Effect of Oral *Sacchromycesboulardii* 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 *Sacchromycesboulardii* 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 followings treatments; Group1; used as a negative control.Group2; was infected with enterotoxigenic *E.coli* culture and use as positive control. Group3; was dosed with *S.boulardii*; Group4; 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 antitoxinactivity [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 2.5×10^7 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 1×10^9[9].

Experimental design

Twenty albino male mice were randomly divided into eight groups designated as 1, 2, 3, and 4. Each group consisted of 5mice, 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 2.5×10^7cfu/ml *E. coli* culture and use as positive control.

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

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

Mice were dosed with a single dose 0.1 ml of 1×10^9cfu/ml *S. boulardii* culture daily by oral administration for 7 consecutive days. After 7 days of treatment, at the 8^{th}day of experiment period, each mouse was given 0.1 ml of 2.5×10^7 *E. coli* culture by oral administration. After the 6^{th} 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 finalpreparation [11]. The staining method was performed by using hematotoxilin and eosin [12].

Results

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

Results indicated in Fig.(1B), intestinal sections taken from mice infected with *E. coli* showed shedding and necrosis of intestinal mucosa and villi inside the lumen of the intestine.

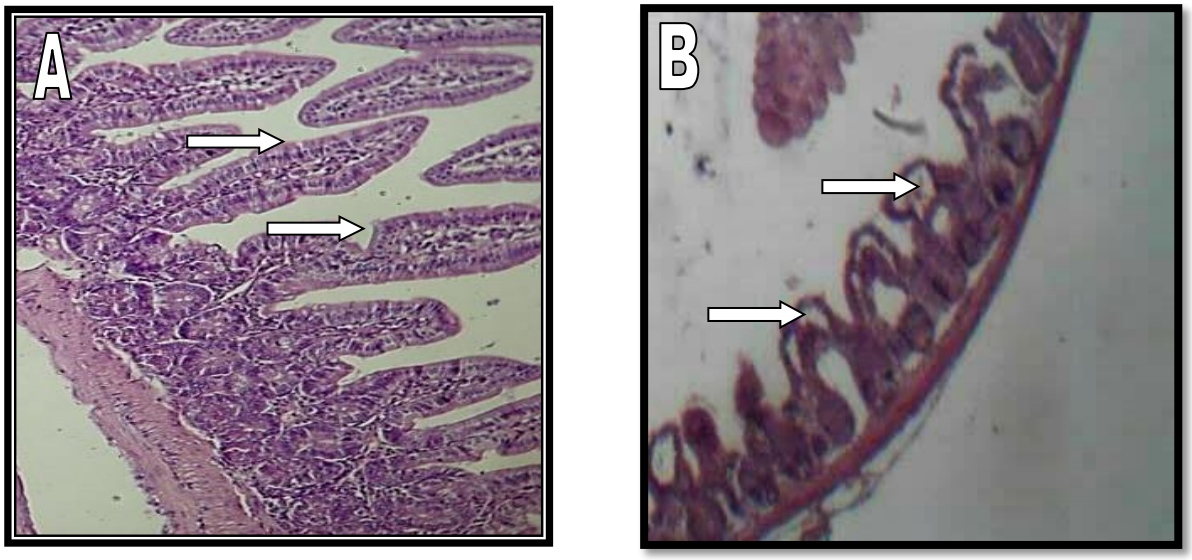

Fig. (1) (A) Section of intestine of normal mice showing normal structure appearance of intestinal vili
(B) Section in small intestine in mice infected with E. coli showing infiltration in the lamina properia in the middle of the villi with odema(arrows) (HE) ×100.
Group 3 was fed on the basal diet and was also dosed with 0.1 ml of S. boulardii figure 2Cshowing 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.

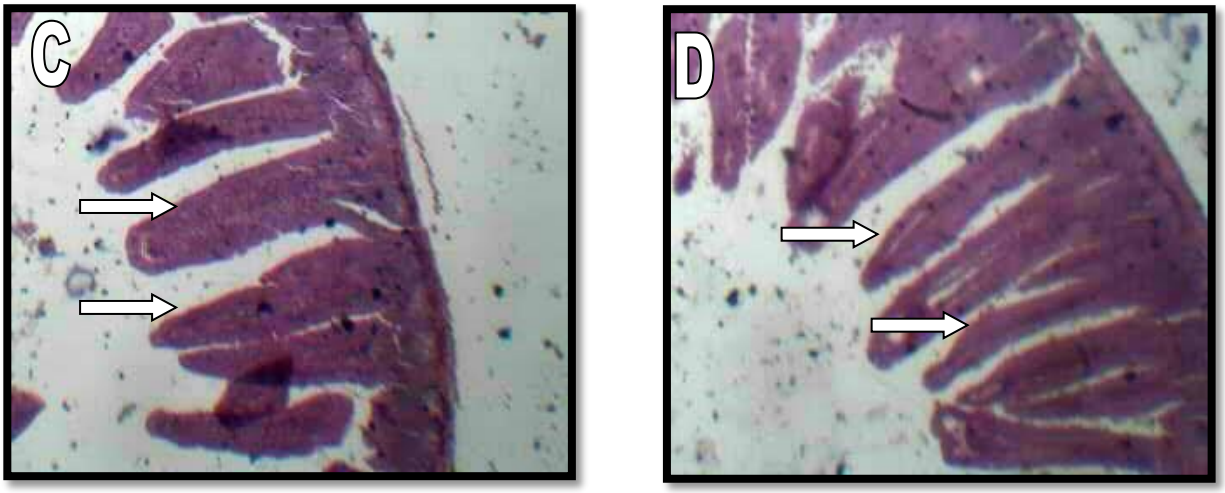

Fig. (2) (C) Section in small intestine in mice treated with S. boulardii showing normal villi appearance.
(D) Section in small intestine of mice treated with S. boulardii and infected with E. coli showing the villi look like normal appearance.

Discussion

Results indicated that the intestinal villus pattern of mice infected with ETEC*E.coli* was markedly eroded; this might be causedby 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; STa andSTb[13, 14]. The bacterium colonize the GI tract by means of a fimbrialadhesin, 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 alkalinehosphatase, 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. boulardi* 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 byendotoxin 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 ($P \leq 0.05$). This effect was not due to a bactericidal action but was correlated with a reduction in EspB and Tir protein secretions; respectively a translocator 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.

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الخلاصة

اجريت الدراسة لتقييم التغيرات النسيجية في امعاء الفئران المعالجة بخميرة *Sacchromyces boulardii* والمصابة ببكتريا الـ *E. coli* المنتجة للسم الداخلي. درس التأثير على ٢٠ فارة بيضاء والتي قسمت الى اربعة مجاميع وقد تضمنت كل مجموعة على ٥ فئران متساوية بالاعمار والاحجام. عدت المجموعة (١) سيطرة سالبة والمجموعة (٢) تم إصابتها ببكتريا الـ *E. coli* المنتجة للسم الداخلي (سيطرة موجبة) والمجموعة (٣) جرعت بخميرة *Sacchromycesboulardii* والمجموعة (٤) جرعت بخميرة *Sacchromycesboulardii*) وتم إصابتها ببكتريا الـ *E. coli* المنتجة للسم الداخلي. اظهرت نتائج الدراسة النسيجية ان اصابة الفئران ببكتريا *E. coli* 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 منع التأثير السام لبكتريا *E. coli* في الفئران المصابة.