Cytogenetic and Biochemical Studies On the Protective Role of *Rhodotorula glutinis* And its Autoploidy Against the Toxic Effect of Aflatoxin B_1 in Mic

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Abstract: The present study was designed to investigate the effect of *Rhodotorula glutinis* and its autoploidy on cytogenetic and biochemical analyses and to evaluate the protective role of these yeasts against aflatoxin B_1- in mice. Eight groups of male mice were used. Three of them were treated with three strains (wild type G1 and two autoploidy (G2 and G3) of *Rhodotorula glutinis*. In addition, one group was treated the suspension of growth medium of yeasts (served as control),three groups were treated with the G1, G2 and G3 after an hour of injection with the aflatoxin. Cytogenetic analyses revealed that the treatment with the wild type of yeast (R.g, G1) and its two mutants (G2 and G3) had improved the genetic materials in normal somatic and germinal animal cells by decreasing chromosome aberrations and increasing the mitotic and meiotic indices compared to control group. On the other hand, the chromosomal aberrations were more frequent of mitotic and meiotic indices were depressed in the animals treated with aflatoxin alone. In contrast, the frequencies of the chromosome aberrations were significantly decreased and mitotic and meiotic indices were increased in animals treated with the wild type (G1) and its two autoploidy (G2 and G3) plus aflatoxin B_1. Biochemical results showed that the treatment with yeast strains especially the treatment with two autoploidy G2 and G3 did not induce changes in liver and kidney functions in normal animals. The treatment with the three strains wild type G1 and two autoploidy G2 and G3 had enhanced the TP compared to control group. The treatment with aflatoxin B1 significantly increased the liver enzymes (GGT, ALT and AST), kidney function markers(uric acid and creatinine) and significantly decreased the TP compared to control group. In contrast, the treatment with yeast strains plus aflatoxin B_1 succeeded in diminishing the elevated value of liver enzymes and kidney functions and normalized TP level. [Nature and Science. 2010;8(5):28-38]. (ISSN: 1545-0740).

Keywords: Aflatoxin,*Rhodotorula glutinis*,autoploidy,chromosome aberrations,biochemistry.

1-Introduction

Fungal deterioration of stored seeds and grains is a chronic problem in the Egyptian storage system because of the tropical hot and humid climate. Harvested grains are colonized by various species of *Aspergillus*, under such conditions leading to deterioration and mycotoxin production. *Aspergilli* are the most common fungal species that can produce mycotoxins in food and feedstuffs. Mycotoxins are well known for their health - hazardous effects in human beings and animals (Probst *et al.,* 2007; Reddy and Raghavender, 2007; Reddy *et al.,*2009). Among all the mycotoxins, particularly aflatoxin is the most toxic form for mammals. This aflatoxin (AF) is a group of structurally similar polysubstituted coumarins produced by the common moulds *Aspergillus flavus* and *Aspergillus parasiticus*. The main biological effects of aflatoxins are carcinogenicity, immunosuppression, and teratogenicity (Betina, 1989; Abdel- Wahhab *et al.*, 1998; Santos *et al*., 2001; Reddy *et al*., 2009). Moreover, aflatoxin is a potent mutagenic food component and has been found to be an inhibitor factor in mitosis (Hall *et al*., 1988 ; Abdel- Wahhab *et al*., 1998). It is metabolized by the mixed function oxidase system to a number of hydroxylated metabolites and to aflatoxin 8, 9 epoxide which binds to DNA, forming covalent adducts (Busby and Wogan, 1984) and disturbs DNA replication causing chromosomal aberrations (Sinha and Prasad, 1990). Many trails were conducted for minimizing the effects of mycotoxins on human and animal health as well as increasing the animal productivity and performance. In animals, many additives such as specific types of clay could be added to their food to bind or reduce the harmful effects of mycotoxins (Ramos *et al*., 1996 and Abdel – Wahhab *et al*., 1998). The situation is different

in humans, especially after the discovery of yeasts which could be added to their food in order to maintain their health by contributing in fighting toxicants and illness (Koleva *et al*., 2008). Recently, an antagonistic yeast strain of *Rhodotorula glutinis* have been reported as an effective biocontrol agent (in vivo) against post-harvest decay of fruits and vegetables (Qin *et al*., 2003; Zhang *et al*., 2007; Zhang *et al*., 2008; Malisorn and Suntornsuk, 2008). Moreover, the yeast strain of *Rhodotorula glutinis* and its autoploidy have been reported as an effective biocontrol agent (in vivo) and (in vivo) against grey mould of greenhouse sweet pepper (Haggag, *et al*., 2005). However, there are no informations available (to our knowledge) about the use of *Rhodotorula glutinis* or its autoploids as protective agents on mammalian cells (in vivo) against the toxic effect of mycotoxins. Therefore, the aim of the present study is to investigate the protective role of *Rhodotorula glutinis* and its two autoploidy against aflatoxin B_1- intoxication in mice by using biochemical and cytogenetic analyses.

2. Materials and Methods

2.1. Materials

2.1.1. Mice: Random bred of forty - eight adult male Swiss albino mice weighing about 25 grams were used. These animals were obtained from animal house laboratory, National Research Centre, Cairo, Egypt. Apparently health acceptable animals were randomly assigned into eight groups (6 mice in each).

***Environmental conditions*:**

Chosen mice were kept in stainless steel wire mesh cages on a bedding of wood chips. They were housed in an ambient temperature of 25± 3 ^{0}C, on a light/ dark cycle of 12/12 hours and supplied with mice chew and fresh water ad-libitum.

2.1.2. Mycotoxins:

Aflatoxin B_1 was purchased in a pure crystalline form from Food Toxicology and Contaminants Department, National Research Centre, Egypt. The toxin was dissolved in corn oil.

2.1.3. Types of used yeasts:

The strains of yeast G2 (Col- 1R1) and G3 (Col-1R3) have been genetically improved and isolated from the wild type yeast strains *Rhodotorula glutinis* (G1) after colchicines treatments, (Haggag, *et al*. 2005). Briefly, wild type yeast isolates were cultured in flasks containing Yeast Nitrogen Base (YNB) liquid medium supplemented with 5% glucose and incubated at 30ºC for 18 h. Then, colchicine was added to the yeast cell suspensions to give final concentrations (0.2%). The treated cell suspensions were incubated for further 18 h. The treated cells were inoculated on YMPG medium plates and incubated for 2 weeks at 30ºC. A glance on colonies grown on the agar plates showed that large colonies were always composed of large cells than that of small colonies. Consequently, large cells from about 270 initial large colonies of *R. glutinis* were selected. The types of used yeasts and their properties were shown in Table (1). The growth of the tested yeasts was estimated by measuring the optical density at 610 nm. Hemocytometer was used to count yeast cells. The cell diameter was measured microscopically using an eyepiece micrometer. Protein content in the supernatant was determined at 595 nm by Bradford(1976) method using bovine serum albumin as a standard. Protein content was determined at 595 nm.

Table 1 shows the properties of *Rhodotorula glutinis* strains (wild type and its two mutants).

GROUPS	*R. GLUTINIS* (WILD TYPE) G1	G2	G3
Asperigillus niger inhibition (inhibition zone, mm)	127	22.4	24.6
Aperigilus flavus inhibition (inhibition zone, mm)	13.6	18.9	21.7
Cell diameter (µm)	6.20 (4.12-7.15)	8.51 (6.15-11.2)	8.30 (6.00-10.0)
Cell volume (μm^3)	158.61 (90.05-230.30)	352.44 (287.25-404.3)	333.51 (255.3-307.4)
DNA content %	100 $156.1\ \mu g / 10^8$ cells	225.08	194.85
Growth rate (h^{-1})	0.0771	0.1122	0.1140
Cell concentration (OD 610 nm)	1.490	1.975	1.910
Total Protein $\mu g^{-1} / ml^{-1}$	216.8	300.7	367.8

2.2 Methods:

2.2.1. Preparation of mycotoxin and strains of yeast:

The strains of yeast (G1, G2 and G3) and growth medium were suspended in gum accacia (5% w/v).

2.2.2. Experimental design:

Eight groups of adult mice (6 animals in each) were used in this work. Three groups of them were given orally with three strains (G1, G2 and G3) of yeast (A strain/ A group). Dose of 50.000 cells of each strain/ 1 ml of solution/ 100 gm of body weight were administrated daily to each animal for 15 days. In addition, one group of animals was given orally with the suspension of growth medium (1 ml of solution/ 100 gm body weight) daily to each animal for 15 days. This group was served as control. Also, one group of

animals was given orally with aflatoxin B_1 (1 mg/ 1 kg body weight) daily to each animal for 15 days.

Moreover, three groups of animals were given orally with three strains (G1, G2 and G3) of yeast (A strain/ A group). Then after an hour of injection, the animals in each group were given orally with the toxin (1 mg/ kg b.w). The strains of yeast and toxin were given daily for 15 days.

2.2.3. Cytogenetic analyses: Two hours before sacrifice, the animals were injected with 0.5 mg of colchicine.

2.2.4. Chromosome analysis in somatic cells: Femurs sere were removed and bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared by using Preston *et al*., (1987) method. Fifty metaphase spreads per animal were analyzed for studying the chromosome aberrations.

2.2.5. Mitotic index: The mitotic activity of bone marrow cells was investigated by recording the number of dividing cells/ 1000 cells/ animal" the mitotic index".

2.2.6. Chromosome analysis in germ cells: spermatocyte cells were prepared according to **Brewen and Preston (1978)** for meiotic chromosomal analysis.

2.2.7. Meiotic index: The meiotic activity of spermatocyte cells carried out by recording the number of dividing cell/ 1000 cells/ animal"meiotic index".

2.2.8. Biochemical studies: At the end of experimental period (after 15 days) blood samples were collected from all animals from the retro-orbital venous plexus for biochemical analyses. These analyses included, serum GGT (Rosalki *et al*., 1970), ALT, AST and creatinine **(**Thefeld *et al*., 1974), total proteins (Bradford, 1976) and uric acid (Haisman and Muller, 1997).

2.2.9. Statistical analysis:

The obtained results of cytogenetic examinations or biochemical analysis were statistically analyzed by ANOVA (one or two-way) using Excel 2003 Microsoft Crop (11.5612.5606), Redmond, WA software package.

3. Results

3.1. Cytogenetic analyses:

3.1.1. Chromosome examination in bone marrow cells:

Cytogenetic results showed that the frequencies of total structural chromosome aberrations were low in the three animals groups which treated with the three yeast strains G1, G2 and G3 compared to those found of the control group (5.5, 5.5 and 5.25 vs. 6.0% respectively). However, statistical analysis for the frequencies of chromosome aberrations showed that there were no significant differences between the three yeast groups and control group. When comparing the cytogenetic results between the three yeast groups with each other, it can be seen that the proportion of frequencies of chromosome aberrations were similar and there were no significant differences between the three

3.1.2. Mitotic index: The mitotic activity (Table 2) was significantly increased in G1 and G3 groups compared with those found in the control. While mitotic activity in G2 was approximately similar with those found in the control (9.03 vs 9.95 respectively) and there were no significant differences between the two groups. Also, there were no significant differences for mitotic activities between G1 and G3. However, mitotic activity was significantly decreased in G2 compared to those found in G1 or in G3.

3.1.3. Chromosome examination in spermatocyte cells: .

Chromosome aberrations in spermatocyte cells (Table 3) consisted of autosomal univalent, sex univalent and translocation. Total chromosomal aberrations (especially autosomal univalent) were lowered in the three animal groups which treated with the three yeast strains (G1, G2 and G3) than those observed in the control group. The G3 group followed by G2 group had the lowest proportion of the total structural chromosome aberrations. However, statistical analysis showed that there were no significant differences for the frequencies of chromosome aberrations between control group and the three animal groups that treated with three yeast strains. Also, there were no significant differences for the cytogenetic results between the three groups (G1, G2 and G3) with each other.

3.1.4. Meiotic activity: The meiotic activity was significantly increased in the three animal groups which treated with the three yeast strains (G1, G2 and G3) compared to those found in the control group (11.65; 8.47 and 10.98 vs. 6.93 respectively).

On the other hand, the meiotic activity was approximately similar with those found in G3 group and there was no significant differences between the two groups. However, the meiotic activity was significantly increased in G2 group compared to those found in G1 or G3.

3.2. The protective role of yeast strains against toxic effect of aflatoxin B_1 in animal cells:

3.2.1 Chromosome examination in bone marrow cells:

The cytogenetic results (Table 2) showed that the frequencies of total structural chromosome aberrations in mice treated with AFB1, were more than those observed in the control group (12.25 vs 6.6, respectively). Statistical analysis showed that there were highly (0.001) significant differences between the AFB1 and control groups.Moreover, the frequencies of deletions and centric fusions were highly significant in AFB1 group compared to those found in control group. On the other hand, cytogenetic examination showed that the frequencies of total structural chromosome aberrations were lowered in mice treated with AFB1, plus three yeast strains (G1, G2 and G3) than those found in mice treated with AFB1, alone (9.25; 7.75 and 8.75 vs 12.25 respectively). AFG2 group followed by AFG3 had the lowest proportions of total structural chromosome aberrations. Statistical analysis showed that there were significant differences between AFG1 , AFG2 , AFG3 groups and AF group. When comparing the cytogenetic results between groups of AFG1, AFG2 or AFG3 with each other, it was found that the frequencies of total structural chromosome aberrations were significantly decreased in AFG2 group than those found of AFG1 group. However, there were no significant differences between AFG1 and AFG3 groups. Also, the treatment with G2 plus AF significantly decreased the frequencies of deletions compared to AFG1 or AFG3 groups. Moreover, the treatment with G3 plus AF significantly decreased the frequencies of deletions than the treatment with G1 plus AF. On the other hand, statistical analysis showed that there were no significant differences for the frequencies of each of gaps, Breaks, centric fusion and end to end association between the three groups AFG1, AFG2 and AFG3 with each other.

3.2.2. Mitotic index: cytogenetic examination observed that the mitotic activity has been increased in mice treated with AF plus G1 or G2 or G3 compared to those found in mice treated with AF alone (9.25, 8.96 and 9.0 vs. 8.87 respectively). However, statistical analysis showed that there were no significant differences for mitotic activity between AF group and AFG1 or AFG2 or AFG3 groups. Also, there were no significant differences for mitotic activity between animal treated with AF plus G1 or AF plus G2 or AF plus G3 with each other.

3.2.3. Chromosome examination in spermatocyte cells:

Cytogenetic results showed that the mice which treated with AF has more frequencies of autosomal univalent (AU), six univalent (SU), translocation (Tr) and total structural chromosome (TSA) aberrations than the control group. Statistical analysis showed that there were significant differences between AF and control groups for the frequencies of AU, Tr and TSA. However, there were no significant differences for the frequencies of SU between AF and control groups.

On the other hand cytogenetic examination found that the treatment of with G1 or G2 or G3 plus AF significantly decreased the frequencies of each of total structural chromosome aberrations and autosomal univalent comparing with the treatment of AF alone (4.0, 5.0 and 4.5 vs. 875, respectively or 2.25, 1.5 and 2.0 vs. 5.25, respectively).

Also, the treatment with G1 or G3 plus AF significantly decreased the frequencies of translocation comparing with the treatment of AF alone. However, there were no significant differences for the frequencies of translocation between animals treated with G2 plus AF and those treated with AF alone. Moreover, there were no significant differences for the frequencies of sex univalent between animals treated with G1 or G2 or G3 plus AF and animals treated with AF alone.

3.2.4. Meiotic activity: Cytogenetic examination showed that the meiotic activity decreased in animals treated with AF than those observed in the control group (5.75 vs. 6.93, respectively). However, this decrease was not significant. On the other hand, the meiotic activity in animals treated with G1 or G2 or G3 plus AF significantly increased than those found of animals treated with AF alone (11.05, 8.46 and 10.52 vs. 5.75, respectively).

The animals treated with G1 plus AF followed by G3AF had the highest proportion of meiotic activity and there were no significant differences between the two groups. However, the meiotic activity was significantly decreased in AFG3 compared to those found in AFG1 or AFG3 groups.

3.3. Biochemical analysis:

Biochemical analysis of serum (table 4) showed insignificant changes in liver markers (GGT, ALT, AST) in normal groups treated with G2 and G3 when compared with the control values. While treatment with G1 significantly elevated GGT, ALT and AST activities. AST values did not changes by all treatment with G1, G2 and G3. However, serum TP level was normal in groups of animals treated with G1 and G3 ; whereas, G2- treatment significantly increased TP value than the other treated groups.

Kidney functions revealed that the tested yeast Rh1(G1) and its two mutants (G2 and G3) had no effect on creatininee concentration. As well as, uric acid values did not altered in groups of mice administrated G2 and G3 when compared with the control group. While, G1 showed significant elevation in uric acid level comparing with the control or with the two mutants (G2 and G3).

AFB1- treated group had significant increase in liver enzymes: GGT, ALT, AST and kidney functions:creatinine and uric acid. Moreover, AFB1-administration caused significant decrease in TP value when compared with the normal control animals or with the combined treated with the three yeasts.

Generally, the combined treatment of AFB1 with G1, G2 and G3 significantly inhibited the liver enzymes, as well as, they decreased the elevated values of creatinine and uric acid in kidney functions (table 1).

Mice treated with the wild yeast suspension concomitant with AFB1 (G1+ AFB1) significantly decreased GGT, ALT, AST and creatinine than AFB1 – treatment alone, while it had no effect on TP and uric acid values.

Furthermore, the treatments with G2 and G3 in combination with AFB1 nearly have similar effects on liver and kidney functions; both of them significantly decreased the elevated values induced by AFB1(GGT, ALT, AST, creatinine and uric acid) and significantly increased serum TP towered the normal values when compared with AFB1- treatment alone.

Table (2): Effect of treatment of *Rhodotorula glutinis* (G1) and its two autoploidy (G2 and G3) with or without aflatoxin B_1 on chromosomes and mitotic index in bone marrow cells of mice.

Treatment	Structural abnormalities					Total structural chrom. Aberrations		Mitotic Index
	Gaps	Deletions	Breaks	Centric fusions	End to end associations	including gaps	excluding gaps	
Control	1.25 ± 0.2 ^{A}	4.0 ± 0.4 ^{A}	6.93 ± 0.32 ^{A}	6.93 ± 0.32 ^{A}	6.93 ± 0.32 ^{A}	6.93 ± 0.32 ^{A}	6.93 ± 0.32 ^{A}	9.95 ± 0.52 ^{A}
G1	1.25±0.3 ^{A}	4.0 ± 0.4 ^{A}	11. 65± 0.43 ^{C}	11. 65± 0.43 ^{C}	11. 65± 0.43 ^{C}	11. 65± 0.43 ^{C}	11. 65± 0.43 ^{C}	11. 2± 0.34 ^{B}
G2	1.0±0.4 ^{A}	3.75± 0.3 ^{A}	8.47± 0.51 ^{B}	8.47± 0.51 ^{B}	8.47± 0.51 ^{B}	8.47± 0.51 ^{B}	8.47± 0.51 ^{B}	9.03± 0.88^{A}
G3	0.75±0.2 ^{A}	3.75± 0.3 ^{A}	10.98± 0. 4 ^{C}	10.98± 0. 4 ^{C}	10.98± 0. 4 ^{C}	10.98± 0. 4 ^{C}	10.98± 0. 4 ^{C}	11.32± 0. 53 ^{B}
A F	1.5±0.28 ^{A}	7.75± 0.62 ^{C}	5.75± 0.72 ^{D}	5.75± 0.72 ^{D}	5.75± 0.72 ^{D}	5.75± 0.72 ^{D}	5.75± 0.72 ^{D}	8.87± 0. 38 ^{A}
A F 1+ G1	0.5±0.3 ^{A}	7.5± 0.5 ^{C}	11.05± 0.67 ^{C}	11.05± 0.67 ^{C}	11.05± 0.67 ^{C}	11.05± 0.67 ^{C}	11.05± 0.67 ^{C}	9.25± 0. 25 ^{A}
A F 2+ G2	1.5±0.3 ^{A}	4.25± 0.3 ^{A}	8.47± 0.32 ^{B}	8.47± 0.32 ^{B}	8.47± 0.32 ^{B}	8.47± 0.32 ^{B}	8.47± 0.32 ^{B}	8.96± 0.41 ^{A}
A F 3+ G3	0.5±0.3 ^{A}	5.75± 0.47 ^{B}	10.52± 0.16 ^{C}	10.52± 0.16 ^{C}	10.52± 0.16 ^{C}	10.52± 0.16 ^{C}	10.52± 0.16 ^{C}	9.0± 0.42 ^{A}

ANOVA single way. The different alphabetical superscripts are significantly different at $p \leq 0.05$.

Table (3): Effect of treatment of *Rhodotorula glutinis* (G1) and its two autoploidy (G2 and G3) with or ithout flatoxin B_1 on chromosomes and meiotic index in spermatocyte cells of mice.

Treatment	Structural abnormalities			Total chromosomal aberrations	Meiotic index
	Autosomal univalent	Sex univalent	Translocation		
Control	1. 25± 0.25 ^{AB}	1. 5± 0.28 ^{A}	0.5± 0.28 ^{A}	3.25± 0.25 ^{AB}	6.93 ± 0.32 ^{A}
G1	1. 0± 0.4 ^{AB}	1. 5± 0.28 ^{A}	0. 5± 0.28 ^{A}	3. 0± 0.4 ^{AB}	11. 65± 0.43 ^{C}
G2	1.0± 0.4 ^{AB}	1.25± 0.25 ^{A}	0.5± 0.28 ^{A}	2.8± 0.62 ^{AB}	8.47± 0.51 ^{B}
G3	0.75± 0.25 ^{A}	1.25± 0.47 ^{A}	0.5± 0.28 ^{A}	2.5± 0.64 ^{A}	10.98± 0. 4 ^{C}

A F	5.25± 0.47 ^{C}	2.0± 0.57 ^{A}	1.5± 0.28 ^{B}	8.75± 0.47 ^{E}	5.75± 0.72 ^{A}
A F 1+ G1	2.25± 0.47 ^{B}	1.25± 0.25 ^{A}	0.5± 0.3 ^{A}	4.0± 0.4 ^{BCD}	11.05± 0.67 ^{C}
A F 2+ G2	1.5± 0.28 ^{AB}	2.0± 0.4 ^{A}	1.5± o.3 ^{B}	5.0± 0.4 ^{D}	8.47± 0.32 ^{B}
A F 3+ G3	2.0± 0.4 ^{AB}	2.0± 0.4 ^{A}	0.5± 0.3 ^{A}	4. 5± 0.28 ^{CD}	10.52± 0.16 ^{C}

ANOVA single way. The different alphabetical superscripts are significantly different at $p \leq 0.05$.

Table(4): Effect of treatment of *Rhodotorula* glutinis (G1) and its two autoploidy (G2 and G3) with or without Aflatoxin B_1on serum biochemical analysis in mice.

Group	Control	G1	G2	G3	AF	AF+G1	AF+G2	AF+G3
GGT IU/ ml	1.42 ± 0.15 ^{A}	2.8 ± 0.2 ^{B}	1.46 ± 0.13 ^{A}	1.93 ± 0.07 ^{A}	5.74 ± 0.24 ^{C}	4.1 ± 0.17 ^{D}	3.84 ± 0.18 ^{D}	3.92 ± 0.19 ^{D}
ALT IU/ ml	28.7± 0.76 ^{A}	37.3 ± 1.01 ^{B}	28. 37± 0.77 ^{A}	29. 06± 0.93 ^{A}	54. 25± 1.61 ^{C}	41. 81± 1.89 ^{D}	40. 73± 1.37^{D}	39. 15± 1.47 ^{BD}
AST IU/ ml	49.4± 1.42 ^{A}	51.7± 1.47 ^{A}	49.3± 1.01 ^{A}	48.8± 1.03 ^{A}	99.34± 2.33 ^{B}	83.98± 1.18 ^{C}	80.22± 2.01 ^{C}	64.48± 1.09^{D}
TP IU/ ml	6.99± 0. 08 ^{AE}	7.24± 0.18 ^{A}	8.09± 0. 2 ^{B}	7.17± 0. 16 ^{A}	5.85± 0. 26 ^{C}	6.37± 0. 2 ^{CD}	6.78± 0. 21 ^{AD}	6.5± 0. 25 ^{DE}
Creatininee IU/ ml	0.45± 0.01 ^{A}	0.52± 0.02 ^{AD}	0. 51± 0.02 ^{A}	0.49± 0.02 ^{A}	1.87± 0.11 ^{B}	0.82± 0.02 ^{C}	0.73± 0.02 ^{C}	0.64± 0. 01 ^{CD}
Uric acid IU/ ml	3.0± 0.1 ^{A}	4.1± 0.23 ^{B}	3.07± 0.19 ^{A}	3.22± 0.18 ^{A}	5.87± 0.16 ^{C}	5.17± 0.19 ^{C}	4.42± 0.17 ^{B}	4.33± 0. 25 ^{B}

T.C.A= total chromosome aberrations
M.I.= mitotic activity

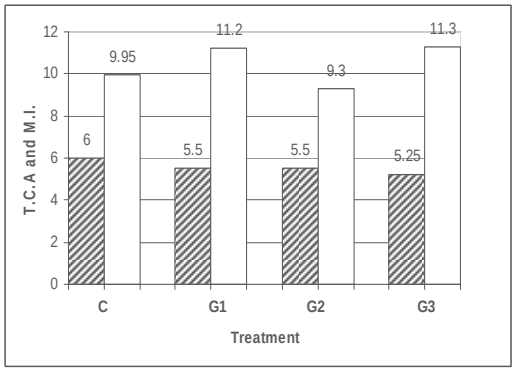

Fig.1: The effect of treatment with the three strains of yeasts on chromosome aberrations and mitotic activity in normal somatic animal cells.

C= control. G1= wild type strain of yeast.

G2= Co1-1R1. G3= CoI-1R3.

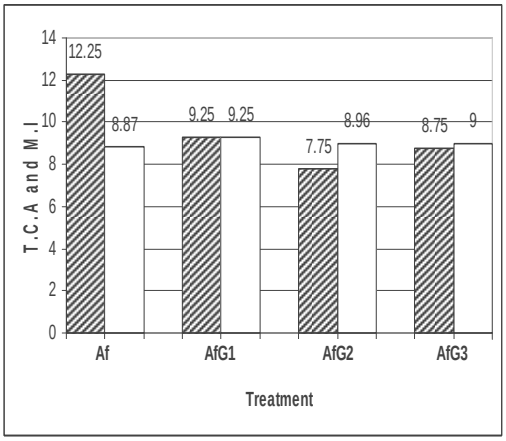

Fig. 2: The effect of treatment with the three strains of yeasts as protective agents against the mutagenecity of Af in somatic animal cells.

C= control. G1= wild type strain of yeast

G2= Co1-1R1. G3= CoI-1R3.

Af = aflatoxin. Af G1= aflatoxin plus G1.

Af G2= aflatoxin plus G2. Af G3= aflatoxin plus G3.

T.C.A= total chromosome aberrations
M.I.= meiotic activity

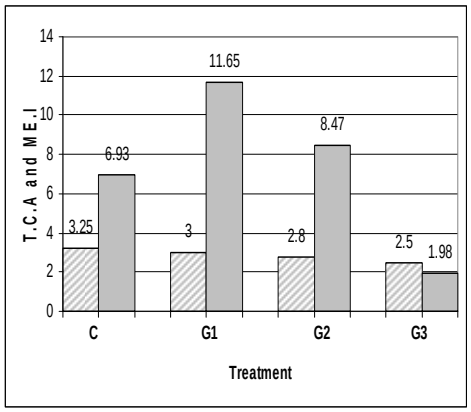

Fig.3: The effect of treatment with the three strains of yeasts on chromosome aberrations and meiotic activity n normal germinal animal cells.

C= control. G1= wild type strain of yeast

G2= Co1-1R1. G3= CoI-1R3. Af = aflatoxin

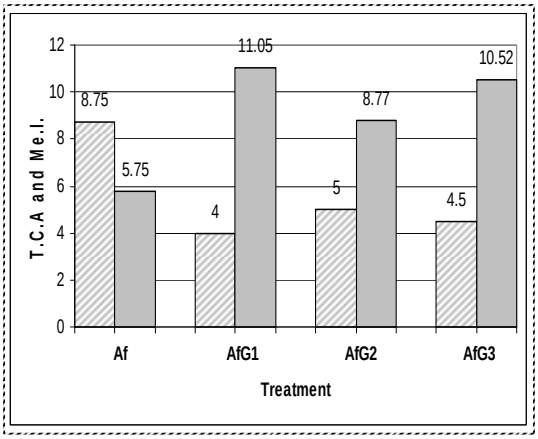

Fig.4: The effect of the three strains of yeasts on chromosome aberrations and meiotic activity in germinal animal cells.

C= control. G1= wild type strain of yeast

G2= Co1-1R1. G3= CoI-1R3.

Af = aflatoxin. Af G1= aflatoxin plus G1.

Af G2= aflatoxin plus G2. Af G3= aflatoxin plus G3.

4. Discussion

In the present study, cytogenetic analyses revealed that the treatment with wild type strain of yeast (R.9, G1) and its mutants (G2 and G3) in normal somatic and germinal animal cells has improved the genetic materials by decreasing chromosome aberrations and increasing the mitotic and meiotic indices. To our knowledge the effect of R.9 (G1) or its mutants on cytogenetic and biochemical analyses in animal cells (in vivo) is not previously studied. Also, the biochemical results found that the treatment with R. yeast strains especially the treatment with G2 and G3 did not induce changes in liver and kidney functions in normal animals. The treatment with the three strains (G1, G2 and G3) has enhanced the TP compared to control group. However, the safe treatment especially with G2 and G3 strains and some enhancement of liver and kidney functions as well as the improvement of genetic materials by treatment with the three strains of yeast which showed in the present study may be due to that the yeast cells contain high amounts of carotenoids, vitamins, minerals and essential amino acids (Hussein *et al*., 1996). These constituents are considered as antioxidant agents (Omara *et al*., 2009). The antioxidant defense system has both enzymatic and nonenzymatic components that prevent free radical formation, remove radicals before damage can occur, repair oxidative damage, eliminate damaged molecules and could be effective means for preventing changes in liver and kidney functions as well as they are protective agents against inducing mutagenic effects in genetic materials (Chandra and Sarchielli, 1993; Kubena and Mc Murray, 1996; Cramer *et al*., 2001; Nicolle *et al*., 2003; Devaraj *et al*., 2008).

In previous cytogenetic studies, the carotenoids were safe on mammalian genetic materials (Agarwal *et al*. 1993 ; Farag *et al*., 2001). Agarwal *et al*. (1993) showed that the treatment with β-carotene did not induce depression of mitotic index in material and foetal cells of animals treated with β-carotene compared with control group. Farag *et al*., (2001) demonstrated that the treatment with β-carotene did not cause inducing chromosome abnormalities or depression in mitotic index in maternal and foetal cells of mice.

Also, in another studies, carotenoids have been found to be protective agents on the liver and kidney functions (Nicolle *et al*., 2003; Devaraj *et al*., 2008) from oxidative stress. Nicolle *et al*., (2003) investigated the effect of 3- week supplementation of the diet with carrot on lipid metabolism and antioxidant status in rats. They observed a significant decrease of cholesterol level together with a reduction of the level of triglycerides in liver. Also, the results revealed that carrot consumption improved the antioxidant status by decreasing the urinary excretion of thiobarbituric acid reactive substances (TBARS), reducing TBARS levels in heart and increasing vitamin E plastimatic level as compared to control. Devaraj *et al*., (2008) tested the effects of different doses of purified lycopene (natural

carotenoid) supplementation on biomarkers of oxidative stress in healthy volunteers. Their results revealed a significant decrease in DNA damage and a significant decrease in urinary 8- hydroxyl deoxaguanosine (8-ohdG) at 8 weeks versus baseline with 30 mg hycopene/ day.

Few literatures demonstrated that some vitamins associated with diets have shown to improve immune status in healthy individuals. Meydani *et al.* (1997) showed that Vitamin E supplementation improved response to delayed- type hypersensitivity tests in healthy individuals > 65 y of age. Moreover, in previous study, Duthie *et al*. (1996) showed a significant decrease in endogenous oxidative base damage in the lymphocyte DNA of both smokers and nonsmokers with a 20- wk daily supplement of vitamin C (100 mg) and B- carotene (25 mg).

Concerning the effect of some amino acids such ascorbic acid on oxidation condition, some studies showed that the addition of ascorbic acid - rich foods to a controlled experimental diets led to inhibition of endogenous formation of N-nitroso compounds (oxidative and mutagenic agent) in humans (Knight and Forman, 1987; Bartsch *et al*., 1988).

In the present study, the treatment with aflatoxin B_1 significantly increased the liver (GGT, ALT and AST) and kidney (uric acid and creation) functions and significantly decreased the TP compared to control group. In contrast, the treatment with the yeast strains (G1, G2 and G3) together with aflatoxin B_1 succeeded in diminishing the elevated values of liver enzymes (GGT, ALT,AST) and kidney function tests (uric acid and creatinine) and normalized TP level. On the other hand, the cytogenetic results showed that the chromosome aberrations were more frequent and the mitotic and meiotic indices were depressed in the animals treated with aflatoxin B_1 alone. In contrast the frequencies of the chromosome aberrations were significantly decreased and mitotic and meiotic indices were increased in animals treated with AFB1 concomitant with yeast strains (G1, G2 and G3).

As known the liver is considered the principal target organ for aflatoxin. The activity of ALT and AST are sensitive indicators for acute hepatic necrosis (Kaplan, 1987) and decrease in concentration of TP as indicators of the alternation in protein synthesis observes in aflatoxicosis (Kubena *et al*., 1993; Abo-Norage *et al*., 1995; Abousadi *et al*., 2007).

Changes in liver and kidney functions induced by aflatoxin have been documented previously by Heathcote and Hibbert (1979) and Abdel- Wahhab *et al*. (1998). Some studies showed that aflatoxins have induced oxidative damage and caused for generation of free radicals which reacted with cellular component and led to pathological changes in liver and kidney functions (Vasankari *et al*., 1997; Abdel- Wahhab *et al*., 1998; Abousadi *et al*., 2007).

Ito *et al*., (1989) and Abdel-Wahhab *et al*., (1998) had previously reported that aflatoxin B_1, has a potent activity to induce chromosome aberrations in bone marrow of rats. The mutagenicity of aflatoxin arising from the toxin molecules might form covalent N7 guanine- adducts which disturb DNA replication (Bonnett and Taylor, 1989), resulting in anomalies in the chromosomes (Sinha and Prasad, 1990).

The protective effect of yeast which observed on animal cells (in vivo), in the present study has not been discussed previously. However, as reported above, the protection role of the yeast may be due to that the yeast cells contain antioxidant components such, carotenoids, vitamins, minerals and essential amino acids (Hussein *et al*., 1996) that interrupts the free radical- initiated chain reaction of oxidation or scavenge and disable free radicals before they react with cellular components (Vasankari *et al*., 1997). β-carotene showed protective activity against AFB1-induced hepatotoxicity (Khorshid *et al*., 2008), hepatic inflammation, fibrosis and attenuating cirrhosis (Seifert *et al*.,1995) in rats. The protective mechanism of β-carotene may also enhance immunity and down regulation of key cytokines (He *et al*., 2004). Kim (1995) reported that carotenoids have protective effect on oxidant-induced liver injury, improved the cell viability of hepatocytes, increased catalase activities and glutathione levels in hepatocytes from chronically ethanol-fed rats.

Moreover, many previous studies provided the presence of B-D- glucans in adequate percentage on the cell wall of yeasts, which had immuno – modulatory effect (Vetvicka 2001; Brown and Gordon 2003). As well as, B-D-glucans has antioxidant activity, scavenging the free radicals, reduced DNA-oxidative damage (Oliveira *et al*., 2009 and Sener *et al*. 2007).

Furthermore, in another studies (in vitro) an antagonistic yeast strain of *Rhodotorula glutinis* has been reported as an effective biocontrol agent against post- harvest decay of apples (Qin *et al*., 2003), pears (Zhang *et al*., 2008; Zhang *et al*., 2009), strawberries (Zhang *et al*., 2007), sweet cherries (Tian *et al*., 2004) and oranges (Zhang *et al*., 2005). Also, an antagonistic yeast strain of *Rhodotorula glutinis* and its autoploidy have been reported as an effective biocontrol agent (in vitro) and (in vitro) against grey mould of greenhouse sweet pepper (Haggag, *et al*., 2005).

The mode of action of the yeast strains (G1, G2 and G3) or their constituents antioxidant compounds against mutagenic or toxic effect of aflatoxin B_1 in animal cells (in vivo) may be due to binding with the

mutagens or inhibition of activation of enzymes of cytochrome systems- meiated N- hydroxylation with consequently enhancement of liver and kidney functions and reduction of abnormalities of genetic materials (Wang *et al*., 2004; Devaraj *et al*., 2008). It is well known that some of economically important yeasts are high ploidy. The increment of ploidy leads to a larger cells than that of the wild strain. Colchicine treatment of cells is an easy and simple method for breeding a microorganism possessing a high ploidy (Jibiki *et al.*,1993). So that, in the present study, when *R. glutinis* strains treated by 0.2% colchicine many ploidy level increments have been generated and showed increased in their cells diameter, size and density as well as DNA contents and growth rate. Convincing experimental evidence in support of the existence of a ploidy-driven mechanism of gene regulation was reported by Galitski *et al*. (1999). The aneuploidy can confer selective advantages by increasing the copy number of beneficial genes and protecting against lethal or deleterious mutations. The increases of yeast number and other unicellular organisms have been induced by a process of cell growth followed by division (Guijo *et al*., 1997). However, the present biochemical and cytogenetic results showed approximately similar effects of *Rhodotorula glutinis* and its autoploidy on normal animal cells (in vivo) or their use as protective agents against toxic effect of aflatoxin B_1 in mice.

5. Conclusion

In conclusion, the present study adds evidence that the treatment with yeast Strains *of Rhodotorula glutinis* is safe on animal cells and could be protective agent against toxic effect of aflatoxin (in vivo).

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