Saccharomyces boulardii as effective probiotic against *Shiegella flexneri* in mice

دراسة القدرة العلاجية التعزيزية لخميرة *Saccharomyces boulardii* ضد بكتريا *Shiegella flexneri*

Nedhaal S. Zbar **Lamyiaa F. Nashi** **Shahlaa M. Saleh**

College of Science/Al-Nahrain University

نضال سهيل زبار **لمياء فنجان ناشي** **شهلاء مهدي صالح**

كليه العلوم/ جامعة النهرين

Abstract

This study was designed to evaluate the ability of *Saccharomyce buolardi* as effective probiotic against *Shiegella flexneri.* Mice treated with *S. boulardii and infected with Sh. flexneri,* then serum levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) of treated mice were measured and histological sections were made from liver to evaluate protective effect. Results showed that mice treated with *S. boulardii* exhibited no significant p≤0.05 differences in serum level of AST and ALT 131,67 respectively U/L in comparison with their levels in serum of control group 113.2, 72.86 U/L. Mice infected with *Sh. flexneri* showed a significant increase in serum level of AST and ALT 198, 101 U/L in comparison with their levels 113,72 U/L in control group. Mice treated with *S. boulardii* and infected with *Sh. flexneri* showed a significant decrease in serum level of AST and ALT in comparison with their levels in mice infected with *Sh. flexneri* 80.13,78.26 U/L vs. 198 and 101 U/L respectively. Histopathological study showed that infection with *Sh. flexneri* caused a necrosis, degenerative changes and inflammatory cells infiltration as compared with control, while treatment with *S. boulardii* prevented the histopathological effect of *Sh. flexneri*.

Key words: *Shiegella .flexneri*, probiotic, *Saccharomyces boulardi*

المستخلص

صممتْ الدراسة الحالية لتَقييم القدرةِ العلاجية التعزيزية لخميرة *Saccharomyces buolardi* ضدّ بكتريا الشيغلا فلاكسناريا . جرعت الفئران بجرعات من هذه الخميرة . وأصيبت ببكتريا *Sh. flexneri*، تم قياس مستويات مصلِ Aspartate aminotransferase (AST) و Alanine aminotransferase (ALT) في الفئرانِ المُعَالَجةِ واخذت مقاطع نسيجية من الكبد لتَقييم التأثيرِالوقائي. اظهرت النَتائِجَ بأنّ الفئرانِ التي عولجت مَع الخميرة *Saccharomyce sbuolardi* اعطت إختلافات غير معنوية في مستوى مصلِ (AST) و (ALT)131 و67 على التوالي وحدة /1لتر(p≤0.05 بالمقارنة بمستوياتهم في مِصل المجموعة القياسيةِ 113.2،72.86 وحدة/ 1لتر. اما ألفئران التي اصيبت بجرثومة الشيغلا فلاكسناريا ، اظهرت زيادة معنوية 113 و72 وحدة/1لتر بالمقارنة مع مجموعة السيطرة. اظهرت الفئران التي جرعت بالخميرة *S.buolardi* وأصيبت بجرثومة الشيغلا فلاكسناريا انخفاضا معنويافي مستوى مصل (AST) و (ALT) بالمقارنة بمستوياتهم في الفئرانِ المصابة مَع *Sh. flexneri* 80.13 و78.26،وحدة/1لترمقابل 198،101 وحدة/1لترعلى التوالي . اظهرت دراسةَ المقاطع النسيجية بأنّ االاصابة بـ *Sh. Flexneri*سببت تنخروتغييرات إنحلالية وترشحا لخلايا الالتهابية كماهومُقارن بمجموعة السيطرةِ ، بينما الفئران المعالجة بالخميرة لم تظهر التأثيراتَ النسيجية المرضية الناتجة من بكتريا الشيغلا فلاكسناريا .

الكلمات المفتاحية : *Shiegella flexneri* ، القدرة العلاجية التعزيزية ، *Saccharomyces boulardi*

Introduction

The term “probiotic” has been firstly defined by Fuller as “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance” [1]. This definition has been extended to health and probiotics were redefined as “live micro-organisms that, when administered in adequate amounts, confer a health benefit to the host” [2]. *Saccharomyces boulardii* is a thermophilic, nonpathogenic yeast administered in Western Europe for the prevention and treatment of a variety of diarrheal diseases [3]. *Shigella flexneri* causes the most infectious form of bacterial dysentery, shigellosis that causes acute mucosal inflammation in the colonic and rectal epithelium of humans and higher primates. Destruction of the epithelial layer causes watery diarrhoea and severe abdominal pain, which progresses into the characteristic bloody mucoid stool [4].

S. flexneri is highly infectious, requiring as little as 100 cells to cause disease in adult volunteers. This low infective dose is in part attributed to *S. flexneri’s* ability to survive the low acidity of the host’s stomach, via an up-regulation in acid resistance genes [5]

This study had been performed to assess the efficacy of *S.boulardii* in the prevention and the treatment of *Sh. flexneri* infection.

Materials and methods

Microbial Isolates

The isolate *Sh. flexineri* was supplied by Micro. Lab. Dept. of Biotech., College of Science, Al-Nahrain Univ. and previously isolated from stool sample of infant suffering from dysenteri. *S. blourdii* was bought as commercial lyophilized yeast (Ultra-Levure®, BIOCODEX, and France).

Bacterial infection

The isolate *Sh. flexineri* was grown overnight at 37°C in brain heart infusion broth (DIFCO). This activated culture was centrifuged at 3,000 rpm for 5 min, washed with sterile phosphate-buffered saline PBS, pH 7.4, and suspended in PBS to a final concentration of 1×10^9 cfu/ml. Mice were infected by the oro-gastric route with 0.1 ml of the bacterial suspension [6].

***S. boulardii* culture**

S. boulardii was grown on Sabouraud agar (DIFCO) medium for 48hrs at 28°C, and then cells were harvested and washed 3 times with PBS. Cells re suspended in PBS to final concentration of 1×10^9 cfu/ml [7].

Experimental design

Twenty albino male mice were randomly divided into four groups designated as 1, 2, 3, and 4. Each group consists of 5 mice, and subjected to the following treatments: Group1**;** this group was used as a negative control. Group2**;** this group was doused with 0.1 ml of 1×10^9cfu/ml *Sh. flexineri* culture and use as positive control. Group3; this group *Sh. flexineri* represents mice treated with 0.1ml of 1×10^9cfu/ml *S. boulardii* and infected with *Sh. flexineri* culture and finally Group4, which represents mice treated with 0.1ml of 1×10^9cfu/ml *S. boulardii* culture. Mice were fed with a single dose 0.1 ml of 1×10^9cfu/ml *Sacchromyces* culture daily by oral administration for 7 consecutive days. After 7 days treatment, at the 8^{th}day of experiment period, each mouse was challenged with 0.1ml of 1×10^9cfu/ml *Sh. flexineri* culture by oral administration. After 6th day infection, serum samples were collected to evaluate ALT and AST enzymes liver activity and histopatholological effect [8].

Histological study

Mice were sacrificed by cervical dislocation to evaluate histological effect. Pieces were taken from liver 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 [9].The staining method was performed by using hematotoxilin and eosin [10].

Enzyme assay

The enzyme activity of AST and ALT were evaluated in the mouse serum following the enzymatic colorimetric method of Reitman and Frankel [11]. For this purpose a commercial kit (Randox Company) was used.

Results

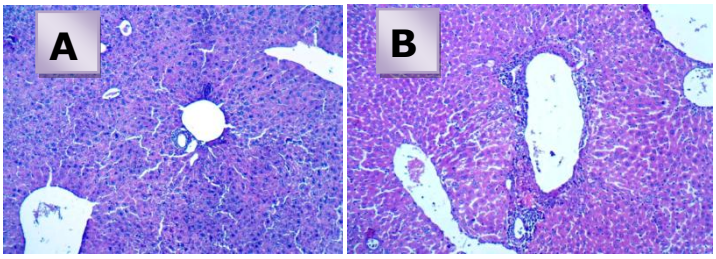
The protective effect of *S. boulardi* on liver enzyme function and the histopathological changes of liver in mice infected with *Sh. flexineri* were observed as follows:

Results indicated in Table (1) showed a significant increase in AST level 198.2 u/ml in mice infected with *Sh.flexineri* $P\leq0.05$ as compared with negative control 113.20 u/ml. Mice feeding with *S.boulardi* and infected with *Sh. flexineri* showed a significant decrease in AST level as compared with positive control 80.12 vs. 113 u/ml. A slight increase in AST level in mice feeding with *S.boulardi* as compared with negative control 131.13 vs. 113 u/ml.

Table (1): Effect of *Sacchromyces boulardii* on the liver functional enzyme activity (AST and ALT) in mice infected with *Shiegella flexneri.*

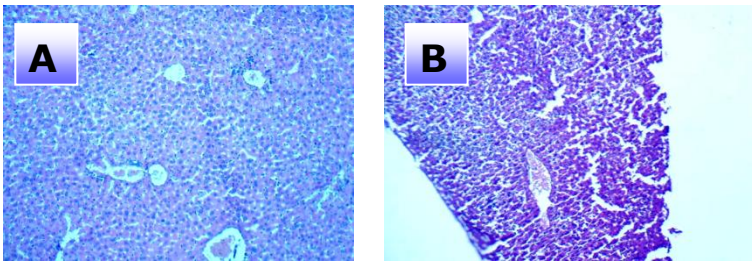
Mice group	AST (Mean±SE *)	ALT (Mean±SE *)
Negative control	**.20±6.95 b113**	**72.86±10.79 c**
Positive control	**198.20±12.43 a**	**101.20±44.94 a**
Treated group	**.13±26.36 b80**	**78.26±31.84 b**
Yeast group	**131.13±66.28 ab**	**67.05±32.77 c**

Morever a significant differences was observed in ALT level in mice treated with *S.boulardii* and mice treated with *S.boulardii* and infected with *Sh.flexineri* as compared with positive and negative control (67.05 and 78.26 vs 101.20 and 72.98. respectively) U/ml and no significant difference was recorded between mice treated with *S. boulardii* and negative control 67.05 vs. 72.86 U/ml. Histological study revealed that liver of mice infected with *S. flexneri* showing certain focal area necrosis with inflammatory cells infiltrate especially at portal area as compared with negative control that exhibited normal looking appearance of parenchymal hepatic tissue, portal area and central vein.



Fig(1): A- liver section of negative control mice showing normal looking appearance of parenchymal hepatic tissue, portal area and central vein while B- liver section of mice infected with *S.flexneri* (Positive control), showing certain focal area necrosis with inflammatory cells infilterate especially at portal area (arraws) (X 200)(H&E).

The liver histological section of mice treated with *S. boulardii* and infected with *Sh. flexneri* showing normal looking appearance with mild inflammatory cells infiltrate since look-like normal hepatic tissue appearance especially near the portal area was observed in the liver section of mice treated with *S. boulardii.*



Fig(2): A-liver section of mice treated with *Saccromyces boulardii* and infected with *Shiegella flenxeri* showing normal looking appearance with mild inflammatory cells infiltrate.B- liver section of mice treated with *Saccromyces boulardii* showing normal looking tissue structure with mild inflammatory cells infiltrate with dispersed cell necrosis.

Discussion

The activity of aspartate aminotransferase and alanine aminotransferase was significantly higher in mice challenged with *Sh flexneri*, as mentioned by Cheesbrough [12] who reported that severe bacterial infections can cause cellular injury that may increase AST level in the serum and result revealed that treatment mice with *S. boulardii* and challenged with *Sh flexneri* led to improvement the liver function activity, this might be due to a reduction of the endotoxin mediated liver damage [12].

Different histopathological changes were observed in liver groups of mice, the histopathological profile showed protective effect after treatment with *S. boulardii*. Its known that *S. boulardii* has several mechanism of action directed against the host as well as pathogenic microorganism which include regulation of intestinal microbial homeostasis, interference with the ability of pathogens to colonize and infect the mucosa, modulation of local and systemic immune responses, stabilization of

the gastrointestinal barrier function and induction of enzymatic activity favoring absorption and nutrition. Based on these findings, we assured that *S. boulardii* prevent a variety of human diarrheal diseases due to its production of SAIF (Saccharomyces anti-inflammatory factor) a small molecular weight, water soluble molecule that inhibits the activation of NF-jB, a transcription factor that plays a central role in human inflammatory responses [13,14 and 15]. Moreover some researcher found that *S.boulardii* triggers release of secretory IgA and enhanced phagocytic and and killing activity of neutrophils.

Refrences

1. Fuller, R. (1989). Probiotics in man and animals. J Appl Bacteriol. 66: 365-378.
2. FAO/WHO. (2001). Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria (Cordoba, Argentina: Food and Agriculture organization of the United Nations and World Health Organization).
3. McFarland, L.V. and P. Bernasconi. (1993). Saccharomyces boulardii: a review of an innovative biotherapeutic agent. Microb. Ecol. Health Dis. 6:157-171.
4. Jennison, A.V. and Verma, N. K. (2004). Shigella flexneri infection: pathogenesis and vaccine development. FEMS Microbiology Reviews. 28:43-58.
5. Small, P., Blankenhorn, D., Welty, D., Zinser, E. and Slonczewski, J.L. (1994) Acid and base resistance in Escherichia coli and Shigella flexneri: role of rpoS and growth pH. J. Bacteriol. 176, 1729–1737.
6. Lilian, M., Elizabeth, N., Leda, Q. and Jacques, R. (2001). Protection by Lactobacillus acidophilus UFV-H2B20 against experimental oral infection with *Salmonella enterica* subsp. Enterica Ser. Typhimurium gnotobiotic and convential mice. Braz. J. Microbiol. 32 (1).
7. Mahzounieh, M., Karimi, T., and Marjianian, R., (2006). The preventive of Saccharomyces boulardii in pathogenesis of Salmonella typhimurium in xperimentally infected rats. Pak. J. of Bio. Scien. 9(4): 632-635
8. Lin, W.H, Yu, B, Lin, CK., Hwang, W.Z, Tsen, H.Y. (2007). Immune effect of heat-killed multistrain of *Lactobacillus acidophilus* against *Salmonella typhimurium* invasion to mice. Appl Microbiol. 102(1):22-31.
9. Bankroft, L. (1980). Basic histological techniques; 4th ed; an Arboer science publishers.: 130-145.
10. Junqueria, L. and Carneiro, J.R.O. (2005). Male Reproductive System. In: Basic Histology. Janson, M.; Harriet and Petery J., Stamford. 11th ed. Pp: 418- 434.
11. Reitman, S. and Frankel, A.S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetatic and glutamic pyruvic transaminases Am J Clin Pathol. 28:56-58.
12. Cheesbrough, M. (1999). District laboratory practical manual in tropical countries. Vol 11. Cambridge University Press.
13. Xavier, R. J., and Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. Nature *448*, 427-434.
14. Czerucka, D., Piche, T. and Rampal, P. (2007). Review article: yeast as probiotics *Saccharomyces boulardii*. *Aliment Pharmacol Ther*. 26: 767–778.
15. Pothoulakis, C. (2009). Review article: anti-inflammatory mechanisms of action of Saccharomyces boulardii. Aliment Pharmacol Ther. 30: 826–833.