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Saccharomyces boulardii as effective probiotic against *Salmonella typhimurium* in Mice

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ABSTRACT

This study was designed to investigate the protective role of *Sacchromyces bouladii* on intestinal section of mice infected with *Salmonella typhimurium*. Mice were divided into four groups. Acontrol group is uninfected with bacteria represent (negative control) , a second group was infected with bacteria S.typhimurium 0.1 ml (2.5×10^7 cfu/ ml) only represent (positive control) , the third group Induced mice received oral dose of *S. boulardii* 0.1ml (1×10^9 cfu/mL). Treated mice received *S. boulardii* (1×10^9 cfu/mL) orally for 7 days, followed by *Salmonella* infection. At the end of the experimental period the histological Results showed that administration of *S. typhimurium* alone resulted in a necrosis, degenerative changes and inflammatory cells infiltration in intestinal sections as compared with normal section taken from uninfected mice, while pretreatment with the *S. boulardii* ameliorate this effect.

Introduction:

In human, Salmonella spp. Are responsible for over one billion infections annually, with consequences ranging from self-limiting gastroenteritis to typhoid fever [1]. To initiate disease, Salmonella first adheres to and then induces its own uptake into intestinal epithelial cells through a specialized mechanism involving injection of virulence factors into host cells by a type III protein secretion system (TTSS) [2].

Gram-negative Salmonella sp. Is acommen bacterial enteropathogen and is widely used in laboratory studies aimed toward understanding the basis of mucosal immune responses and diseases such as gastroenteritis and typhoid.Most laboratory studies are carried out using S.Typhimurium in mice, where adisseminated infection with some similarities to human typhoid is observed.Typhoid fever affects more than 20 million individuals and causes more than 220,000 deaths annually [3,4].

In recent years, worldwide interest for the use of functional foods containing probiotic microorganisms for health promotion and disease prevention has increased significantly and according to the Food and Agriculture Organization and the World Health Organization, a probiotic is “a live microorganism which, when administered in adequate amounts, confers a health benefit to the host”. [5] Lyophilized *Saccharomyces boulardii* is probiotic yeast used worldwide for the prevention and treatment of a variety of diarrheal diseases [6]. In the case of infectious diarrhea, administration of *S. boulardii* to animals provides protection against intestinal lesions caused by several diarrheal pathogens [7].

Indeed, controlled clinical trials have shown that oral administration of S boulardii could treat or prevent gastrointestinal diseases such as antibiotic-associated diarrhea [8], recurrent Clostridium difficile-associated diseases [9], traveller• diarrhea [10] , children acute diarrhea [11], bowel disease such as Crohn's• disease and ulcerative colitis [12,13] and irritable bowel syndrome [14]. This study was carried out to evaluate the treatment role of Sacchromyces bouladii on mice infected with Salmonella typhimurium.

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Materials and Methods:

Microbial isolates:

S.typhimurium was supplied by Central Public Health Lab.Which was previously isolated from stool sample of infant suffer from diarrhea, S. boulardii was bought as commercial lyophilized yeast (Ultra-Levure®, BIOCODEX, France).

Bacterial culture:

S.typhimurium strain was grown overnight at 37°C in brain heart infusion broth. This activated culture was centrifuged at 3,000 rpm for 5 min, washed with sterile phosphate-buffered saline (PBS, pH 7.4), and resuspended in PBS to a final concentration of 2.5 × 107 bacteria/ml [15].

Determination of the S. typhimurium susceptibility to antibiotics.

Disk diffusion test was used for testing susceptibilities of isolates to different antibiotics. Ten ml of nutrient broth medium were inoculated with bacterial isolates, the cultures were incubated at 37°C to mid log phase (18hrs). 100µl of inoculated broth then transferred to Muller-Hinton agar plates. A sterile cotton swab was used in three different planes to obtain an even distribution for inoculating triplicate plates [16].

With sterile forceps, the selected antibiotic disks were placed on the inoculated plate.All plates were incubated at 37°C for 24 hrs in an inverted position. Then diameter of inhibition zone was noted and measure by a ruler in mm.

Experimental design:

Twenty adults albino male mice were randomly divided into four groups designated as 1, 2, 3, and 4. Each group consists of 5mice, and subjected to the following treatments according to [17].

Group1: This group was used as a negative control.

Group2: This group was dosed with 0.1ml. of 2.5 × 107cfu/ml S.typhimurium culture and considered as positive control.

Group3: This group was dosed with 0.1ml of 1 × 109cfu/ml S. boulardii culture.

Group4: This group was dosed with 0.1ml of 1 × 109cfu/ml S. boulardii culture, and infected with 0.1ml of 2.5 × 107 cfu/ml culture of S.typhimurium.

Mice were dosed with a single dose(0.1 ml) of 1 × 109cfu/ml S. boulardii culture daily by oral administration for 7 consecutive days. After 7 days treatment, at the 8thday of experiment period, each mouse was given 0.1 ml of 2.5×107 Salmonella culture by oral administration. After 6th day of infection with S.typhimurium, mice were sacrificed by cervical dislocation and collected to evaluate histological effect Pieces were taken from intestine and put in petridishes containing physiological salty solution to remove the fatty tissues and sticky bundles, then the organ was kept in test tubes containing 10 % formalin solution then wash, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness preparation [18]. The staining method was performed by using hematotoxilin and eosin as a routin work for histological studies[19].

Results and Discussion:

Susceptibility to different antibiotics as shown in table 1 revealed that S. typhimurium was sensitive to Carbencillin, Ciprophloxacin and Rifampicin and resistant to other antibiotics.

Results indicated that mice intestinal sections taken from the control group showed normal structure appearance of villi without any pathological changes as shown in figure 1A . While, in intestinal sections taken from mice infected with S. typhimurium showed shedding and necrosis of intestinal mucosa and villi inside the lumen of the intestine figure 1 B Mice fed with S.boulardii showed normal villi appearance, while mice infected with S. typhimurium and pretreated with S.boulardii revealed shortening of the intestinal villi with thinning of the intestinal mucosa and ameliorate cytotoxic effect of Salmonella as shown in figure 1 C and D. Susceptibility of S.typhimurium to different antibiotics exhibited their resistance to different antibiotics used in this study. These results were close to those of CDC [20] who found that S. typhimurium isolates resisted chloramphenicol, ampicillin, streptomycin, and tetracycline the drug resistance genes can be transferred among many species of enteric bacteria.

Results showed that intestinal section of mice infected with Salmonella resulted in widening and odema in the villi with slight exist of inflammatory cells. This might be attributed to its ability to destroy M cells found within pyer's patches. It's known that S. typhimurium grows primarily inside the macrophages

of liver and spleen. It has been shown that within 30 min of infection, invasive S. typhimurium entered M cells found within the follicle-associated epithelium (FAE) of Peyer's patches. At 60 min, internalized bacteria were cytotoxic for the M cells and the dead cell formed a gap in the FAE, which allowed bacteria to invade adjacent enterocytes or rapidly migrate to a number of sites in the body, including the spleen and liver, where they replicated inside phagocytic cells[21].

Results indicated that pretreatment with S.boulardii reduce the effect of Salmonella in intestinal pattern of mice. This protective effect could be due to immunomodulation or competition for nutrients or adhesion sites.The antagonism effect of Sacchromyces against Salmonella and E.coil mentioned by Gedek [22] who reported that the mannose in the cell wall may cause the yeast to act as a decoy for the attachment of pathogens the yeast acts as a pathogen adherent microflora (PAM) and binds organisms such as Salmonella that may enter the gastrointestinal tract before the bacteria can attach to the chicken's intestinal wall.

Rodrigues et al [23] reported that the immunomodulation effect of S. boulardii has been shown to increase intestinal secretory IgA production. sIgA is thought to inhibit the close association of pathogenic bacteria with the mucosal epithelium, and so to reduce bacterial penetration and more efficient clearance of S. bouladii against E.coli B41 in mice was correlated with earlier production of IFN-γ and IL-12 which are involved in the T-helper 1 response.

Another study proved that S. boulardii interferes with the host cell signalling pathways and decreases the expression of inflammation-associated cytokines such as interleukin 8 (IL-8), IL-6, IL-1β, tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) [24], [25] and [26].

Table 1: Susceptibility of *Salmonella typhimurium* to antibiotics.

Antibiotic (Symbol)	Susceptibility
Amikacin(AK), Amoxicillin(Ax), Ampicillin(Am), Ceftazidime (CAZ), Cephalxin (CL), Co-trimoxazole(SXT), Chloramphenicol(C) Gentamycin(CN), Kanamycin(K), Neomycin (N), Tetracycline(T), Tobramycin (Tob)	R
Carbencillin(Py), Ciprophloxacin(Cip), Rifampicin (Rif)	S
Ceftriaxone	Int

R: resistant, S: sensitive, Int: intermediate.

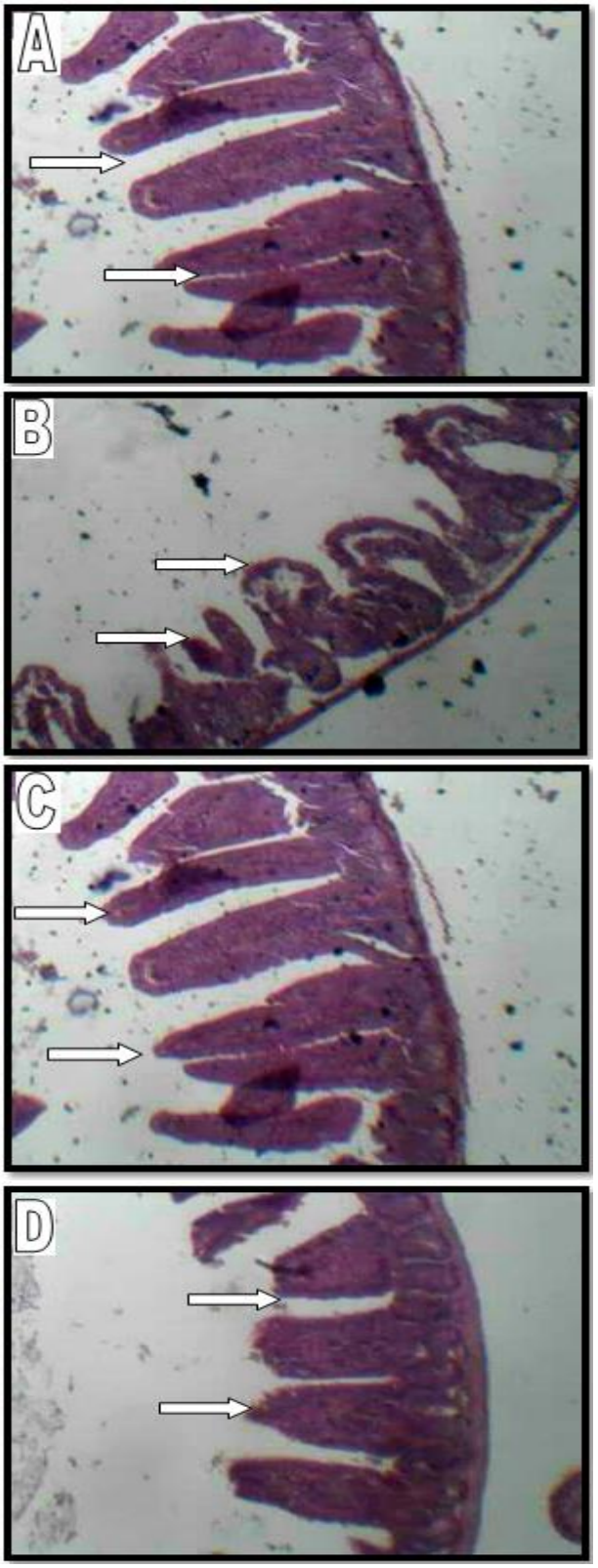

Figure 1: A Section of intestine of normal mice showing normal structure appearance of intestinal villi B Section in small intestine in mice infected with S.typhimurium showing infiltration in the lamina properia in the middle of the villi with odema C Section in small intestine in mice treated with S. boulardii showing normal villi appearance D

Section in small intestine of mice treated with S. boulardii and infected with S.typhimurium showing the shortening of the intestinal villi with thinning of the intestinal mucosa (arrows) (HE) ×100.

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الدور العلاجي لخميرة *Sacchromyces boulardii* في الفئران المصابة ببكتريا *Salmonella typhimurium*

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الخلاصة :

اجريت هذه الدراسة لتقييم الدور الوقائي لخميرة *Sacchromyces boulardii* في امعاء الفئران المصابة ببكتريا ال *S.typhimurium* , درس التأثير على 20 فارة بيضاء والتي قسمت الى اربعة مجاميع ,,مجموعة سيطرة غير مصابة بالبكتريا تمثل (negative control) ومجموعة ثانية تم إصابتها ببكتريا ال *S.typhimurium* 0.1ml (2.5×10^7 cfu/ml) فقط تمثل (positive control) اما المجموعة الثالثة جرعت بخميرة *S. boulardii* 0.1ml (1×10^9 cfu/mL) فقط, والجموعة الرابعة هي المجموعة المعاملة جرعت بخميرة *Sacchromyces boulardii* لمدة 7 ايام بعد ذللك تمت إصابتها ببكتريا *S.typhimurium* 0.1ml(2.5×10^7cfu/ml) (1×10^9 cfu/mL) 0.1ml . اظهرت نتائج الدراسة النسيجية ان اصابة الفئران ببكتريا *S.typhimurium* تسبب بتنخرات وتغيرات انحلالية وارتشاح الخلايا الالتهابية في امعاء الفئران مقارنة بالمقاطع الطبيعية الماخودة من الفئران الغير مصابة. كما اظهرت النتائج ان معالجة الفئران بالخميرة ادى الى خفض التأثير المرضي لبكتريا *S.typhimurium* في الفئران المصابة.