivo Studies

cerevisiae, LGG and LC 705 concomitant with AFB1 resulted significant decrease in BUN values than AFB1-treatment alone but still increased than normal groups. Creatinine had similar results as obtained with BUN values, in which the combined treatments of probiotic bacteria (LGG & LC 705) with AFB1 resulted in a significantly decreased creatinine level than AFB1 alone; except the group treated with S. cerevisiae plus AFB1, its creatinine value was normalized and showed non-significant changes when compared to the control group.

GSH levels were depleted in rats administered AFB1 alone, and it was increased significantly by the combination with LGG, LC 705 and S. cerevisiae to become more than normal control values (AFB1+ S. cerevisiae > AFB1+ LC 705 > AFB1+ LGG).

**Figure 2**

Table (2) : Effect of different biologically active microorganisms on Aflatoxicosis (AF) in rats administered 2 mg/Kg.b.wt./Os of media containing AFB1 alone and/or ( ), GG (LGG) and LC705 (LC 705). (n= 6 rats / group, means ± SE of the ANOVA – one way.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal groups</th>
<th>AFB1- treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/ml)</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>35.67±</td>
<td>33.3±</td>
</tr>
<tr>
<td>LC 705</td>
<td>32.5±</td>
<td>31.67±</td>
</tr>
<tr>
<td>S.cerviae</td>
<td>61.67±</td>
<td>38.17±</td>
</tr>
<tr>
<td>AFB1</td>
<td>43.12±</td>
<td>41.5±</td>
</tr>
<tr>
<td>AST (IU/ml)</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>46.37±</td>
<td>42.3±</td>
</tr>
<tr>
<td>LC 705</td>
<td>42.3±</td>
<td>41.67±</td>
</tr>
<tr>
<td>S.cerviae</td>
<td>52.27±</td>
<td>51.82±</td>
</tr>
<tr>
<td>AFB1</td>
<td>48.58±</td>
<td>45.1±</td>
</tr>
<tr>
<td>GOT (IU/ml)</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>0.99±</td>
<td>0.92±</td>
</tr>
<tr>
<td>LC 705</td>
<td>0.9±</td>
<td>0.86±</td>
</tr>
<tr>
<td>S.cerviae</td>
<td>2.95±</td>
<td>1.51±</td>
</tr>
<tr>
<td>AFB1</td>
<td>1.33±</td>
<td>0.84±</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>36.33±</td>
<td>41.67±</td>
</tr>
<tr>
<td>LC 705</td>
<td>42.3±</td>
<td>41.67±</td>
</tr>
<tr>
<td>S.cerviae</td>
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<td>51.82±</td>
</tr>
<tr>
<td>AFB1</td>
<td>48.58±</td>
<td>45.1±</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>22.55±</td>
<td>23.47±</td>
</tr>
<tr>
<td>LC 705</td>
<td>21.2±</td>
<td>21.5±</td>
</tr>
<tr>
<td>S.cerviae</td>
<td>40.00±</td>
<td>38.18±</td>
</tr>
<tr>
<td>AFB1</td>
<td>39.5±</td>
<td>39.5±</td>
</tr>
<tr>
<td>Creatinine</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>LGG</td>
<td>0.54±</td>
<td>0.48±</td>
</tr>
<tr>
<td>LC 705</td>
<td>0.48±</td>
<td>0.48±</td>
</tr>
<tr>
<td>S.cerviae</td>
<td>0.48±</td>
<td>0.48±</td>
</tr>
<tr>
<td>AFB1</td>
<td>0.48±</td>
<td>0.48±</td>
</tr>
</tbody>
</table>

Histochemical staining revealed that liver sections stained with Feulgen stain showed normal content of DNA in the nucleus (Fig. 1 E). The liver of rats treated with AFB1 only showed decrease in DNA content (Fig. 1 F). Liver sections examined after treatment with LGG, LC 705 and Yeast plus AFB1 showed improvement in the DNA content as compared with AFB1-treated groups (Figs. 1 G and H).

Histopathological results supported the obtained data from biochemical studies. Histopathological examination of the liver revealed the following changes. The control groups presented liver with normal architecture (Fig.1 A). No histopathological changes were observed in the liver of rats treated with S.cerevisiae, LGG or LC 705. Aflatoxine treated group showed mononuclear cellular infiltration around the portal tracts. Histopathological observations include a large amount of necrosis, vacuolar degeneration, with loss of normal architecture, mild fibrosis and fatty changes (Fig. 1 B). In groups treated with LGG, LC 705 and S. cerevisiae plus AFB1, their liver exhibited an almost normal architecture with little amount of inflammatory cells and congestion of the central vein (Fig. 1C and D).

Figure (2 A) shows normal histological structure of the kidney of control rat. Kidneys of groups treated with LGG, LC 705 and Yeast showed normal kidney histology. AFB1 treated group caused vacuolar degeneration, cellular swelling, and pyknotic nuclei were observed in the epithelial cells of proximal convoluted tubules. Dilated and congested blood vessels are a prominent feature, together with many large areas of interstitial hemorrhage, in AFB1-treated animals (Fig. 2 B). Microscopic examination of renal tissue in animals treated with LGG, LC 705 and Yeast plus AFB1 showed normal appearance of kidney tissue and degeneration of some proximal convoluted tubules. Mild congestion of blood vessels was also observed.
Saccharomyces Cerevisiae And Probiotic Bacteria Potentially Inhibit Aflatoxins Production In Vitro And In Vivo Studies

Figure 3
Fig.1A: Section in the liver of control rat showing central vein (CV), hepatocytes with nucleus (N) and blood sinusoids(S). B: Sections in the liver of rat treated with AFB1 showing loss of normal architecture, cellular infiltration (Li) and fatty changes (F). C and D: Sections in the liver of rat treated with LGG, LC 705 and plus AFB1 showing almost normal architecture with little amount of inflammatory cells and congestion of central vein. E: Section in the liver of control rat showing normal content of DNA in the nucleus. F: Sections in the liver of rat treated with aflatoxin B1 showing decrease in DNA content. G and H: Sections in the liver of rat treated with LGG, LC705 and plus AFB1 showing increase in DNA content compared with AFB1-treated group.

Figure 4
Fig 2 A: Section in the kidney of control rat showing glomerulus (G), and renal tubules (T) B: Sections in the kidney of rat treated with AFB1 showing vacuolar degeneration (long arrow), interstitial hemorrhage (arrow head), and hemorrhage of glomerulus (G). C and D: Sections in the kidney of rat treated with LGG, LC 705 and plus AFB1 showing almost normal architecture with little amount of degeneration of some proximal convoluted tubules (arrow head). Mild congestion of blood vessels was also observed in D (arrow head). E: Section in the kidney of control rat showing normal content of DNA in the nucleus. F: Sections in the kidney of rat treated with aflatoxin B1 showing decrease in DNA content in glomerulus and renal tubules. G and H: Sections in the kidney of rat treated with LGG, LC 705 and plus AFB1 showing increase in DNA content in glomerulus and renal tubules compared with AFB1-treated group.

DISCUSSION
During the past decades, several studies have suggested that Lactobacillus rhamnosus strain GG (LGG) and L. rhamnosus strain LC-705 (LC705) and yeast (Saccharomyces cerevisiae)
Saccharomyces Cerevisiae and Probiotic Bacteria Potentially Inhibit Aflatoxins Production In Vitro and In Vivo Studies

have the ability to bind mutagens using in vitro methods (Shetty et al., 2007). In vitro studies have shown that probiotic bacteria and S. cerevisiae significantly reduced germination of Aspergillus flavus spores (Smith 1978). Subsequently, they inhibited mold growth and decreased mycelial dry weight. These findings may due to the presence of aromatic compounds produced by S. cerevisiae such as: organic acids, esters, alcohols, aldehydes, lactones and terpenes (Janssens et al., 1992) and probiotic metabolites (lactic, acetic formic, propionic acids and hydrogen peroxide) could to reduce the fermentation process (Lindgren and Dobrogosz 1990).

Several investigators report that LGG and LC-705 significantly inhibit ~ 99% of AFB1 production in vitro (El-Nezami et al., 1998, Reddy et al., 2009); whereas Oatley et al. (2000) observed that Bifidobacteria bound to ~ 60% of AFB1 when added to the growth media. Moreover, Madrigal-Santillan et al., (2006) found that S. cerevisiae had potent adsorbent capacity without modification in AFB1 molecule when added to AFB1-contaminated corn. In vivo studies examine, the oxidative stress induced by AFB1-administration which caused significant alteration in the studied biochemical parameters including, reduced GSH level and ~ 30% of a mortality rate amongst AFB1-treated animals (Anwar et al., 2008, Gursoy et al., 2008 and Zhou et al., 2001). Histopathological results demonstrated that livers and kidneys of animals treated with AFB1 showed congestion of blood vessels, cytoplasmic vacuolization of the hepatocytes, fatty degeneration and leukocytic infiltrations (Gursoy et al., 2008; Sakr et al., 2006). Moreover, total protein contents and nucleic acids (DNA and RNA) in the liver and kidney tissues were significantly depleted by AFB1 treatment. These findings are due to AFB1 binding to DNA and chromosomal proteins, consequently it inhibit RNA synthesis (Appleton and Campbell, 1983; Lin et al., 1978) and altered enzymatic processes such as glyconeogenesis, Kreb’s cycle and fatty acid synthesis (Lesson et al., 1995 and Garcia et al., 2009). Co-administration of probiotic bacteria (LGG and LC705) and S. cerevisiae together with AFB1 significantly reduced the toxic effect induced by the mycotoxin. No deaths were recorded among these groups, there was significant increase in GSH level, and liver and kidney functions were improved. Similarly, liver and kidney structure was preserved as demonstrated by histopathological examination.

Reduced glutathione (GSH) is the main component of endogenous non-protein sulphydryl pool that scavenges free radicals in the cytoplasm (Shaw et al., 1990). However, antioxidants restore the cellular defense mechanisms, block lipid peroxidation, and thus protect against the oxidative tissue damage (Toklu et al., 2006).

Many previous studies suggest that the presence of B-D-glucans in adequate percentage on the cell wall of S. cerevisiae spp., were responsible for the immuno-modulatory effect (Vetvicka 2001, Brown and Gordon 2003). As well, B-D-glucans have antioxidant activity, scavenging the free radicals and reducing DNA-oxidative damage (Oliveira et al., 2009 and Sener et al. 2007). The therapeutic effect of S. cerevisiae is attributed to its release of protease that causes cleavage of the toxins and diminishes their binding capacity to receptors located on the colonic brush-border membrane (Castagliuolo et al., 1999), This findings supported our results in preventing aflatoxicosis in the current experimental study.

The potential function of the tested probiotic strains (LGG and LC705) may be due to their binding ability to the toxins or metabolically transforming them into non-toxic degradation products (Hooper et al., 2001; Zhou et al., 2001). Yan et al. (2007) found that LGG prevent cytokine-induced apoptosis in intestinal epithelial cells, the intestinal epithelial tight junction (TJ) prevents the diffusion of toxins from gastrointestinal lumen into the tissue (Anderson et al., 1995). Moreover, AFB1-oral administration disrupts TJ and adherents junction proteins due to the presence of free radicals and inflammatory process induced by the mycotoxin ingestion (Gratz et al., 2006, Seth et al., 2008, Shifflett et al., 2005).

CONCLUSION

Probiotic bacteria and S. cerevisiae inhibited A. flavus growth in vitro and they diminished aflatoxicosis in vivo study.

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References

Lactobacillus rhamnosus Strain GG Modulates Intestinal


39. Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg,
Saccharomyces Cerevisiae And Probiotic Bacteria Potentially Inhibit Aflatoxins Production In Vitro And In Vivo Studies

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