

Full Length Research Paper

Ultra-structure of the yeast *Trichosporon mycotoxinivorans* and its effect on some biological parameters in mice

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In this research work, the ultra-structure of yeast, *Trichosporon mycotoxinivorans* was studied by scanning and transmission electron microscopy. Effects of yeast on biological parameters in mice including body weight, mortality rate and compounds in blood (glucose, total protein, cholesterol, and insulin) were estimated. Blood constituents (White blood cells (WBC), Red blood cells (RBC) haemoglobin (HGB), hematorit (HCT) and platelets (PLT), mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined. The results indicated that the ultra-structure of the organism is found to be similar to most of the basidiomycetes yeasts. *T. mycotoxinivorans* yeast increases weight of mice and reduces the rate of mortality. It did not have any negative effect on the compounds and blood constituents in mice. These results suggest that this yeast could be used as a probiotic in animal feeding to reduce the risks of mycotoxins.

Key words: Ultra-structure, mycotoxinivorans, mycotoxinis, trichosporon, blood constituents.

INTRODUCTION

Mycotoxins are the potentially toxic and immunogenic by products of the life cycles of certain fungi. As knowledge of the effects of mould by products continues to emerge, it is apparent that mycotoxins can damage any target organ in the human body and cause not only cancer, but several symptoms and diseases via multiple mechanisms (Ueno, 1983; Scott, 1989). Recently strategies such as enzymatic or microbial detoxification have been used for reduction of potency of certain fungal toxins (Binder, 2007). A *Trichosporon mycotoxinivorans* is one of the Basidiomycetous yeasts that is associated with termites, and is characterized by its ability to detoxify ochratoxin A and zearalenon (ZON). It has no negative influence on

performance and wellbeing of chicken (Molnar et al., 2004). Politis et al. (2005) found that the dietary inclusion of *T. mycotoxinivorans* blocked the detrimental effects of ochratoxin A on several immune properties in broilers. According to Molnar et al. (2004) most of *Trichosporon* strains were isolated from several sources from the environment, and are not pathogenic strains except *Trichosporon asahii*, *Trichosporon asteroides*, *Trichosporon cutaneum*, *Trichosporon inkin*, *Trichosporon mucoides* and *Trichosporon ovoides*, which are known in the medicine, since they can cause opportunistic infections or induce summer-type hypersensitivity pneumonitis. Padhye et al. (2003) reported that the first known case of human infection is caused by *Trichosporon loubieri*. The strain of *Trichosporon debeurmannianum* was isolated from human bronchial secretions, while the *Trichosporon dermatis* was isolated from an infected human skin lesion. Recently, Hickey et al. (2009) reported that the *T. mycotoxinivorans* was a

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newly recognized human pathogen that was associated with cystic fibrosis. Vekiru et al. (2010) studied the degradation of ZON by *T. mycotoxinivorans*, a basidiomycete yeast which is used as a microbial feed additive against mycotoxins, were found, and that a nonestrogenic ZON metabolite (ZOM-1) was the main product of this ZON degradation. The aim of this research was to study the ultra-structure of *T. mycotoxinivorans* and their effects on biological parameters in mice including body weight, mortality rate and blood compounds in mice as a partial biological evaluation.

MATERIALS AND METHODS

Yeast and its preparation

(*T.mycotoxinivorans* HB 1230) was purchased from Universität für Bodenkultur, Vienna. Preparation of the yeast suspension for cultivation of yeast, 50 ml of malt extract broth (MEB) was inoculated in a 100 ml Erlenmeyer flask with 0.5 ml from a culture cell, which was stored at 40°C. The flask was closed with a cotton-wool swap and incubated at 25°C for 3 days. For preparation of suspension of the yeast, cells were collected from the exponential phase; yeast cells were separated by centrifugation at 6000 xg for 10 min and washed three times with 10 ml saline solution (0.89% NaCl). The yeast pellet was re-suspended in 5 ml saline solution. The total plate count was estimated by standard plate count (SPC) method (Pringle and Mor, 1975).

Ultra-structural study

Ultra-structure of *T. mycotoxinivorans* was studied by scanning and transmission electron microscopy (SEM and TEM) according to Tronchin and Bouchara (2006).

Procedures and experiments

Adult healthy male and female albino mice *Mus musculus domesticus* (body weight 26 ±1 gm) were used in this study. A 120 albino mice which comprised of 60 males and 60 females were divided into three groups for each sex. The first group was maintained on a standard diet as control group, the second group was dieted standard diet plus 0.250 ml saline solution (orally) while the third group was fed standard diet plus 0.250 ml suspension of the yeast (orally). The experiment lasted for four weeks. Blood samples were collected at the end of the experiment from orbital sinus according to Riley (1960), with the addition of ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. The blood plasma was separated by centrifugation and stored at -20°C.

Biological parameters

Body weight was recorded at weekly basis, while mortality was recorded daily.

Blood compounds

Total protein, total cholesterol, insulin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated according to Dumas et al. (1981), Allain et al. (1974),

Starr et al. (1978), Tietz (1995), Bergmeyer et al. (1986), Reichling and Kaplan (1988), Epstein et al. (1986) and Moss and Henderson (1999), respectively.

Blood constituents

WBCs, RBCs, HGB, HCT, PLT, MCV, MCH and MCHC were automatically determined by vet-animal blood counter (ABC, ABX-France).

Statistical analysis

The experiment was designed as completed random design (CRD). The data were expressed as means ± standard error. Statistical analyses of blood compounds and constituents data were performed by analysis of variance (SAS, 2002).

RESULTS

Ultra-structure

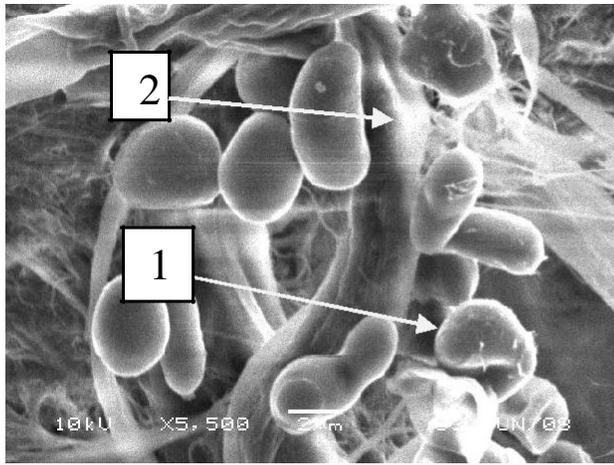
Ultra-structure of *T. mycotoxinivrans* was studied by SEM and TEM in order to know some the fine structures that characterize the yeast. In Figure 1A to B Scanning electron micrograph for *T. mycotoxinivorans* shows that it appears as cells globose, ovoidal, ellipsoidal and elongate. Monopolar budding and cell division in initial phase (A-3) and end phase (B-3) were observed. The slim layer, cell wall, cytoplasm, nuclear membrane, nucleus and nucleolus were shown in Figure 2A. The Basidiomycetes-type budding, four collars of dark material and ruff around the budding site were shown in Figure 2B, In Figure 2C and D giant cells with septum transversum were appeared.

Biological parameters

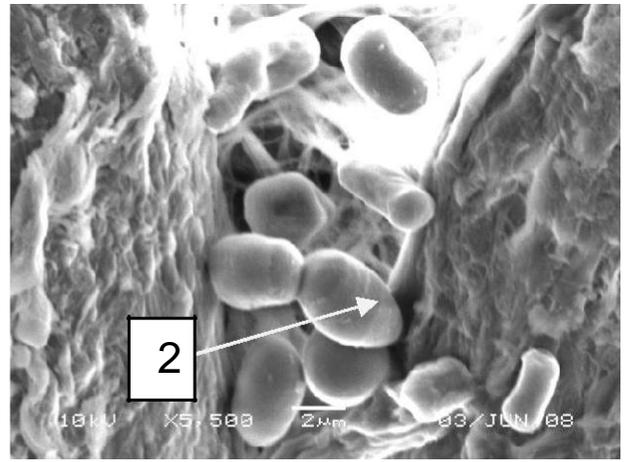
Body weight and mortality were recorded weekly and daily, respectively. In Figure 3 the mean weight (gm) of the control and treated mice groups at the last fourth week is presented. The mean weight in the treated groups with the yeast showed a significant increase ($P<0.05$) in treated female or male groups (32.9±3.8 and 34.4±3.6 gm, respectively) compared to the control or treated groups with saline solution in female (28.6±4.2 and 28.4±2.2 gm, respectively) or male groups (28.7±3.8 and 31.3±3.6 gm, respectively). The mortality has decreased in female and male groups which were treated with yeast (0 and 30%, respectively) compared to the control or treated groups with saline solution (Figure 3). The mortality was 10 and 20% in female control or treated with saline groups, respectively, while it reached 50% in male control group.

Blood compounds

Total protein in blood of female group ranged from 5.22

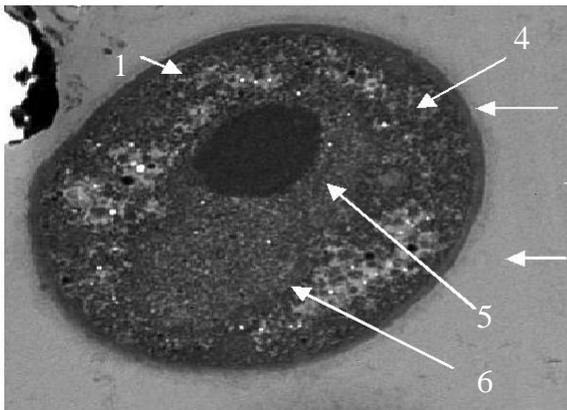


A



B

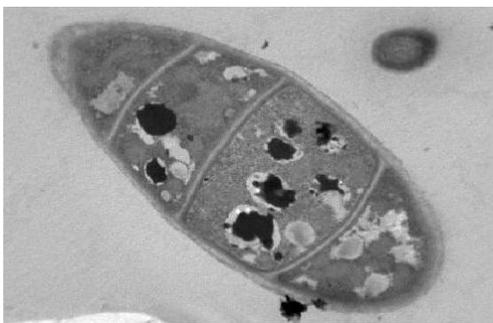
Figure 1. Scanning electron micrograph of a *Trichosporon mycotoxinivorans* cell (x5500)- (1), monopolar budding, (2) cell division.



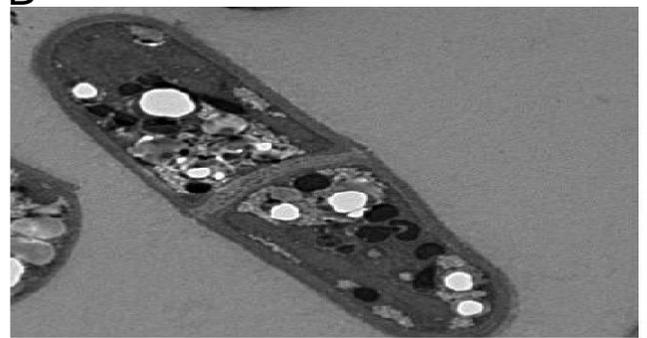
A



B



C



D

Figure 2. *Trichosporon mycotoxinivorans* transmission electron micrograph showing slim layer (1), cell wall (2), cytoplasm (3), nuclear membrane (4), nucleus (5) and nucleolus (6) (A) (x15000), Basidiomycetes-type budding (B) (x10000), and giant cells with septum transversum (C-D) (x10000).

to 6.05 (g/dl) without significant differences, and in blood of male group 4.5 to 5.7 (g/dl) also without significant differences (Figure 4A). The mean of glucose

concentration (mg/dl) in the control and treated groups can be seen in Figure 4B. In female or male groups there were no significant differences ($P < 0.05$) between control

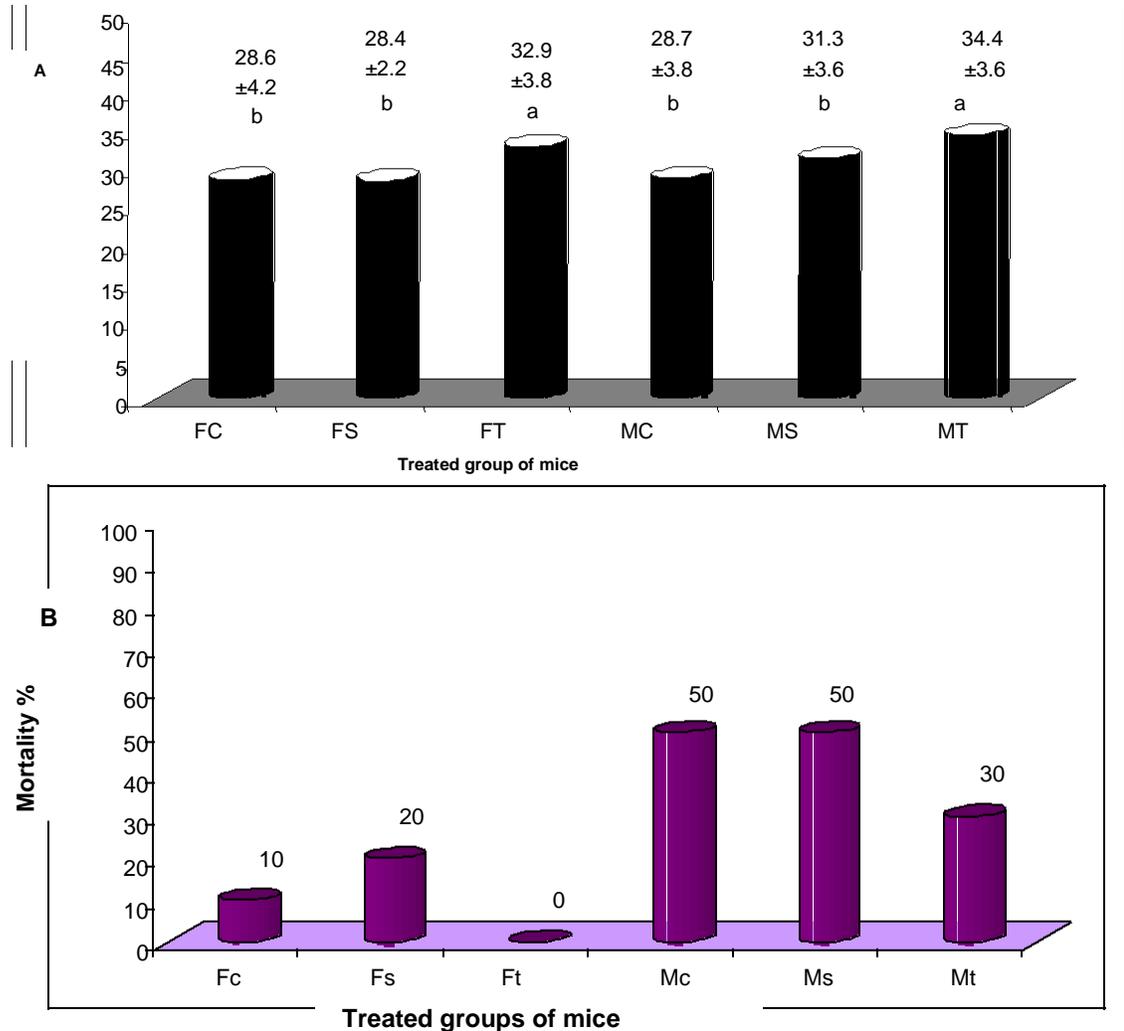


Figure 3. Means body weight (gm); **A**, and mortality rate (%); **B** in control and treated mice \pm S.E ($n = 6$). Means with different letters notification are significant at $P < 0.05$. Fc = Female control group, Fs = Female treated group with saline solution, Ft = Female treated group with *T. mycotoxinivorans*, Mc = male control group, Ms = male treated group with saline solution, and Mt = male treated group with *T. mycotoxinivorans*.

group and treated group with the yeast. The mean of Insulin level (U/l) in blood plasma of control and treated mice can be seen in Figure 4C all concentrations were between 2.6 and 6.7 (U/l), without significant differences in all groups. The treated groups with the yeast (female or male groups) have shown significant decrease in the mean of total cholesterol level (60.25 and 76.65 mg/dl, respectively) compared to the control or treated group with saline solution Figure 4D.

Blood constituents

The results for the blood constituents determination are shown in Table 1. There was no significant differences in WBCs concentration in male groups (Control= 3.91 ± 0.27 , treated with saline solution = 14.01 ± 0.26 , treated with the

yeast = 14.38 ± 0.53 ($10^3 / \text{mm}^3$)), but in female groups control group and treated groups (with saline solution or the yeast) differed significantly ($P < 0.05$). The concentration of RBCs in mice blood of male group (9.9 ± 0.21 $10^6 / \text{mm}^3$) that were treated with the yeast has shown significant increase ($P < 0.05$) compared to the other groups. The results in Table 1 showed no significant differences in concentration of HGB (g/dl) between all groups. The treated group with yeast in male group has a significant increase in HCT ($48.2 \pm 0.48\%$) compared to another groups, but no significant differences in male group in MCH (pg), also between control groups and treated groups with the yeast in female groups. The concentration of MCV (μm^3) of mice blood in male group has no significant differences, also no significant differences between control groups and yeast treated group in female groups (50.96 ± 3.1 and 48.38 ± 4.1 μm^3).

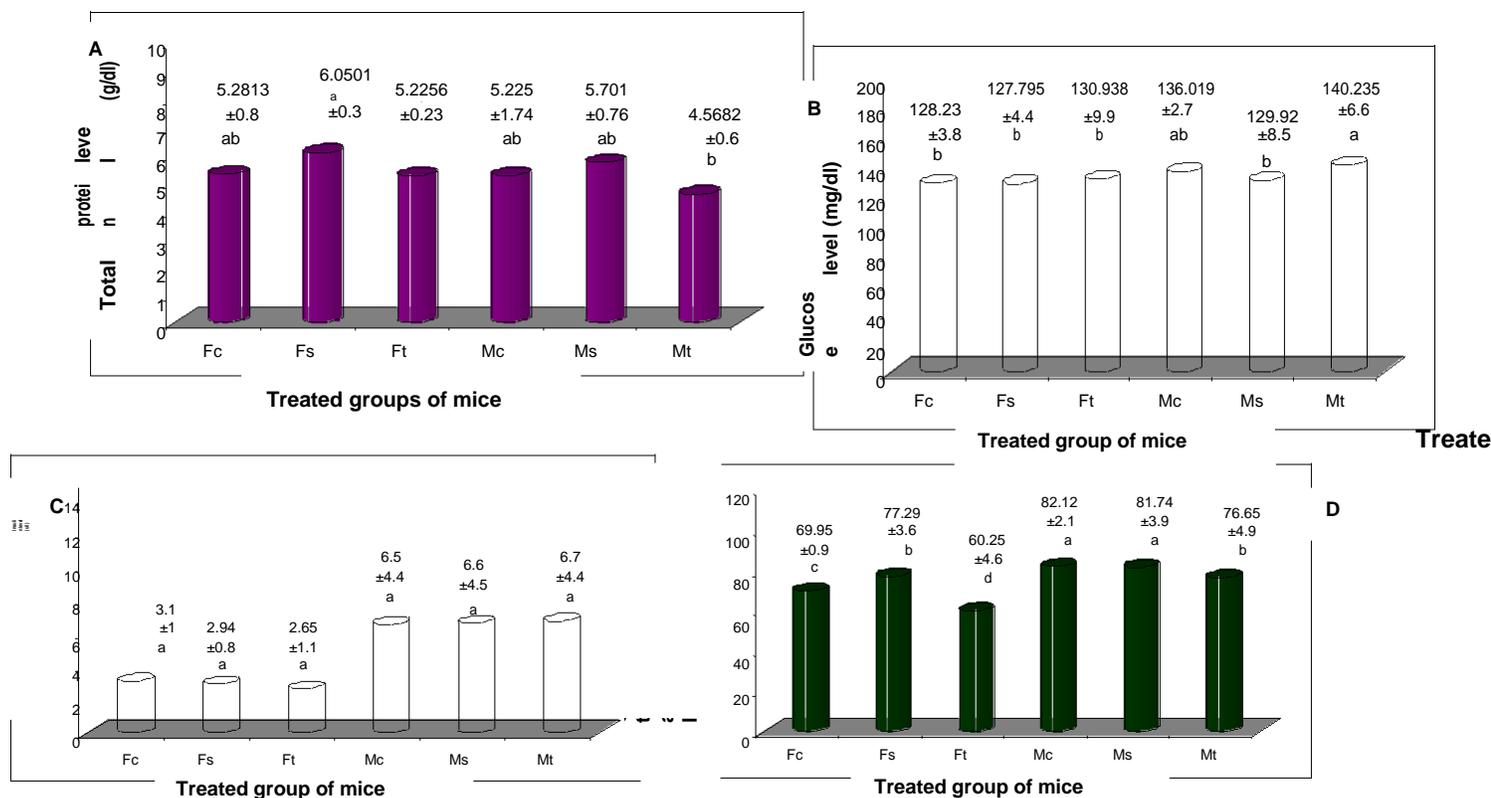


Figure 4. Means of total protein level (g/dl), Glucose level (mg/dl), Insulin Level (U/l), and total cholesterol level (mg/dl) in blood plasma of control and treated mice \pm S.E ($n = 6$). Means with different letters notification are significant at $P < 0.05$. Fc = Female control group, Fs = Female treated group with saline solution, Ft = Female treated group with *T. mycotoxinivorans*, Mc = male control group, Ms = male treated group with saline solution, and Mt = male treated group with *T. mycotoxinivorans*.

Table 1. Means \pm S.E ($n = 6$) of measured blood constituents in control and treated mice.

Blood constituents	Treated group					
	Fc	Fs	Ft	Mc	Ms	Mt
White blood Cells (WBCs) (10^3 /mm ³)	12.16 \pm 0.38c	13.08 \pm 0.37b	12.8 \pm 0.33b	13.91 \pm 0.27a	14.01 \pm 0.26a	14.38 \pm 0.53a
Red blood Cells(RBCs) (10^6 /mm ³)	8.81 \pm 0.26cb	7.8 \pm 0.5d	8.6 \pm 0.63c	9.31 \pm 0.06b	8.93 \pm 0.48cb	9.9 \pm 0.21a
Haemoglobin (HGB) (g/dl)	11.4 \pm 0.54a	11.31 \pm 0.49a	11.56 \pm 0.55a	11.19 \pm 0.99a	11.15 \pm 0.58a	11.4 \pm 0.48a
Hematocrit (HCT) %	40.91 \pm 1.28cd	40.08 \pm 0.33d	41.4 \pm 0.58c	47.36 \pm 0.67b	46.4 \pm 0.67b	48.2 \pm 0.48a
Mean Corpuscular Haemoglobin (MCH) (pg)	12.27 \pm 1.09bc	14.58 \pm 1.44a	13.48 \pm 0.75ab	12.72 \pm 1.71bc	12.52 \pm 0.59bc	11.51 \pm 0.27c
Mean cell volume (MCV) (μ m ³)	50.96 \pm 3.1b	51.56 \pm 3.2a	48.38 \pm 4.1ab	51.99 \pm 3.6a	46.6 \pm 2.2a	48.7 \pm 0.9ab
Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dl)	24.06 \pm 0.9a	28.23 \pm 1.2a	27.94 \pm 1.3a	27.27 \pm 2.5b	24.12 \pm 1.3b	23.64 \pm 0.9b
Platelets(PLT) (10^3 /mm ³)	664.8 \pm 73.5b	709.6 \pm 33.5ab	659.3 \pm 66.4b	726 \pm 21a	698 \pm 10.9ab	705 \pm 8.7ab

Means with different letters in the same row differ significantly ($P < 0.05$), whereas those with similar ones are not significantly different. Fc = Female control group, Fs = Female treated group with saline solution, Ft = Female treated group with *T. mycotoxinivorans*, Mc = male control group, Ms = male treated group with saline solution, and Mt = male treated group with *T. mycotoxinivorans*.

respectively). Differences in the mean concentration of MCHC (g/dl) were not significant in female groups (control=24.06±0.9, treated with saline solution=28.23±1.2 and 27.94±1.3 g/dl) or male groups (control=27.27±2.5, treated with saline solution=24.12±1.3 and 23.64±0.9 g/dl). Significant differences in the mean concentration of PLT ($10^3/\text{mm}^3$) were not noticed in female group (control=664.8±73.5, treated with saline solution=709±33.5 and 659.3±66.4 $10^3/\text{mm}^3$) or male groups (control=726±21, treated with saline solution=698±10.9 and 705±8.7 $10^3/\text{mm}^3$).

DISCUSSION

Budding is the most common mode of vegetative reproduction in yeast and fission yeasts, divide exclusively by forming a cell septum, which constricts the cell into two equal-sized daughters. The type of budding is very important in identification of yeast, the monopolar budding (buds originate at only one pole of the mother cell) common in *Malassezia* spp. (Walker and White, 2005). Basidiomycetes-type budding (ruff around the budding site) characterize basidiomycetes yeasts for example, *Filobasidium floriforme*. Several generations of budding have produced a ruff around the budding site (Moore, 1998). In the present study, it could be observed that *T. mycotoxinivorans* have monopolar and basidiomycetes-type budding. Similar result was observed in previous study by Moor (1998) on Basidiomycete-type budding of *Filobasidiella* (*Cryptococcus*) *neoformans*. Giant cell that characterize *trichosporon* genus was also observed with septum transversum, these cells may be used to characterize specific species. Hickey et al. (2009) indicated that the *T. mycotoxinivorans* form giant cell but differed in the shape from cells formed by *T. loubieri*.

T. mycotoxinivorans increases body weight of treated mice and decreases mortality rate in treated mice, that is agreed with the results of Molnar et al. (2004) and Politis et al. (2005), it might be the yeast which has played a positive role in enhancement of the immune system in mice or it has a mechanism of detoxification such as biotransformation of mycotoxins, Vekiru et al. (2011) confirmed the capability of the *T. mycotoxinivorans* in production of non-estrogenic zearalenone (ZOM-1) by biotransformation of estrogenic zearalenone. Vekiru et al. (2010) suggested that *T. mycotoxinivorans* can be fermented, concentrated, freeze-dried and stabilized without losing its viability, its utilization as a feed additive for mycotoxin detoxification seems practicable. The level of compounds in blood of mice which was studied in this work were normal, according to Mitruka and Rawnsley (1981), which means that *T. mycotoxinivorans* has no negative influence on total protein, glucose, insulin and total cholesterol. The concentration of blood constituents WBCs, RBCs, HGB, HCT, PLT, MCV, MCH and MCHC in treated mice was normal, according to Russell and

Bernstein (1966); and Mitruka and Rawnsley (1981), which indicated that this yeast has no adverse influence on blood constituents in mice.

In conclusion, these results suggest that there are some ultra structure of *T. mycotoxinivorans* which could be used as characterization features such as Basidiomycetes-type budding and giant cells appearance with septum transversum. These results indicated that there was no negative influence of *T. mycotoxinivorans* on blood compounds and constituents in mice and it has a role in improving health status of mice.

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