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The Preventive Effect of *Sacharomyces boulardii* in Pathogenesis of *Salmonella typhimurium* in Experimentally Infected Rats

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Abstract: The present research studied the capacity of *Saccharomyces boulardii* (*S. boulardii*) to antagonize *Salmonella typhimurium* (*S. typhimurium*) in the intestinal tract and humoral response of rats after challenging with *S. typhimurium*. Sixty conventional male rats were divided at random into 4 groups. The *S. boulardii* suspensions contained 10^6 , 10^7 and 10^8 cells mL^{-1} were administered (orally) to 3 groups (A, B and C) of rats, respectively for 5 continues days. The rats of group D received Saline instead of *S. boulardii* and used as control. All of rats challenged with 10^7 CFU of pathogenic *S. typhimurium* at fifth day. The viable *Salmonella* were counted at g^{-1} of faeces of rates at 0, 1, 3, 5 and 7 days after challenge. Titer of anti- salmonella antibodies were determined. Present experiments showed that *S. boulardii* could reduce the bacterial colonization in experimental animals. There was a significant reduction at number of *S. typhimurium* between control and test groups. A daily dose of 10^6 yeast cells was resulted in the most reduction in bacterial shedding and high titers of anti salmonella antibodies. The mortality rates were 0 and 46.4% in yeast treatment and control animals, respectively. *S. typhimurium* viable cells were not detected in the organs of yeast-treated rats. We conclude that *S. boulardii* has a preventive effect in pathogenesis of *S. typhimurium*.

Key words: *Saccharomyces boulardii*, *Salmonella typhimurium*, probiotic, rat, pathogenesis

INTRODUCTION

Diarrhoea caused by infectious agents is responsible for large economic losses in animal production. One of the most frequent genus of bacterial contaminants of domestic animals is *Salmonella*. Especially, in the case of *Salmonella* infections, these may be asymptomatic and production losses are insignificant. However, colonized food animals can serve as a source of infection for humans causing a disturbance of the gastrointestinal microbiota. Antibiotics have traditionally been the most frequently administered agents to counteract this type of infections. However, the current trend is to totally eliminate the prophylactic use of antibiotics in farming. The use of several antibiotics has already been banned. Therefore, in an effort to prevent or cure these disorders, alternative forms of therapy are searched. have been suggested as alternative therapies for the treatment or prevention of gastroenteritis (Laukova *et al.*, 2004).

The yeast of *Saccharomyces boulardii*, has originally been isolated from lychee fruit in Indochina and used first

in France to treat diarrhoea, beginning in the 1950s (McFarland and Bernasconi, 1993). *S. boulardii* is effective in the prevention and treatment of many forms of diarrhoea in humans, especially antibiotic-associated diarrhoea and colitis. Recent studies showed that *S. boulardii* administration significantly reduced the frequency of diarrhoea in patients administered antibiotic therapy and that in combination with vancomycin or metronidazole. It reduced the number of relapses of *C. difficile* infection (Castagliuolo *et al.*, 1999). Although, *S. boulardii* is not a constituent of the normal gut flora, it survives undigested in the lower gastrointestinal tract after oral administration and can be retrieved from faeces (Roffe, 1996). Besides of preventive and therapeutic effects, Gil de los Santos *et al.* (2005) showed that this probiotic improved feed efficiency in broilers. Agawane and Lonkar (2004) found that use of *S. boulardii* in field conditions could reduce the adverse effect and economic losses of ochratoxicosis in broilers.

This study was conducted to determine the influence of the yeast on salmonella population as well as their

inhibitory effect in pathogenic action of salmonella in rat after oral administration.

MATERIALS AND METHODS

The present research work has conducted at department of pathobiology, Shahrekord University, Shahrekord, Iran, in 2004. The yeast, *S. boulardii* was cultured on Sabouraud-dextrose Agar medium (Merck) at pH 6 and was incubated at 30°C for 48 h. At the end of fermentation, yeasts were recovered and suspended in a sterile aqueous solution of 0.05 M NaCl isotonic. We prepared 3 suspension (A, B and C) contained 10⁶, 10⁷ and 10⁸ cells per mL and stored them at 4°C for 5 days.

A clinical isolate of *S. typhimurium* was selected for resistance to tetracycline by successive cultures on MacConkey agar (Biolife) containing 65 µg tetracycline mL⁻¹ medium. Strain cultures in brain heart infusion (BHI-Difco) incubated at 37°C for 18 h were used as inocula. All groups received 10⁷ Colony Forming Units (CFU) of *S. typhimurium*. The number of CFU in the inocula and in the feces was determined by plating 0.1 mL of the suspensions and of their respective decimals dilutions onto sterile normal saline, in MacConkey agar supplemented with 65 µg tetracycline mL⁻¹ (Andreatti Filho *et al.*, 2000). The rats were challenged by intraesophageal inoculation of 1 mL of the bacterial suspension.

Sixty conventional male rats who didn't shedding non-lactose fermenting bacteria, weighting 200±20 g divided into 4 groups (A, B, C and D). Throughout the study period, each rat was placed in an individual decontaminated cage in a controlled heat environment with free access to unmedicated commercial feed and water.

Rats in 3 groups (A, B and C) received *S. boulardii* suspensions as a single daily dose contained 10⁶, 10⁷ and 10⁸ cells per ml (orally) from day 1 to 5, respectively. The rats of group D received saline instead of *S. boulardii* and used for control. All of rats challenged with 10⁷ CFU *S. typhimurium* at fifth day. A faecal pellet was removed directly following its emission from each of the animals of a given cage. For each experiment, individual counts were

made on one or two occasions for faeces from each of the animals of a given cage in order to establish possible individual variations.

Immediately following sampling, faeces were homogenised 10 times (W/V) in normal saline, then diluted in a decimal manner. *S. typhimurium* was counted by inoculation of dilutions on MacConkey agar medium with incubation for 2 days at 37°C. The numbers of salmonella per gram of feces of each rat were determined on 0, 1, 3, 5 and 7 days after challenging by pathogenic strain of *S. typhimurium*. Titers of anti-salmonella antibodies in rats sera were determined by agglutination tube test on 7, 14 and 21 days after challenge with salmonella.

Means of viable salmonella numbers excreted from feces and anti-salmonella antibodies titers compared with one-way ANOVA test among four groups of rats.

RESULTS AND DISCUSSION

The mortality rate of control rats (group D) that receiving normal saline and experimentally infected with *S. typhimurium* was 46.8% at first week after challenge. These animals showed signs of diarrhea and upon autopsy were found to have typhoid nodules in livers. *S. typhimurium* were found in the liver of these dead rats. In contrast, 5 days administration of *S. boulardii* prior to challenge with *S. typhimurium* resulted in 100% protection in three groups of rats (A, B and C). Mortality was significantly higher (p<0.05) in the control group (46.8%) when compared to the probiotic treated group (0%). The means of *Salmonella typhimurium* numbers (expressed per gram of faeces) in four groups of rats after challenge were shown in Table 1.

S. typhimurium concentration increased rapidly over the first 1 day after challenge and then decreased gradually. The differences in the mean values among the treatment groups (A, B and C) are greater than would be expected by chance; there was a statistically significant difference (p<0.001). A decrease was apparent between days 5 and 7 and no live cells could be detected on fifth day in group A and seventh day in group B and C as well. Sixty six percent of rats in group A had no excretion of salmonella at all. Nevertheless some rats in group D excreted low bacteria after seventh day.

Table 1: The means of *Salmonella typhimurium* numbers (expressed per gram of feces) in four groups of rats after challenge with *S. typhimurium* (A, B, C = Yeast treated and D = Control groups)

Bacterial counts (g ⁻¹)																
Groups	Day 1				Day 3				Day 5				Day 7			
	A1	B1	C1	D1	A2	B2	C2	D2	A3	B3	C3	D3	A4	B4	C4	D4
Mean	27717	32478	92673	234667	2020	10327	81400	204857	0	514	1933	11023	0	0	0	176
SD	100245	64084	130541	77447	5109	16734	121683	383937	0	1026	3670	20159	0	0	0	100
SE	25883	16547	33705	19996	1319	4321	31418	102612	0	265	948	5591	0	0	0	33

Table 2: Geometrical Mean Titers (GMT) of anti-salmonella antibodies in sera of four groups of rats after challenge with *S. typhimurium* (A, B, C =Yeast treated, D = Control groups)

Week (s)	Groups			
	A	B	C	D
1	14	0	14	0
2	208	18	96	15
3	144	48	136	-

The Geometrical Mean Titers (GMT) of anti-salmonella antibodies in four groups of rats were shown in Table 2. The most titer was found in group A and less in group D.

Gastroenteritis in humans and animals is the main cause of acute diarrhea and is a frequent syndrome that can be due to several viral or bacterial pathogens or to parasites. Probiotics can be defined as nonpathogenic microorganisms that, when ingested, exert a positive influence on the health or physiology of the host. Recent studies showed that *S. boulardii* administration significantly reduced the frequency of diarrhoea in patients. In this study the preventive effect of *Saccharomyces boulardii* in rat was very marked. Comparing the total counts of salmonella in feces of rats after 24 h between the group A, B and C (Table 1) and D, differences of log 0.97, 0.8, 0.4 were detected ($p < 0.001$). After 72 h, a lower level of colonization by salmonella in all experimental groups were found, which continued until the end of the experiment (96 h, Table 1). The most effective inhibition was found with administration of 10^6 yeast cells. Although there were no significant difference among experimental groups, it is not clear why this effect was decreased apparently with higher dose of yeast. Our experiments showed that administration of *S. boulardii* completely inhibited mortality. These findings suggest that this yeast has a preventive effect to reduce the levels of undesirable microbes such as salmonellae. In review literature, similar results to the application of the *Saccharomyces boulardii* were obtained in mice, hamster and tissue culture against *C. difficile*, *Yersinia*, *Salmonella*, *E. coli* and *Shigella* (Filho-Lima *et al.*, 2000; Rodrigues *et al.*, 1996). Several studies have documented that *S. boulardii* reduced the levels of *C. difficile* in hamster fecal pellets (Elmer and McFarland, 1987). Corthier *et al.* (1992) reported that 3.3×10^{10} *S. boulardii* ml in drinking water given to *C. difficile* infected mice, resulted in 85% protection.

The mechanisms of this inhibition are not known but antimicrobial substances production might be one possible mechanism of action of probiotics. Other effects of probiotics include competition for nutrients and receptors for adhesion to the host cell surface (Fuller, 1991).

Zbinden *et al.* (1999) reported that *S. boulardii* may have different modes of counteracting with bacterial enterotoxins. The yeast may have an intrinsic bactericidal activity, itself activating via metabolites or internal constituents. The absence of any effect seen *in vitro* would be against this hypothesis. The effect of yeasts might therefore rather be due to a change in one of the three successive stages in pathogenic capacity: adhesion, cytolytical effect and phagocytose

The effect of *S. boulardii* against *Entamoeba histolytica* trophozoites could also be explained by competition between yeast receptor sites and intestinal cells (Ravdin *et al.*, 1985). One of the mechanisms by which *S. boulardii* exerts its immunoprotective effect in the gastrointestinal tract is a stimulation of the intestinal secretion of S-IgA and of the secretory component of immunoglobulins (Buts *et al.*, 1990). We also found higher anti-salmonella antibodies titers in sera of rats that received *S. boulardii* before challenging with *salmonella*. However, there is a risk of fungaemia in immunocompromised patients and further large trials to document safety are needed before use of this agent will be accepted widely (Niault *et al.*, 1999).

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