ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

Histological Effects of *Saccharomyces Boulardii* Probiotic in Some Mice Organs Infection with *Salmonella Typhimurium*

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Abstract

Background and Objective: *Saccharomyces Boulardii*, Eukaryotic probiotic it was unable to proven therapeutic efficacy. This study leads to evaluate the protective effect of three types of probiotic yeast *Saccharomyces Boulardii* in the tissues of some internal organs of laboratory mice before and after infection with pathogenic bacteria *Salmonella Typhimurium*. Materials and Methods: Three types of yeasts were used ,Local Iraqi Isolate (Local *Saccharomyces Boulardii*) LSb ,Mutant Iraqi Isolate (Mutant *Saccharomyces Boulardii*) MSb and commercial strain (Commercial *Saccharomyces Boulardii*) CSb. Forty male white mice were divided into 4 groups , Each group included 10 mice: control, MSb, CSb and LSb ,dosed with 0.2 ml/day for 28 days of yeast inoculums except control treatment fed on the standard diet, half number of animals in each group were sacrificed for histological examination of some organs (liver, spleen and intestine),the remaining mice were infected with 0.2ml of *S. Typhimurium* daily for 8 days , and sub-treated have been derived: Control + St, MSb + St, CSb + St and LSb + St. the histological examination of the indicated organs was performed .Results: Histological indicators of internal organs (liver ,spleen and intestine) of the groups MSb, LSb and CSb, shown that no necrosis or clear lesion or inflammatory sign in those organs .In addition ,histological examination of infected St +control group shown necrosis of hepatocytes , congested central vein and dilated of sinusoids of the liver , necrosis in the lymphoid tissue and inflammatory cells infiltration in the white pulp of the spleen , while the changes in the intestine shown inflammation cells infiltration in the lamina properia and erosion of epithelial cells , compared with the positive effects of MSb + St, CSb + St, and LSb + St in reducing necrosis ,inflammation and cytotoxic effects of *S. typhimurium*, but the MSb + St group was the best .Conclusion: Mutant Iraqi isolate was the efficient in protective the tissues of (liver, spleen and intestine of animals after infections with *S. Typhimurium* bacteria .

Keywords: *Probiotics, Saccharomyces Boulardii, Mutagenesis, Salmonella Typhimurium, Histopathological changes***.**

Introduction

Salmonella discovered by American scientist Daniel Salmon [1], species associated with this genus are causing numerous pathological infections worldwide such as typhoid fever, food poisoning, gastroenteritis, intestinal fever and other diseases and the most important serotype *Salmonella Typhimurium*. Duerr *ET al.*[2]

Reported that S. *Typhymurium* is one of the main causes in gastrointestinal infections associated with contaminated food, as adhesive with endosomal vesicle cells that contain the cells of living bacteria (Saimonella-Containing Vacuole).

Probiotic word is means life, and this contrasts with antibiotic term, which means anti-life [3]. The importance of microorganisms as probiotic was cleared in the 20th century, specifically by the Russian scientist Eli Miechenkov, when he observed the ability of *Lactobacilli* bacteria in fermented milk to get positive effect in gut micro flora of the human [4].

As defined by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), the Probiotics are live microorganisms that, when present in adequate numbers, provide useful health to the host [5].

Despite the widespread use of Prokaryotic probiotic[6], however scientific studies confirmed on the existence of Eukaryotic microorganisms that have positive advantages in enhancing the health and defenses of the host[7,8] ,such as some types of molds and yeasts , and the most important *Saccharomyce Boulardii* yeast , Which is the only species of *saccharomyces* genus that is documented for human consumption[9], as listed in the General Recognized As Safe (GRAS) list prepared by Food and Drug Administration in 1996.

S. Boulardii yeast has proven its therapeutic efficacy by possessing many unique characteristics, that enable it to management the GI effectively[10].Scientific experiments and clinical applications have confirmed its ability to treat diarrhea associated with several factors (diarrhea associated with severe bacterial and viral, travelers' diarrhea and diarrhea caused by antibiotics) .

It also inhibits the adhesion of *Salmonella* bacteria to the epithelial cells of the intestinal membrane by connecting it to the layer of mannose on its surface[11]. Badia *et al.*[12] also referred to the effect of *S. Boulardii* on farms containing a combination of DCs cells and intestinal epithelial cells infected with S. typhymurium , It was found that *S. Boulardii* had contributed to the modulation of primary inflammatory pathways and inhibit the expression of bacterial pathogenesis.

Materials and Methods

Sources of Isolates

The local isolate of *S. Boulardii* (LSb), was supplied as a lyophilized yeast, which is isolated from mangastin tropical fruits by[13], and mutant it chemically by the same researcher [14] , which is consider as a mutant *S. Boulardii* (MSb) ,while commercial strain of *S. Boulardii* (CSb) was supplied by Sarrow Fomalas Company, Los Angelos-USA, *S. Typhimurium* was provided by Central Health Laboratory, isolated from adult patient with diarrhea resulting from food poisoning.

Preparation the Suspension of *Salmonella typhimurium*

The bacterial suspension of *S. typhimurium* was prepared according to McFarland method[15] where it was suspended in phosphate buffered saline with a concentration of (1×10^8 cfu /ml). It was used to challenge the mice orally by gavages needle.

Preparation of Yeasts Inoculums

In order to activated the probotics yeasts of *S. boulardii*, small amount of lyophilized powder of each yeast ,were transferred by a spatula under sterile conditions to the yeast extract dextrose peptone broth medium and incubating at 37° C usually for one to two days.(repeat three times) ,then 100 ml of skim milk 12% recovery (weight/volume) containing 1% of sucrose was prepared and inoculating with 2% of fresh liquid culture of each yeast , then incubated at 37 ° C till clotting ,repeated three times ,it was used for mice oral administration

Experimental Design

Forty male laboratory mice BALB / C aged 12 -14 week and 35-38 g in weight, were divided into four groups, included 10 mice. Where group (control), fed on a standard diet only , group (LSb), administrated with 0.2ml of local *S. Boulardii* inoculom for 28 days, group (MSb) administrated with 0.2ml of mutant *S. Boulardii* inoculom for 28 days, group (CSb) administrated with 0.2ml of commercial *S. Boulardii* inoculom for 28 days, at day 29, five mice from each group were sacrificed by cervical dislocation to remove internal organs (liver, spleen and intestine), according to [16], the remaining mice were infected with 0.2ml of *S. Typhimurium* suspension at concentration (1×10^8 cfu / ml) daily for 8 days, and subgroups were split: St+ control, St +, LSb + St, MSb + St and CSb + St. The mice were sacrificed for histological examination of internal organs referred to.

Results

The histological examination of the liver sections taken from the mice of control group showed normal structure of the liver which is composed of central vein and hepatocytes arranged in rows and regulated as a form of stripes, which contain inside the natural nuclei, in addition to the existence of the sinusoids in natural form as shown in Figure 1: A.

The histological examination of the liver sections taken from the mice of LSb group showed the structure look like normal which

is composed of hepatocytes as a form of stripes, portal vein, and sinusoids as shown in Figure 1:B. The histological examination of the liver sections taken from the mice of MSb group showed the normal appearance of the liver tissues, which is composed of central vein and hepatocytes, with increase in the number of Kuffer cells as shown in Figure 1:C.

Also, the histological examination of the liver sections taken from the mice of CSb group showed the stricture look like normal , which is composed of central vein and hepatocytes, with increase in the number of Kuffer cells as shown in Figure 1:D The histological examination of the spleen sections taken from the mice of control group showed normal structure of spleen which is composed of white pulp and red pulpas shown in Figure 2: E The histological examination of the spleen sections taken from the mice of LSb group showed the normal shape which is composed of white pulp and red pulp as shown in Figure 2: F.

The histological examination of the spleen sections taken from the mice of MSb group showed structure look like normal which is consisted of white pulp and red pulp as shown in Figure 2: G. The histological examination of the spleen sections taken from the mice of CSb group showed the normal shape of tissues which is composed of white pulp and red pulpas shown in Figure 2: H. The histological examination of the intestine sections taken from mice of the control group showed normal structure of the intestine appearance the villi as a finger, as shown in Figure 3: I.

The histological examination of the intestine sections taken from the mice of LSb group showed normal shape of villi like finger with simple hyperplasia cellular as shown in Figure 3: J. The histological examination of the intestine sections taken from the mice of MSb group showed marked hyperplasia of the lymph node in the sub mucosa as shown in Figure 3: K.

The histological examination of the intestine sections taken from mice of the CSb group showed normal structure of the intestine appearance the villi as a finger with goblet cells as shown in Figure 3: L. In another side , histological examination of the liver sections taken from the mice of control +St group showed necrosis of hepatocytes and marked dilated of sinusoids lead to atrophy of hepatocytes , in addition to inflammatory cells in the liver parenchyma with congested central vein as shown in Figure 4: M .

The histological examination of the liver sections taken from the mice of LSb+St group showed returned the liver to the near shape of normal structure which is composed of the central vein and hepatic stripes, but there is a slight expansion in the venous sinuses as shown in Figure 4: N. The histological examination of the liver sections taken from the mice of MSb+ St group showed returned the liver to the shape similar to the normal structure which is composed of the central vein and hepatocytes with kuffer cells as shown in Figure 4: O.

The histological examination of the liver sections taken from the mice of CSb+St group showed returned the liver to the near structure with kuffer cells but there is a simple expansion in the venous sinuses as as shown in Figure4: P The histological examination of the spleen sections taken from mice of the control +St group showed marked necrosis in the lymphoid tissue and inflammatory cells infiltration in the white pulpas shown in Figure 5: Q .

The histological examination of the spleen sections taken from the mice of LSb+St Group showed marked hyperplasia of white pulp at the expense of the red pulp with simple degeneration in the connective tissue as shown in Figure 5: R. The histological examination of the spleen sections taken from the mice of MSb+ St Group showed moderate hyperplasia of white pulp and proliferation of megakaryocytes as shown in Figure 5: S.

The histological examination of the spleen sections taken from the mice of CSb+ St group showed marked hyperplasia of white pulp with simple degeneration in lymphoid tissue as shown in Figure 5: T The histological examination of the intestine sections taken from mice of the control +S t group showed inflammatory cells infiltration in the lamina properia with clear erosion of epithelial cells as shown in Figure 6: U.

The histological examination of the mice intestine sections taken from the mice of LSb+ St group showed returned the intestinal villi as a finger near to the normal shape with simple shortening of intestinal

villi as shown in Figure 6: W .The histological examination of the intestine sections taken from the mice of MSb+St group showed stricture look like normal, where the clear extension of the intestinal villi as a finger as shown in Figure 6: X.

The histological examination of the intestine sections taken from mice of CSb+St Group showed returned the intestinal villi to the near normal shape with simple shortening as shown in Figure 6: Y.

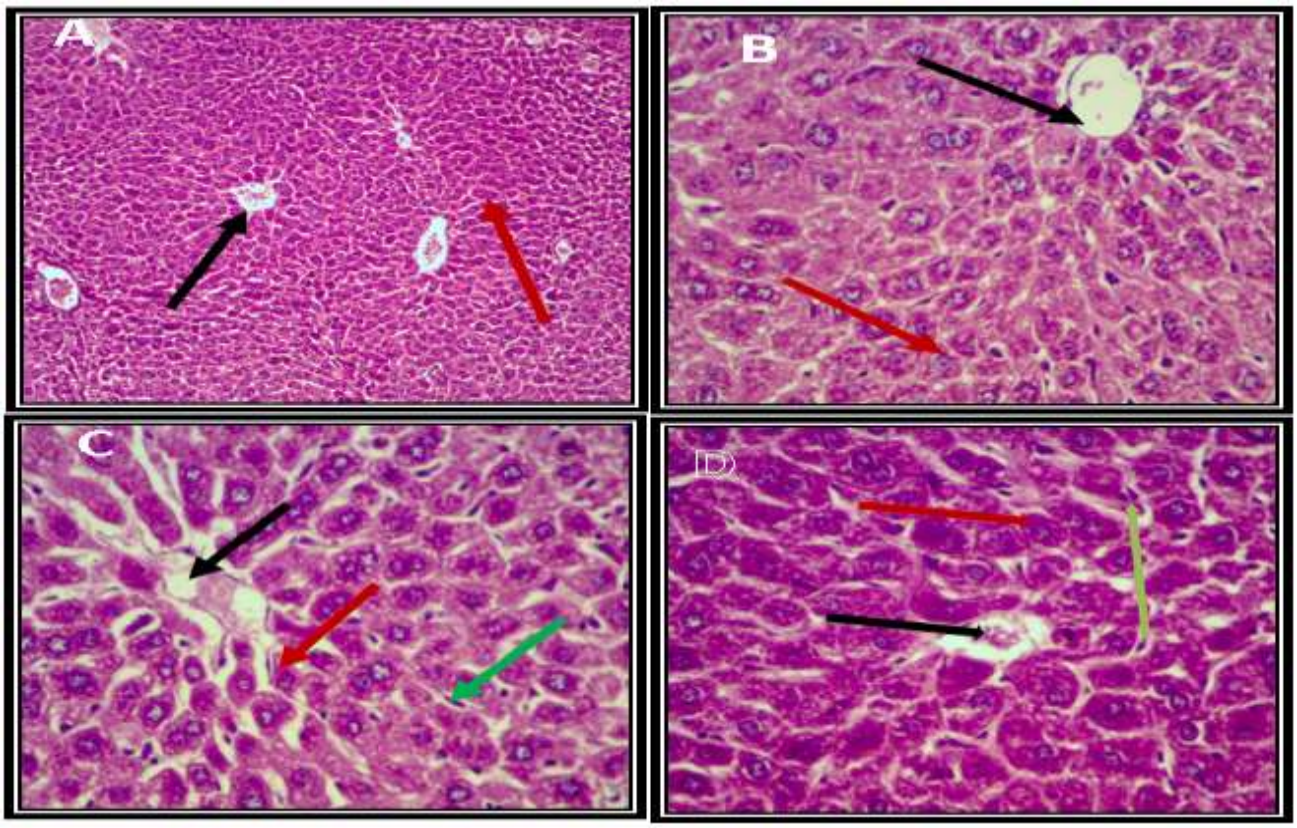

Figure 1: A section of mouse liver (control group) central vein (black arrow) and hepatocytes (red arrow) . B: section of mouse liver (LSb group central vein (black arrow) and hepatocytes (red arrow) . C: section of mouse liver (MSb group) central vein (black arrow) , hepatocytes (red arrow)and kufer cells(green arrow) . D: section of mouse liver (CSb group) central vein (black arrow), hepatocytes (red arrow) and kufer cells (green arrow)

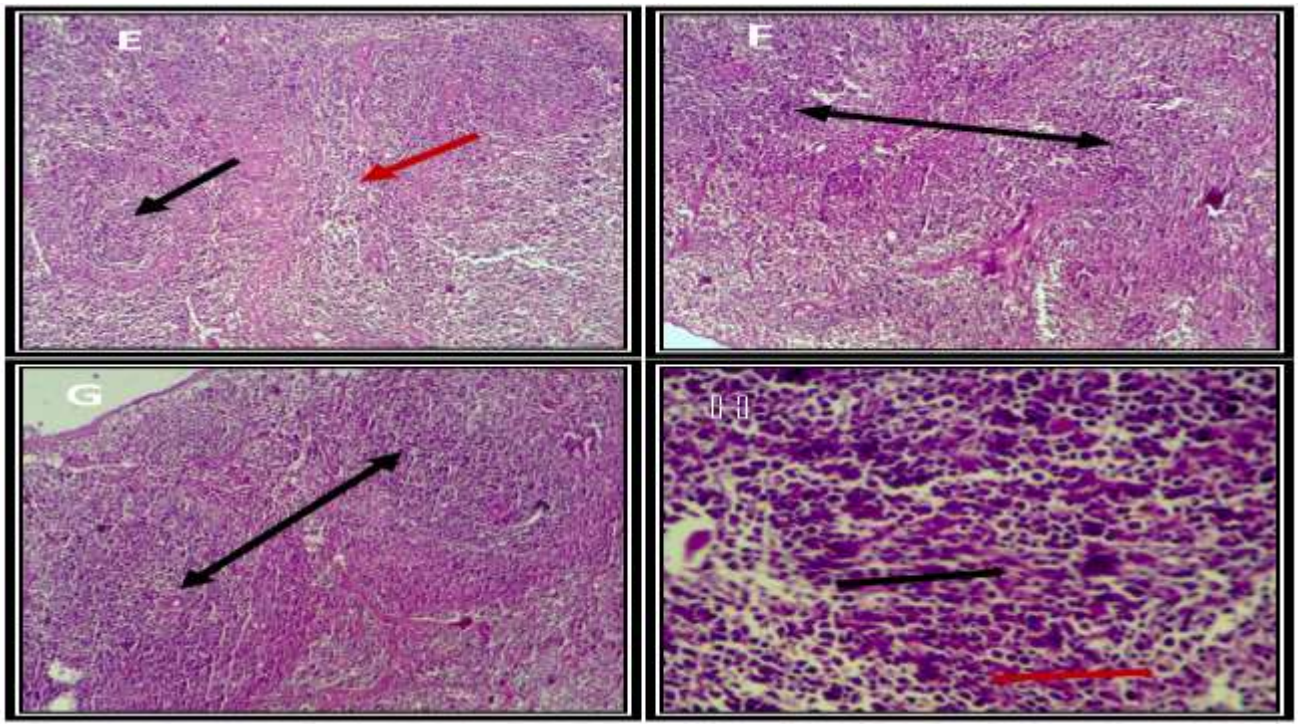

Figure 2: E section of mouse spleen (control group white pulp (black arrow) and red pulp (red arrow). F: section of mouse spleen (LSb group the normal shape of tissue (black arrow). G: section of mouse spleen (MSb group) still preserves structure (black arrow). H: section of mouse spleen (CSb group) white pulp (black arrow) and red pulp (red arrow)

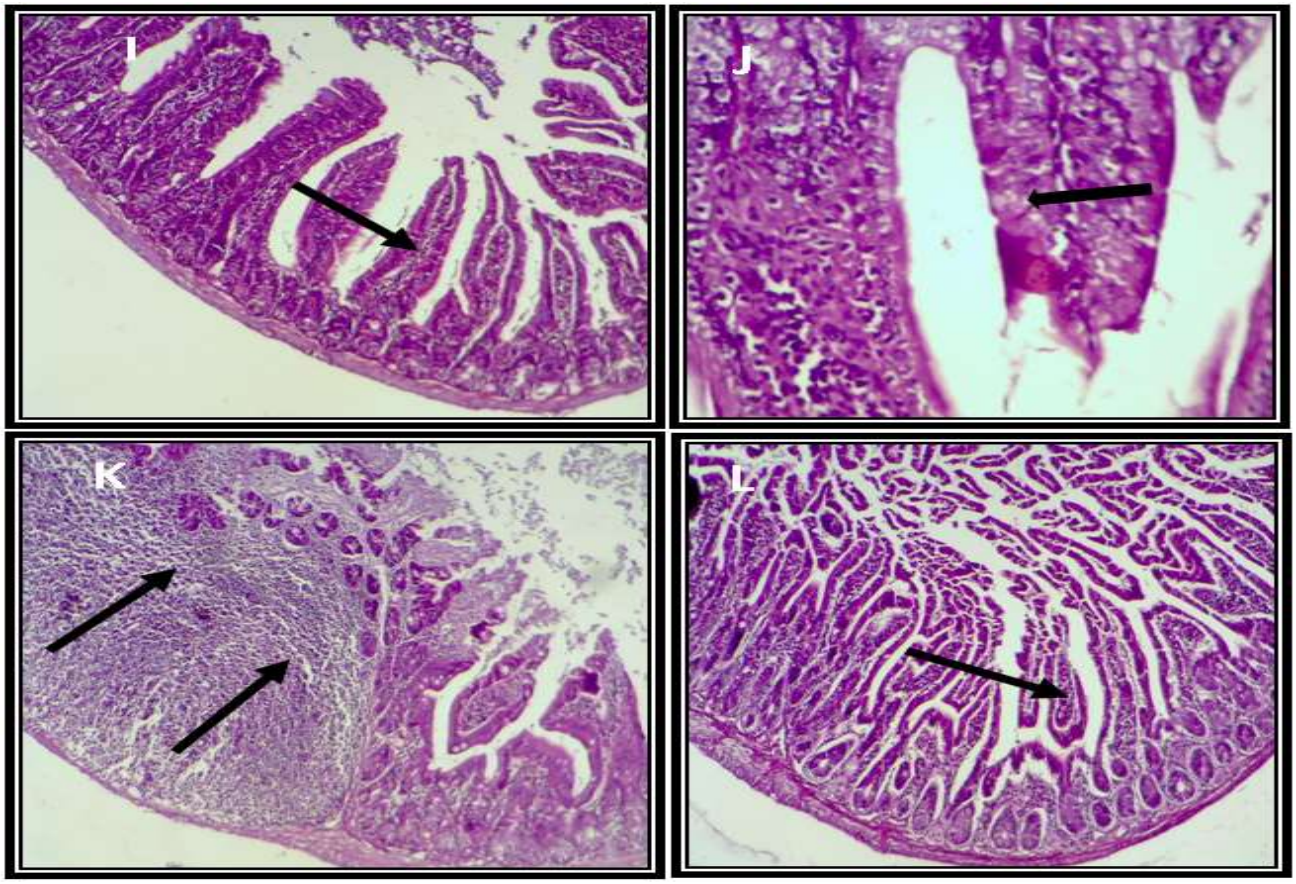

Figure 3: I section of mouse intestine (control group) villi as finger (black arrow). J: section of mouse intestine (LSb group) normal structure of villi like finger (black arrow). K: section of mouse intestine (MSb group) hyperplasia of the lymph node (black arrows) L: section of mouse intestine (CSb group) normal structure of villi with goblet cells (black arrow)

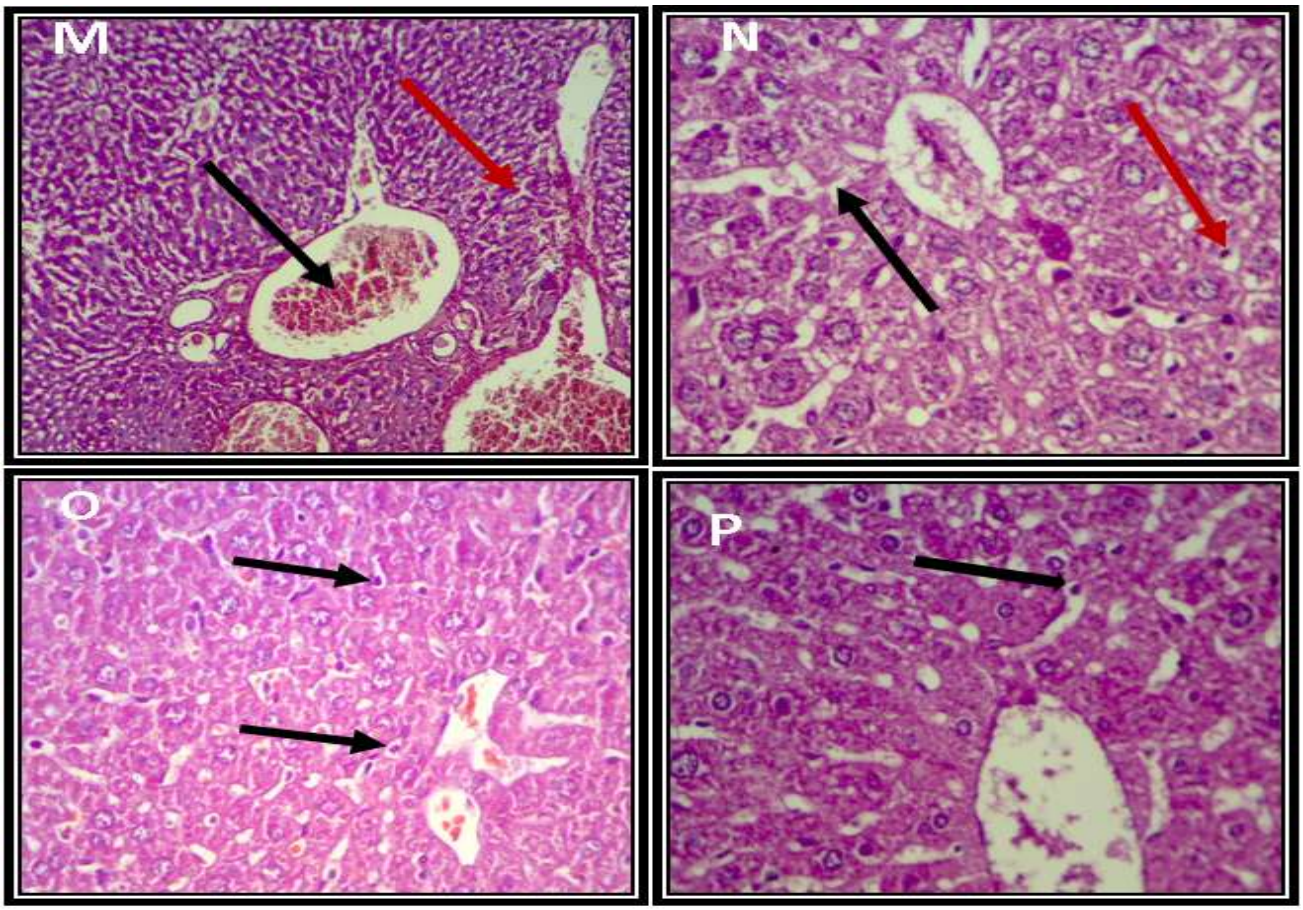

Figure 4: M: section of mouse liver (control +St Group) necrosis of hepatocytes (red arrow) and congested central vein (black arrow). N: section of mouse liver (LSb +St group) returned the liver to the near shape ,but there isa slight expansion in the venous sinuses(black arrow) . O: section of mouse liver (MSb +St group) returned the liver to the shape similar to the normal structure with kuffer cells (black arrows) . P: section of mouse liver (CSb +St Group returned the liver to the near structure but there is a simple expansion in the venous sinuses

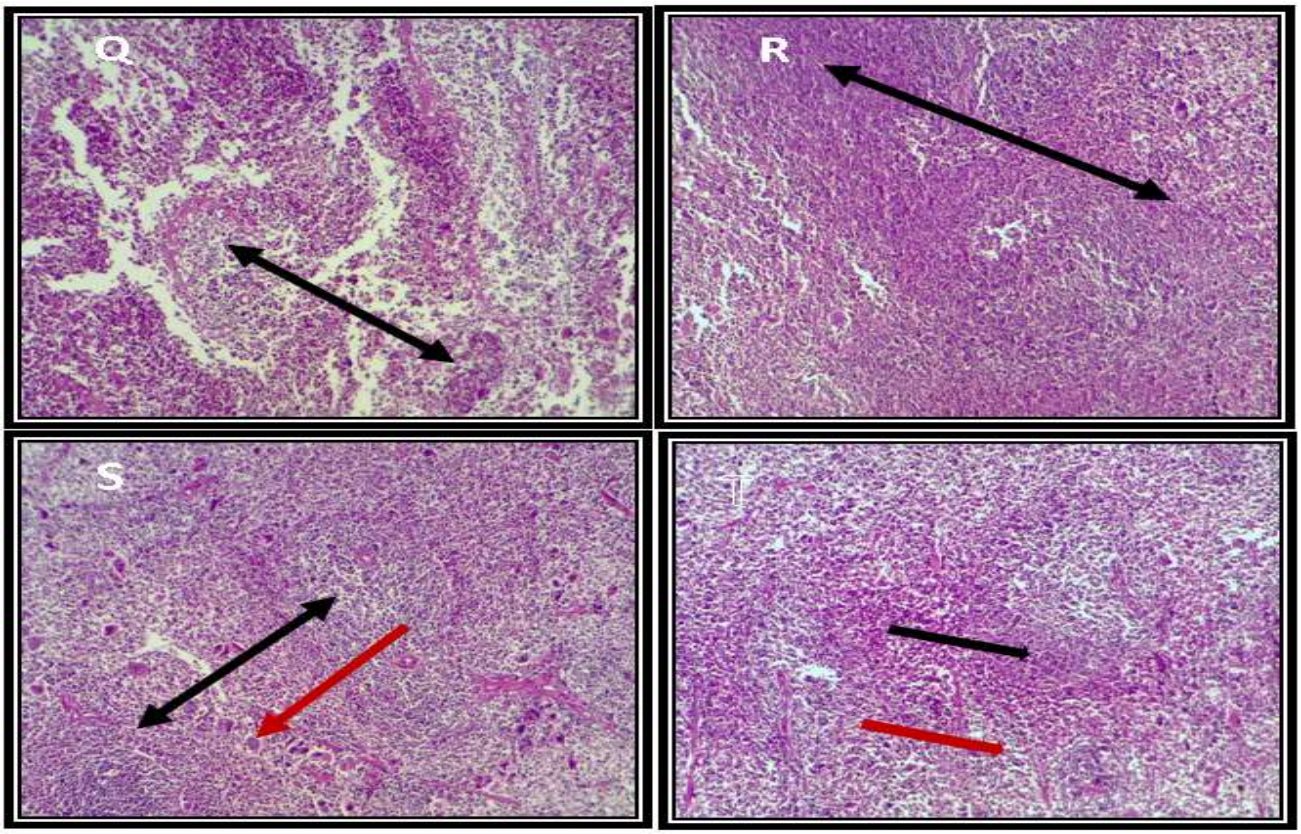

Figure 5: Q: section of mouse spleen (control +St Group) necrosis in the lymphoid tissue in the white pulp (black arrow). R: section of mouse spleen (LSb+St group) marked hyperplasia of white pulp at the expense of the red pulp with simple degeneration in the connective tissue (black arrow). S: section of mouse spleen (MSb+St group) moderate hyperplasia of white pulp (black arrow) and proliferation of megakaryocytes(red arrow). T: section of mouse spleen (CSb+St group) marked hyperplasia of white pulp (black arrow) with simple degeneration in lymphoid tissue(red aroww)

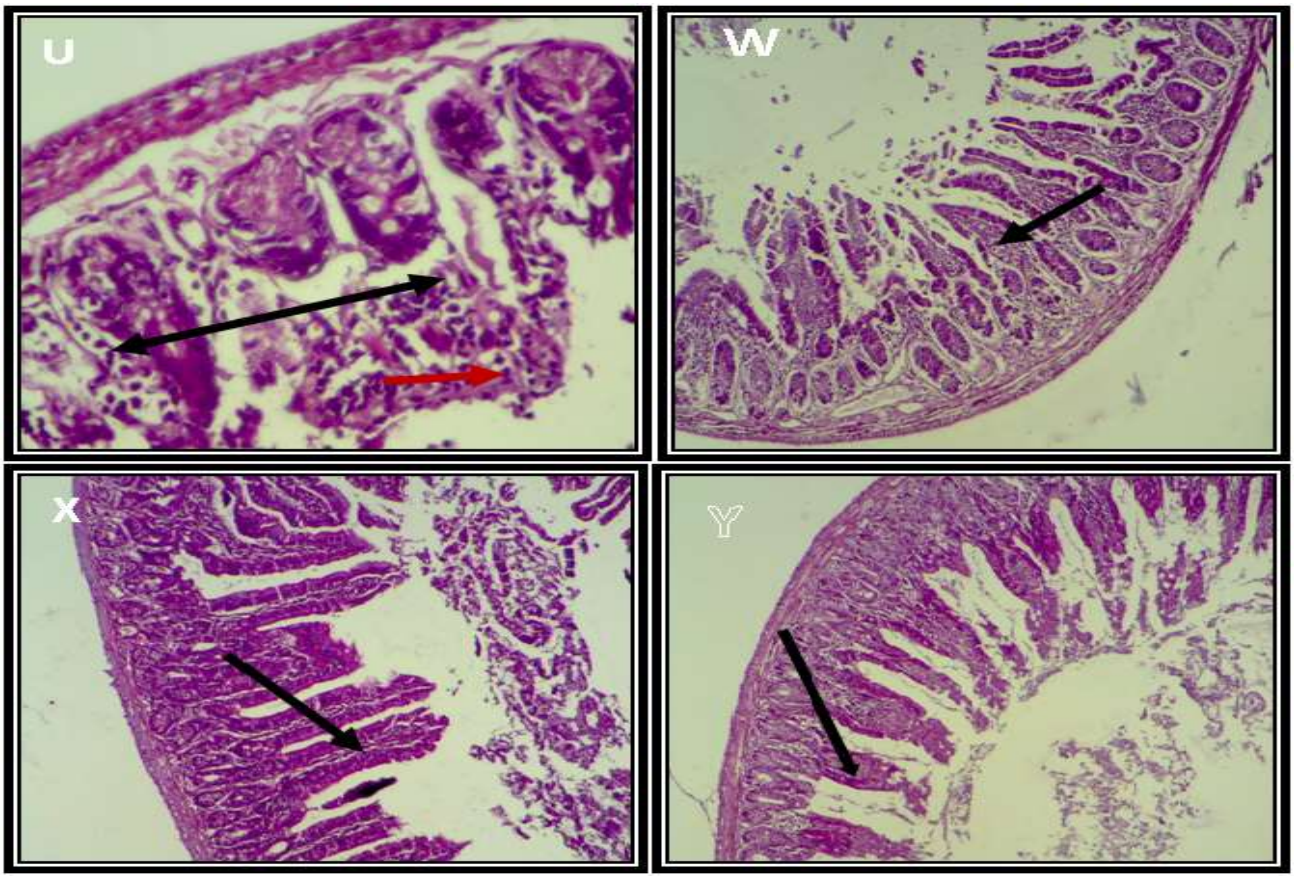

Figure 6: U: section of mouse intestine (control +St group) inflammatory cells infiltration in the lamina properia (black arrow) , clear erosion of epithelial cells (red arrow) . W: section of mouse intestine (LSb +St group) returned the intestinal near to the normal shape with simple shortening of villi (black arrow) . X: section of mouse intestine (MSb +St group) stricture look like normal (black arrow) . Y: section of mouse intestine (CSb +St group) returned the intestinal villi to the near normal shape with slight shortening (black arrow)

Discussion

The results showed that the histological sections of the liver, spleen and intestine of the mice administrated with LSb, MSb and CSb yeasts ,showed no clear lesions in those organs or pathogenic effects ,which refers to the safety of these probiotics. The results showed that the sections of the liver, spleen and intestine of *S. Typhimurium* group had led to many pathological changes in the organs, which were due to the rapid transfer of bacteria through the blood stream to reach the intestine, where it works to hyperplasia the intestinal mucosa, penetrating epithelial cells into the mesenteric lymph node, and M cells that cover the surface of the Payers patches to migrate to important organs such liver and spleen , causing the necrosis of their macrophages, its vital tissues and infiltration of inflammatory cells [17,18].

Martins *et al.* [19] confirmed that the infection of *S. Typhimurium* in mice led to morphological changes in the tissue of the colon and liver causing hepatic degeneration and tissue necrosis, as well as infiltration in monocyte cells. Abed [20] pointed that S.boulardii did not cause any negative effects or symptoms in internal organs, as it was safe, and maintained the natural structure and appearance of the spleen, which is composed of lymphatic cavities. This explains the enhanced immune effects shown by *S. Boulardii* towards lymph organs. Despite the effectiveness of local isolate and commercial strain of *Sacchromyces Boulardii* in mitigating the pathogenesis of *S. Typhimurium* bacteria , however ,the results of histological sections of liver, spleen and intestine of mice administrated with mutant isolate then challenged with *S. Typhimurium*, showed the active and positive effect of this yeast in preventing the deformities , necrosis the tissues, reducing the blood congestion and negative effects resulting from the bacteria in exhaustion the tissues of those organs. This is due to the therapeutic properties, as well as having some of the positive advantages resulting from mutagenic process 14.

The effect of *S. Boulardii* in the preventing of *S. Typhimurium* pathogenesisis either directly by linked the bacteria to the yeast cell wall through the external mannose layer and effect on the virulence factors of the bacteria [21],or by restricting the movement of bacteria[22],or indirectly by interfering with primary cellular pathways that cause inflammation or through nutritional benefits and their role in promoting natural immunity[23].

Conclusion

The three species of probiotic *Saccharomyces Boulardii* shown efficacy in protecting internal organ tissues in mice infected with *Salmonella Typhimurium* ,however mutant isolate was the best ,this refer to the efficiency of mutagenesis process in enhancement therapeutic characteristics in protection the animals.

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