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EFFECTS OF *SACCHAROMYCES BOULARDII* US PROBIOTICS ON SOME HAEMATOLOGY PARAMETERS AND THE LIVER IN MALE RATS INFECTED BY *SALMONELLA ENTERITIDIS*

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Abstract

This study was carried out to investigate the effect of *Saccharomyces boulardii* US probiotics on some blood parameters and histopathological changes in male rats infected by *Salmonella enteritidis*. To re-search this objective, twelve male rats (*albino*) were divided into three groups, housed under same conditions of water, feed, temperature, light and humidity. The first was the control group, while the second was experimentally infected with *S. enteritidis* by injection orally using 1ml / rat of skim milk solution vaccinated by (1.5×10^3cfu) colony forming from *S. enteritidis*. The third group was experimentally infected with *S. enteritidis* and treated after 7 days by oral injection using the skim milk solution. That solution was fortified with count cells 2.5 x 10^6 cfu / ml ,from therapeutic yeast(*Saccharomyces boulardii*). Seven days later blood were obtained from animals by cardiac puncture to measure the total and differential white blood cells count (WBC), lymphocytes, neutrophils, monocytes, eosinophils, basophils. One animal from each group was killed and histopathological examination was done for liver ,to detect the effect of infection on this organ. The results revealed a significant increase in white blood cells count (WBC),and lymphocytes at a significant level (P<005) in infected group while the differential count showed a significant decrease in neutrophils (P< 0.05) in infected group. Also, there were a significant decrease in eosinophils percentage and basophils percentage.

Key words : *Saccharomyces boulardii*, *S. enteritidis*, male rats.

Introduction

Saccharomyces boulardii is a tropical species of yeast first isolated from lychee fruit. *S. boulardii* described that distinct taxonomic, metabolic and genetic properties and sometimes used as a probiotic with the purpose of introducing beneficial active cultures into the large and small intestines (Ali, 2007). As well as conferring protection against pathogenic microorganisms in the host (Rajkowska *et al*., 2012; Toma *et al*., 2005; Soccol *et al*., 2010). It grows at 37°C (McFarland and Bernasconi, 1993). *S. boulardii* was shown to be capable of inhibiting multiplication of enteropathogenic bacteria in vitro and studied the capacity of this yeast to antagonize Salmonella bacteria (Kirpich *et al*., 2015). The protection against *S. enteritidis* obtained in conventional and/ organotobiotic rats previously associated with *S. boulardii* is not due to the reduction of the bacterial populations in the intestines. *S. enteritidis* species are gram-negative, *Salmonella* is an optional, anaerobic bacteria that causes 1.3 billion disease per year (Coburn *et al*., 2007). When supplied orally, *Salmonella* rapidly moves into the intestine and grows in the liver, spleen and intestine (Collin, 1972) and caused food poisoning and inflammation of the intestines, diarrhea and other diseases. *Salmonella enteritidis* is the most numerous anaerobic bacteria commensally inhabitants of the large intestine (Nadim *et al*, 1982). *S. enteritidis* normally inhabit the intestinal tract however some strains have acquired genes that enable them to cause intestinal

infection When ingested can cause diarrhea (Nadim *et al*., 1982). *S. enteritidis* produce active toxins once established in the host but all cases are food and water borne infections (Canani *et al*., 2011). *Salmonella enterica serovar enteritidis* is among the most common that causing salmonellosis. *Salmonella* bacteria are ingested and they pass through a person's stomach ,colonize, small intestine, and large intestine and invaded, intestinal mucosa , proliferate and the lymphoid tissues of the gastrointestinal tract and spread to the bloodstream (McFarland and Bernasconi, 1993). *Salmonella* bacteria was non-endospore-forming bacteria that colonize the intestinal tracts of a wide variety of animal hosts. The primary importance of *Salmonella* spp. is as zoonotic agents and as pathogens in immune compromised rats.

Materials and Methods

Twenty male rats (*albino*) were obtained from animal house of College of Veterinary Medicine, Baghdad University. They were 8-12 weeks old and weighted around 200-250 gm and placed in cages labeled as control, control infected and group infected and treated. They were left for 7 days for a adaptation and fed with standard pellet and provided with distil water. All animals were housed under controlled conditions (temperature, 21±2°C, humidity and a 12-hours light-dark cycle). *S. enteritidis* isolated and was from chicken droppings in (the Veterinary Research Center, Ministry of Agriculture) and identification in the National Center for Salmonella, Ministry of Health.

The pathological dose of the *S. enteritidis* bacteria : Determination of the pathological dose of Salm. enteritidis bacteria was done according to Quinn *et al.* (2004).

The oral administration : The Infection was induced by oral administration of 1 ml of milk solution containing 1.5×10^3 colony forming units of *S. enteritidis* per mil (Canani *et al*., 2011). Control animals received distal water following the same protocol *S. enteritidis* infected animals. Animals were observed daily for activity level food and water intake. Blood were obtained from rats 7 days after inoculation. Blood were collected by cardiac puncture with a sterile needle and syringe and then evacuated in collection tubes containing anticoagulant (Potassium EDTA) anticoagulant tubes were inverted gently several times immediately after sample collection and before use of blood to ensure uniform mixing blood samples were prepared immediately after sample collection and the determine Complete Blood cells Count(CBC) done according to Campbell (1995). The CBC consist of a series of tests that determine number variety percentage concentrations and quality of blood cells. One rat taken from each group (infected and Control) anaesthetized dissected and the visceral organs were harvested sich as spleen. The harvested organs were preserved in 10% formalin solution.

Histological examination : Done according to Almas (1999) Fixation Dehydration Cleaning Wax Infiltration Embedding sample in wax sectioning dewaxing and staining (Haematoxylin and Eosin).

Statistical analysis : Data analysis was performed by using ANOVA and application the S.A.S program (2012) and the data were tested according to the Duncan test (1955). The averages were compared with the probability level of ($p < 0.05$) (SAS, 2012).

Results and Discussion

Blood cells count : These enteropathogenic *S. enteritidis* infections is a very serious disease during the first few weeks of life it occurs in all breeds of dairy calves Pathogenic strains of enteropathogenic *S. enteritidis* are associated with disease of the gastrointestinal tract and with fulminating septicemia of the newborn calves or young animals (Dosogne *et al*., 1997). The objectives of the study are to identify, the effect of *S. enteritidis* infection on some blood parameters which are include; Total White Blood Cells count. In addition to: Histological changes that caused due to *S. enteritidis* infection in liver.

White Blood Cells (WBCs)

The data showed that the white blood cells count in infected group was ($29.00x10^3/mm^3$)significantly increased at level ($p<0.005$) in relative to control value recorded as the control group reached $5.55x10^3$ / mm 3 after 7 days while, in treated group that indicated to significant reductions in the white blood cells at level ($p<0.005$) comparative to control after 7 days. An overall ANOVA of white blood cells data revealed a significant treatment effects at 7 day (table 1).

Neutrophils %

The data showed that the Neutrophils percentage. in infected group was (30.80%) significantly decreased at level ($p <0.005$) in relative to control value recorded 63.82 while,in treated group was (18.50%).

Lymphocyte cells

Lymphocytes (%) percentage the effect oral injection with *Sacch. boulardii* on lymphocyte number of WBCs revealed occurrence of significant increase in its number at level ($p < 0.005$) after 7 day as it reached (75.75%), comparative with control group as it reached (38.25%)

Table 1 : Effect of *Salmonella enteritidis* infection on some blood parameters.

Parameter	Control group M±SE	Infected group M±SE	Treated group M±SE
Total white blood cells count ($x10^3/mm^3$)	5.55±o.19 [b]	29.00±1.87[a]	7.67±0.45[b]
Neutrophils (%)	63.82±3.30[a]	30.80±1.87[b]	18.50±0.69[c]
Lymphocytes (%)	38.25±1,91[c]	56.75±1.32[b]	75.75±2.74[a]

** Significant differences (P<005) Number of animals = 4 for each group. The numbers = mean ± SE.

Table 2 : Effect of *Salmonella enteritidis* infection on some blood parameters.

Parameter	Control group M±SE	Infected group M±SE	Treated group M±SE
Monocytes (%)	3.22±0.21[b]	7.42±0.50[a]	3.22±0.43[b]
Eosinophils (%)	2.95±0.29a	2.37±0.49a	0.15±0.064[b]
Basophils (%)	1.07±0.26[a]	1.02±0.17[a]	0.27±0.08[b]

** Significant differences (P<005) for Monocytes and Eosinophils, While * for Basophils, Number of animals = 4 for each group. The numbers = mean ± SE

after 7 day, while reached in infected group (56.75%) after 7 day. ANOVA performed on the lymphocyte cells revealed a significant treatment effect after 7 day (table 1).

Monocyte cells

The monocyte cells number in WBCs counted following oral injection, displayed significant increase at level ($p<0.005$) as it reach 7.42% in infected group while as it reach 3.22 in treated group, in relative to the control value after 7 days. Where, nor significant between control group and treated group at level ($p <0.005$) and monocyte did not disclose any variation due to the treatment with *Sacch. boulardii* (table 2).

Eosinophil cells

This study was undertaken to examine the eosinophil cells number of WBCs in response to oral injection of endotoxins in *Salmonella enteritidis*. The results revealed non significant at level ($p <0.005$) between control group and infected group, while there were significant between treated group and other group after 7 day from injection. ANOVA of the eosinophil data revealed a significant treatment effect after 7 days (table 2).

Basophil cells

Basophil count where distinguish variation due to the treatment with *Sacch. boulardii* after 7 days of injection. The values of basophil cells in the control group and infected group were 1.07%, 1.02, respectively. Except Monocytes percent an ANOVA test performed on all parameter count resulting revealed a significant treatment effects after 7 days (tables 1, 2).

The results revealed that there are many effects of *S. enteritidis* infection in male rats blood parameters , these effects include significant ($P< 0.05$) increase in total white blood cells count in infected group to reach ($29.00±1.87×10^3$) in compared with ($5.55±o.19×10^3$) in control group, the group treated with therapeutic yeast include significant ($P< 0.05$) decrease in total white blood cells count compared with infected group to reach ($7.67±0.45×10^3/mm^3$) (table 1). This decrease may be due to *Saach. boulardii*. This increase may be due to an therapeutic effect of Yeast (table 2). The experimental increase in the number of white blood cells of rats from the normal range ($1.96\text{-}8.25×10^3/mm^3$) indicates that the animal was disorder while the increase very high, it means the presence of tumor. The results also showed there is an increase in lymphocytes in infected group and treated group comparative with control group (table 1).

Liver histology analysis

The histological analysis of the liver in control group animals showed that liver was normal (Fig. 1).

The liver slice for the control group, whose animals were infected with *Salmonella enteritidis* (fig. 2). All totals fed with standard diet. The histopathological data showed a protective effect against the pathogenic bacteria in yeast-treated rats. Fig. 2 show that the liver of control group animals uninfected. There were proliferation among hepatocytes as well as infiltration of Kupffer cell with congestion. There are different levels from liver damage due to an increase the endotoxins for salmonella bacteria which infected rats, also that the liver was a member of nutrient absorption, metabolism and protection of immunity (Yong-Tao *et al*., 2016). The liver function was affected during the injury, which was associated with intestinal barrier damage (Visschers *et al*., 2013).

Figs. 3a, 3b shows two slices of the liver for one animal infected by *Salm. enteritidis*, which was supported by skim milk solution and fortified with therapeutic *Sacch.*

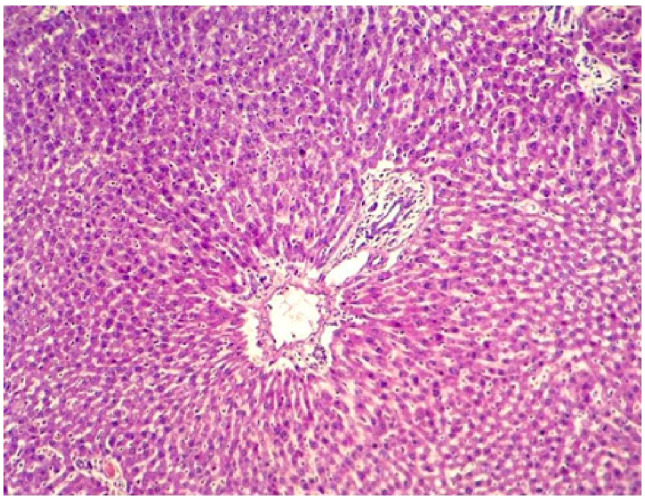

Fig. 1 : Liver slice of the control group animals non-infected with *Salm. enteritidis* (H&E 10X).

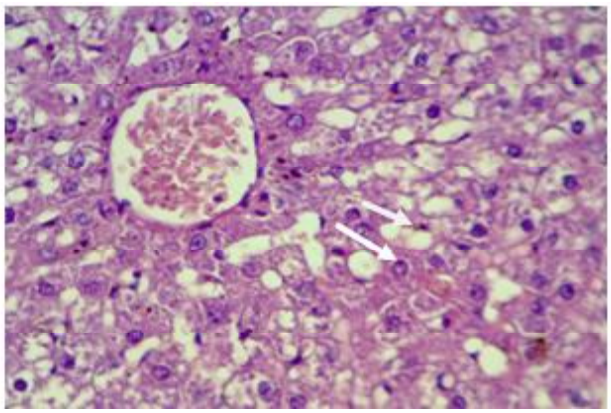

Fig. 2 : The liver slice of the control group animals that infected with *Salmonella enteritidis* (H&E40X).

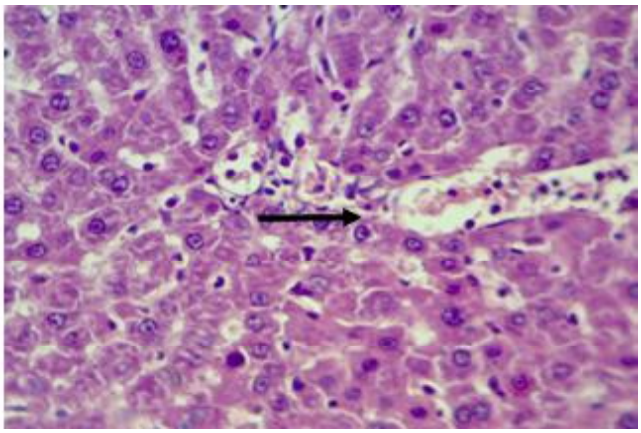

Fig. 3 A : Liver treated group (H&E 40X) as shown.

boulardii yeast. Also, presence proliferation, congestion in the periportal region. There were filtration of mono nuclear cells, which enters the arteries and lymph. As noted presence of apoptosis of monocyte pancreatic cells.

Fig. 3b shows there were a proliferation of fibrous tissue between hepatocytes and infiltration of Kupffer cell, comparative with the control group animals which are not infected. That is the liver appeared naturally, comparative with the infected group, also that group treated with therapeutic yeast. Also showed improvement in the liver for group non-infected and non necrosis in the liver cells. This may be caused by the therapeutic ability of the yeast, which may cause the impairment of toxins or inhibition action bacteria *Salm. enteritidis*. The liver injury was associated with an imbalance of natural microbes in the intestines that cause the growth of pathogenic bacteria and reduce the types of preventive bacteria within the intestines and thus contribute to the risk formation of sepsis and infections (Yong-Tao *et al*., 2016). The functions of liver were significantly affected by ecological changes in the intestinal tract such as liver and biliary abnormalities are common in patients with inflammatory bowel disease, celiac disease or short bowel syndrome (Visschers *et al*., 2013). The intestinal barrier was breaked and the increasing in the internal toxins due to macrophages in the liver. Nitric oxide and cell division also damage or impair liver function (Kirpich *et al*., 2015). The *bifid bacterium* and *Lactobacillus* can reduce the liver injury by restoring the microbial balance of the intestines and increasing colonies resistant to internal pathogens (Xing *et al*., 2006; Bezirtzoglou and Stavropoulou, 2011).

Salmonella enteritidis is the predominant cause significant clinical symptoms of liver injury (Visschers *et al*., 2013). *Saccharomyces boulardii* is used in clinical application for prophylaxis and the treatment of a variety of diseases caused by bacterial infection. The rats used as model of *Salmonella Enteritidis* infection, which included pretreatment with *Sacch. boulardii*, to reveal the protection mechanisms of *Sacch. boulardii* against *Salmonella enteritidis* infection, including the translocation of *Salmonella enteritidis* to the liver and the formation of hepatic tissue lesions in rats after *Salmonella enteritidis* challenge on the 7th day. Compared with *Salmonella enteritidis* infection in rats, that *Sacch. boulardii* yeast decreased *Salmonella enteritidis* translocation to the liver, also abated hepatic tissue injury caused by the infiltration of neutrophilic granulocytes and lymphocytes, by decreasing the translocation of *Salmonella* to the liver (Wu *et al*., 2014; Rodrigues *et al*., 1996).

Conclusion

The oral administration of *Salm. enteritidis* exacerbates acute liver injury. The results revealed an increase in WBC, count and lymphocytes in infectious group and decrease in neutrophils, eosinophils % and basophils %. The histological analysis of the liver in control

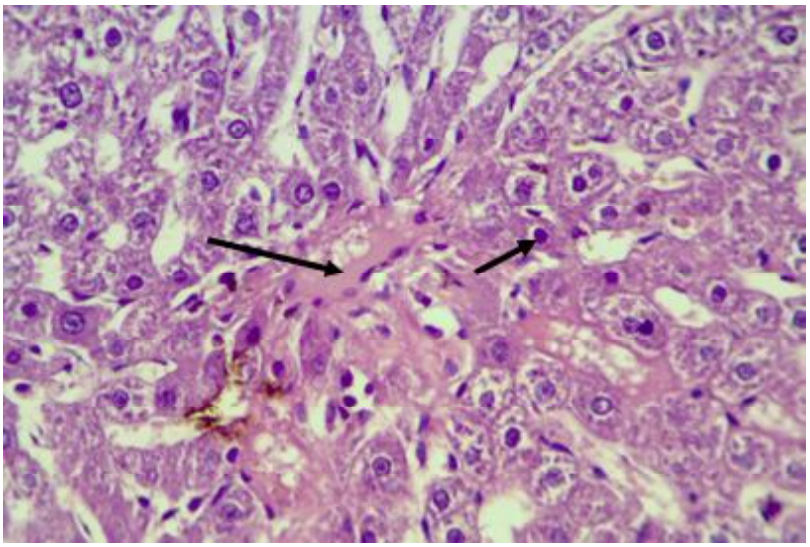

Fig. 3 B : Liver treated group (H&E 40X).

group showed that liver was normal. While the histopathological data showed a protective effect against the pathogenic bacteria in yeast-treated rats while there are different levels from liver damage in infectious rats, in the liver slices for one animal infected, which was supported by skim milk solution and fortified with therapeutic *Sacch. boulardii* yeast. There were proliferation, congestion in the periportal region and filtration of mono nuclear cells, which enters the arteries & lymph and presence apoptosis of monocyte pancreatic cells.

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