Effect of Oral Sacchromyces boulardii Treatment Against Experimental Infection with Enterotoxigenic E. coli in Mice

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Abstract
This study was designed to evaluate histological changes in intestine of mice treated with Sacchromyces boulardii and infected with enterotoxigenic Escherichia coli (ETEC). Twenty albino male mice were divided into four groups, and designated 1, 2, 3, and 4. Each group consisted of 5 mice and subjected to the following treatments; Group1; used as a negative control. Group 2; was infected with enterotoxigenic E.coli culture and use as positive control. Group 3; was dosed with S.boulardii; Group 4; was dosed with S.boulardii culture, and infected with ETEC E.coli. Histopathological study showed that infection of mice with ETEC causes a necrosis, degenerative changes and inflammatory cells infiltration in intestinal sections as compared with normal section taken from uninfected mice, while dosing with S.boulardii prevented the cytotoxic effect of ETEC in mice.

Introduction
Enterotoxigenic E. coli (ETEC) is a type of E. coli and the leading bacterial cause of diarrhea in the developing world, as well as the most common cause of traveler's diarrhea [1]. Each year, approximately 210 million cases and 380,000 deaths occur, mostly in children, from enterotoxigenic E. coli[2].

Disease caused by ETEC follows ingestion of contaminated food or water and is characterized by profuse watery diarrhea lasting for several days that often leads to dehydration and malnutrition in young children [3].

Numerous probiotic agents have been studied for the management of diarrheal disease. In particular, the prevention and management of acute viral diarrhea, the treatment of recurrent Clostridium difficile diarrhea, as well as the control of antibiotic-associated diarrhea seem to be areas of significant potential benefit and few agents, including Lactobacillus rhamnosus GG, Lactobacillus reuteri, and S. boulardii (S. boulardii), seem to be promising agents for the amelioration of the course of acute diarrhea in children when used therapeutically [4].

The yeast S. boulardii is a thermophilic, nonpathogenic administered in Western Europe for the prevention and treatment of a variety of diarrheal diseases [5]. Preclinical and experimental studies of S. boulardii have demonstrated an anti-inflammatory, antimicrobial, enzymatic, metabolic and antitoxin activity [6].

The yeast S. boulardii secretes a 54-KDa protease which has been shown to neutralize certain bacterial toxins; S. boulardii is also able to stimulate an immune response in the intestinal mucosa. It has a trophic effect by enhancing the metabolic function of the mucosa by releasing polyamines, which are implicated in stimulating the enzymatic activity of the colonic mucosa [7]. This study was designed to assess the efficacy of S. boulardii in the treatment of diarrhoea caused by ETEC E. coli and parameter of assessment was studying histological alteration in the intestine of mice infected with E. coli.

Materials and Methods
Microbial isolates
Enterotoxigenic E.coli was supplied by Central Public Health Lab. and previously isolated from stool sample of infants suffering from diarrhea; S. boulardii was bought as commercial lyophilized yeast (Ultra-Levure®, BIOCODEX, France).

Bacterial culture
The bacterial strain E. coli 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 [8].
**S. boulardii culture**

The yeast *S. boulardii* was grown on Sabouraud Dextrose Agar medium for 48hrs at 28 °C, and then cells were harvested and washed 3 times with PBS. Cells resuspended in PBS to a final concentration of $1 \times 10^9$[9].

**Experimental design**

Twenty albino male mice were randomly divided into eight groups designated as 1, 2, 3, and 4. Each group consisted of 5 mice, and subjected to the following treatments according to [10].

Group1: This group was used as a negative control.

Group2: This group was dosed with 0.1ml of $2.5 \times 10^7$ cfu/ml *E. coli* culture and use as positive control.

Group3: This group was dosed with 0.1ml of $1 \times 10^9$ cfu/ml *S. boulardii* culture.

Group4: This group was dosed with 0.1ml of $1 \times 10^9$ cfu/ml *S. boulardii* culture, and infected with 0.1ml of $2.5 \times 10^7$ cfu/ml culture of *E. coli*.

Mice were dosed with a single dose 0.1 ml of $1 \times 10^9$ cfu/ml *S. boulardii* culture daily by oral administration for 7 consecutive days. After 7 days of treatment, at the 8th day of experiment period, each mouse was given 0.1 ml of $2.5 \times 10^7$ *E. coli* culture by oral administration. After the 6th day of infection with *E. coli*, mice were sacrificed by cervical dislocation and collected to evaluate histological effect. Pieces were taken from intestine and put in petridishes contain physiological salty solution to remove the fatty tissues and sticky bundles, then the organ was kept in test tubes containing 10% formalin for about 16-18 hrs for fixation purpose, then they were transferred into tubes containing 70% ethanol alcohol in which they were preserved till the time of the final preparation [11]. The staining method was performed by using hematoxilin and eosin [12].

**Results**

Mice intestinal sections were taken from the control group showed normal structure appearance of villi without any pathological changes as shown in Fig.(1A).

![Fig. 1](image)

**Fig. (1) (A) Section of intestine of normal mice showing normal structure appearance of intestinal villi**

**(B) Section in small intestine in mice infected with *E. coli* showing infiltration in the lamina propria in the middle of the villi with edema(arrow) (HE) ×100.**

**Group 3 was fed on the basal diet and was also dosed with 0.1 ml of *S. boulardii* figure 2C showing the normal appearance of intestine villi. Section of intestine showing normal structure appearance of intestine villi figure 2D in mice treated with *S. boulardii* and infected with *E. coli*.**

![Section of intestine of normal mice showing normal structure appearance of intestinal villi](image)

![Section in small intestine in mice infected with *E. coli* showing infiltration in the lamina propria in the middle of the villi with edema(arrow) (HE) ×100.](image)
Discussion

Results indicated that the intestinal villus pattern of mice infected with ETEC E.coli was markedly eroded; this might be caused by bacterial attachment to the epithelium of the intestinal tract and production of a toxin that acts locally on enterocytes. It is known that ETEC E.coli produces two main types of toxins designated heat-labile (LT) and heatstable (ST) toxins. There are two forms of heat stable enterotoxin; STα and STβ [13, 14]. The bacterium colonize the GI tract by means of a fimbrial adhesin, e.g. CFA I and CFA II, and is noninvasive, but produces either the LT or ST toxin.

Mice treated with S. boulardii revealed a probiotic effect on intestinal sections. The probiotic effect of S. boulardii on the intestine could be due to its ability to adhere to cells in the Jejunum [15, 16], increasing brush border enzyme activity of lactase, alpha-glucosidase, and alkaline phosphatase, and increasing normal enterocyte maturation [17].

The intestinal villus pattern of mice treated with S. boulardii and infected with E.coli showing normal villi appearance. This may be attributed to antagonism effect of S. boulardii. A similar result was obtained by Geddek[18] who mentioned that the outer membrane of S. boulardii is rich in mannose, allowing pathogens such as E. coli, Salmonellae and other type 1 pili (mannose-binding fibers) containing pathogens to bind to this mannose rich membrane. This binding action prevents E. coli and other harmful bacteria from adhering to intestinal cells. This action may also be beneficial for other probiotic species as there is less competition for colonization.

Also, Dahan et al., [19] showed that S. boulardii has the capacity to release factors which neutralize bacterial toxins and decrease the deleterious effects of infectious pathogens. Another study showed that S. boulardii also produces a 63 kDa protein phosphatase that inhibits the LPS toxicity of enterotoxigenic E. coli O55B5 by endotoxin dephosphorylation [20].

The beneficial effect of S. boulardii on C. rodentium-induced colitis was assessed in mice. This improvement effect of S. boulardii was associated with significantly reduced numbers of mucosal adherent bacteria compared with the infected untreated animals (P ≤ 0.05). This effect was not due to a bactericidal action but was correlated with a reduction in EspB and Tir protein secretions; respectively a translocater and an effector protein implicated in the type III secretion system (TTSS) [21].

This result came in accordance with Caetano et al., [22] who mentioned that S. boulardii also exerts a protective effect on epithelial cells infected with E. coli by decreasing the level of intracellular infection and reducing the apoptotic effect of E. coli on intestinal epithelium.
References
الخلاصة

اجريت الدراسة لتقييم التغيّرات النسيجية في امعاء الفئران المعالجة بخميرة Sacchromyces boulardii والمسالحة ببكتريا الـ E. coli المصابة ببكتريا الـ E. coli المدرجة للسم الداخلي. درس التأثير على 200 فارة بيضاء والتي قسمت الى اربعة مجاميع وقد تضمنت كل مجموعة على 5 فئران متساوية بالاعمار والاجتماع. عدت المجموعة (1) سبعة سالبة والمجموعة (2) المدرجة للسم الداخلي (سبعة E. coli تم إصابتها ببكتريا الـ موجبة) والمجموعة (3) جرعت بخميرة Sacchromycesboulardii والمجموعة (4) جرعت بخميرة Sacchromycesboulardii الـ E. coli المدرجة للسم الداخلي. أظهرت نتائج الدراسة النسيجية ان اصابات الفئران ببكتريا E. coli تسبب بتنخرات وتغيرات انحلالية وارتشاح الخلايا الالتهابية في امعاء الفئران مقارنة بالمقاطع الطبيعية المأخوذة من الفئران غير المصابة. كما اظهرت النتائج ان معالجة الفئران بالخميرة منع التأثير السام لبكتريا E. coli في الفئران المصاب.