Hepatoprotective Effect of *Lactobacillus Plantarum* Against *Salmonella Typhimurium* in Mice

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Abstract

This study was designed to investigate the ability of *Lactobacillus plantarum* as hepatoprotective microorganism against *Salmonella typhimurium* in mice. Twelve albino mice were divided into 4 groups designated as CC, PC, SC and PS. Each group consisted of 3 mice, and was subjected to the following treatments; CC; this group was used as a control. PC; this group was dosed with 0.1ml of 10^9 cfu/ml *Lb plantarum* culture.SC; this group was dosed with 0.1ml. of 2.5×10^7cfu/ml *Sal. typhimurium* culture. PS; this group was dosed with 0.1ml of 10^9cfu/ml *Lb. plantarum* culture, and infected with 0.1ml of 2.5×10^7 cfu/ml culture of *Sal. typhimurium,* then serum levels of Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT) of treated mice were measured and histological sections were made from liver to evaluate protective effect of *Lb. plantarum* . Results showed that mice treated with *Lb plantarum* exhibited a significant ($p\leq 0.05$) decrease in serum level of GPT and GOT (43.25 U/L and 95.50 U/L, respectively) in comparison with their levels in serum of control group. Mice infected with *Sal. typhimurium* showed a significant ($p\leq 0.05$) increase in serum level of GPT (87.3 U/L), and a significant increase ($p\leq0.05$) in serum level of GOT (227 U/L) in comparison with their levels (55.23 U/L and 113 U/L) in control group. Mice treated with *Lb. plantarum* and infected with *Sal. typhimurium* showed a significant ($p\leq 0.05$) decrease in serum GPT level (47.5U/L) and significant decrease ($P\leq0.05$) in serum GOT level (121 U/L) in comparison with their levels in mice infected with *Sal. typhimurium*. Histopathological study showed that infection with *Sal. typhimurium* caused a necrosis, degenerative changes and inflammatory cells infiltration as compared with control while, treatment with *Lb plantarum* prevented the histopathological effect of *Sal. typhimurium* on mice liver.

Keywords: *Sal. typhimurium* Probiotic, *Lactobacillus plantarum.*

Introduction

The term probiotic refers to live microorganism that survive passage through the gastrointestinal tract, and have beneficial effect on the host [1]. Probiotics have several mechanisms to exert their beneficial effect, they prevent colonization, cellular adhesion and invasion by pathogenic microorganisms, they exhibit direct antimicrobial activity and stimulate the host immune response [2]. Probiotic bacteria such as Lactic acid bacteria (LAB) may have a potential effect in several gastroenterological conditions especially in the disturbance of intestinal normal flora [3].

Lactic acid bacteria have the ability to inhibit the growth of various gram positive and gram negative bacteria, this due to the production of organic acids such as lactic acids and acetic acids, hydrogen peroxide, bactriocins, bactriocins like substances and possibly biosurfactants [4].

Lb. plantarum is a gram positive bacilli grouped in chains, microaerophilic, grows at 15°C but not at 45 °C and produces lactic acid, *Lb. plantarum* has one of the largest genomes known among the lactic acid bacteria and it is a very flexible and versatile species [5].

Sal. typhimurium causes gastroenteritis in humans and other mammals; when the bacterial cells enter epithelial cells lining the intestine. They cause host cell ruffling which temporarily damage the microvilli on the surface of the cell. This causes a rush of white blood cells into the mucosa, which throws off the ratios between absorption and secretion, and leads to diarrhea. In mice *Sal. typhimurium* causes symptoms resembling typhoid fever in humans [6].

Clinical symptoms that have been treated or potential to be treated with probiotics include diarrhea, gastroenteritis, irritable bowel syndrome, and inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis),

cancer, depressed immune function, inadequate lactase digestion, infant allergies, hyperlipidaemia, hepatic diseases, *H. pylori* infections, and others [7].

Materials and Methods

Bacterial isolates: *Lb. plantarum* and *Sal. typhimuruim* were supplied by microbiology Lab. in the Department of Biotechnology, College of Science, Al-Nahrain University. *Lb. plantarum* previously isolated from infant stool and *Sal. typhimuruim* previously isolated from patients stool infected with Salmonellosis.

The *Lactobacillus* was grown in de Mann, Rogosa and Sharp (MRS) broth (Merck) medium for 18 hrs at 37 ^{o}C. This activated culture was centrifuged at 2000 *g* at 4ºC and resuspended in phosphate-buffered saline with pH 7, the 10^{9}colony forming units (cfu)/ml was obtained by using Mc Farland method and 0.1 ml of this suspension was administered to mice by gavage needle, before infection with the pathogenic bacteria [8].

Bacterial infection: *Sal.* typhimurium was grown in liquid brain heart infusion (BHI) medium (Difco) for 18 hrs at 37ºC. Mice were infected by the oro-gastric route with 0.1 ml of the bacterial suspension containing about 2.5×10^{7} cfu/ml [8].

Experimental Design: Twelve albino male mice were divided into four groups designated as CC, PC, SC and PS. Each group consisted of 3 mice, and subjected to the following treatments:

Group CC: This group fed only on the basal diet (control).

Group PC: This group fed on the basal diet, and dosed with 0.1ml of 10^{9} cfu/ml *Lb. plantarum* culture.

Group SC: This group fed on the basal diet, and dosed with 0.1ml of 2.5×10^{7}cfu/ml *Sal. typhimurium* culture.

Group PS: This group fed on the basal diet, and dosed with 0.1ml of 10^{9} cfu/ml *Lb. plantarum* culture, then infected with 0.1ml of 2.5×10^{7}cfu/ml culture of *Sal. typhimurium*.

Mice fed with a single dose 0.1 ml of 10^{9}cfu/ml *Lactobacillus* 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 0.1 ml *Sal. typhimurium* (2.5×10^{7}) by oral administration. After 6^{th} day infection with *Sal. typhimurium* [9]. Blood was collected by heart puncture by putting the mouse under anesthetic conditions and the needle was at acute angle to avoid rupture of RBCS. Serum was separated by centrifuging at 2000 g for 10 min., and then was stored at 4 ^{o}C until use.

Mice were sacrificed by cervical dislocation and liver from each group was isolated. Pieces were taken from liver (for histopathological study**)** placed in Petri dishes contain physiological salt solution to remove the fatty tissues and sticky bundles, then the organ were put in 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 [10]. The staining method was performed by using hematotoxilin and eosin [11]. Serum level of GOT and GPT was measured as the procedure described by manufacturer according to the method mentioned by Reitman and Frankel [12]**.**

Statistical Analysis

The values of the investigated parameters were analysis of variance (ANOVA) and Duncan test, using the computer programme SPSS version 7.5 given in terms of mean ± standard error, and differences between means were assessed [13]**.**

Results

Results in Table (1) revealed that feeding mice with *Lb. plantarum* caused a significant decrease ($p\leq0.05$) in the level of serum GPT and GOT (43.25U/L, 95.5U/L) as compared with the control (55.23U/L, 113U/L), respectively. Mice infected with *Sal. typhimurium* showed a significant increase ($p\leq0.05$) in the level of serum GPT (87.3U/L) when it was compared with the control (55.23 U/L), also there was a significant increase ($p\leq0.05$) in serum level of GOT (227.00 U/L) as it was compared with the control (113.00 U/L).

Mice fed *Lb. plantarum* and infected with *S. typhimurium* showed that there was a significant decrease ($p \leq 0.05$) in level of serum GPT (47.5 U/L) in comparison with its level in serum of SC group (87.3 U/L), while there was no significant difference when it was compared with the control (55.23 U/L). On the other hand, a significant decrease ($p \leq 0.05$) in serum GOT level (121.3 U/L) when it was compared with its level in blood serum of SC group (227.00 U/L). There was no significant difference when it was compared with the control (113.00 U/L).

Control group (CC) was kept on the basal diet alone (control). Liver sections, taken from control group showed normal structure appearance of hepatocytes cells arrangement

with a central vein Fig.(1-1A). The group of PC fed on the basal diet and was also dosed with 0.1 ml of *Lb. plantarum* Fig.(1-2A) showed the normal appearance of liver tissue with still congestion of blood vessel in portal area **(**2-2A).

Table (1)
Effect of Lb. plantarum on the liver functional enzyme (GOT and GPT) activity in mice before and after infection with Sal. Typhimurium.

Group (n=3)	***GPT (U/L) Mean ± SE***	***GOT (U/L) Mean ± SE***
Control group (CC)	55.23±1.20 a	113.00±2.25 A
Lb. plantarum group(PC)	43.25±2.32	95.5±2.1
Sal. typhimurium group (SC)	87.3±3.24 b	227.00±1.22 B
Lb. plantarum and S. typhimurium group (PS)	47.5±1.25 c	121.3±1.32 C

*Different letters means there is a significant difference ($p \leq 0.05$) as compared with Control.

* n= number of animals per group used during the two weeks of the experiment.

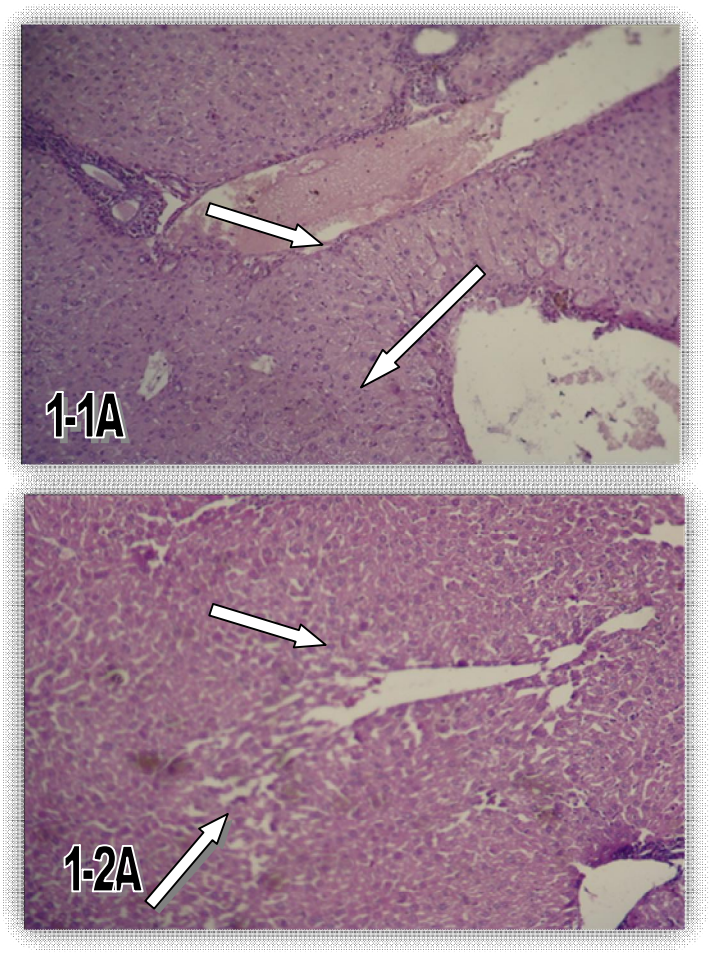

Fig.(1) 1A- Section of liver from CC (control) showing normal structure appearance of hepatocytes cells arrangement with a central vein (arrows). (1-2A) Section of liver PC treated with 0.1 ml of 10^9 cfu/ml Lb.plantarum showing a structure like the normal appearance of hepatocytes cells (arrows) (HE) ×10.

In Fig.(2-1A), liver section taken from group SC showed that a wide spread or increase area of degenerative and necrosis of hepatocytes cells with excessive accumulation or inaccumulation of inflammatory cells with abcess formation as compared with normal section taken from un infected mice (control Fig.(1-1A). Mice treated with *Lb. plantarum* and infected with 0.1ml of 10^9 cfu/ml *Lb. plantarum*, and then infected with 0.1ml of 2.5×10^7 cfu/ml *Sal. typhimurium* revealed that normal look like appearance of liver tissue.

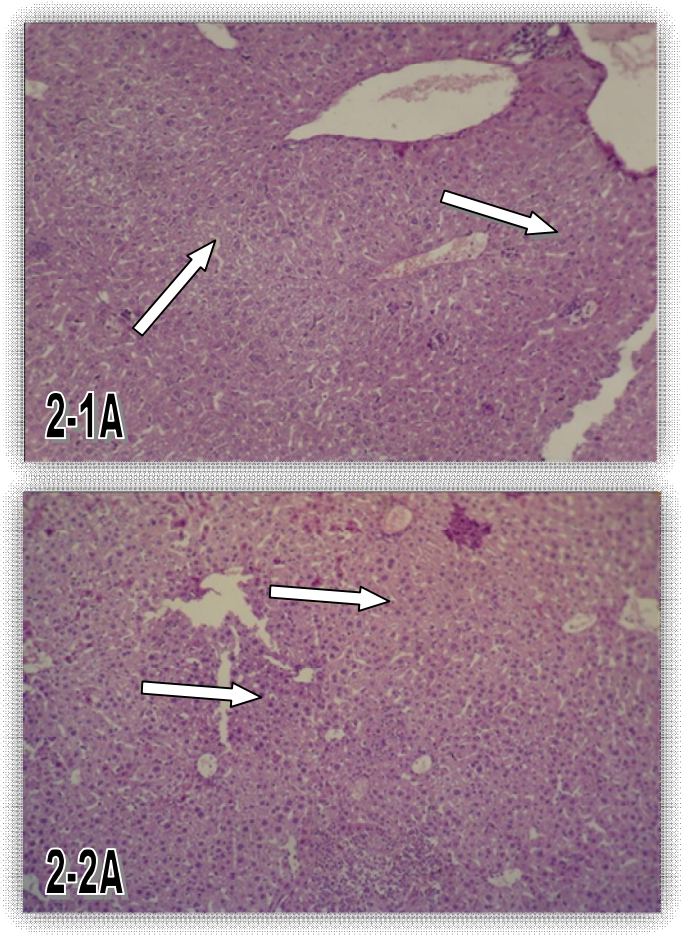

Fig.(2) (2-1A) Section of liver from SC infected with 0.1 ml of 2.5 ×10^7 cfu/ml of Sal. typhimurium, showing a wide spread or increase area of degenerative and necrosis of hepatocytes cells with excessive accumulation or inaccumulation of inflammatory cells with abscess formation (arrows). (2-2A) Section of liver from PS treated with 10^9 cfu/ml Lb. plantarum, and infected with 0.1 ml of 2.5×10^7 cfu/ml of Sal. typhimurium, showing mild degenerative changes and necrosis of hepatocyte cells with dispersed infiltration of inflammatory cells (arrows) (HE) ×10.

Discussion

The decrease in the GOT and GPT activity in mice treated with *Lb. plantarum* indicate that *Lb. plantarum* had a probiotic effect on liver cells and showed improvement in liver function enzymes. The probiotic effect of *Lb. plantarum* on the liver could be due to the production of polyamines from arginine, and these polyamines are known to be important mediators of cell growth and differentiation as mentioned by McCormack and Johanson, [14], and to prevent lipid peroxidation in liver microsomes [15].

A significant increase in serum GOT and GPT activity was observed in mice infected with *Sal.* and this may be due to the cytotoxic effect on liver cells that leads to increase the permeability of liver cell membrane or to cause damages in liver tissue. Thus, causing the release of high quantity of these enzymes into the blood stream as it was mentioned by Bonnefoi [16].

These results agree with Nakoneczna and Hsu [17] who found that multiple microscopic acute abscesses with predominantly polymorphonuclear leucocytes (PMNs) were seen in the liver and the spleen beginning on the 4th day after infection. By the 7th day, these lesions had become enlarged and were gradually transformed into granulomas with central necrosis and peripheral mononuclear cells .

There was no increase in the levels of GOT and GPT in mice feeding *Lb. plantarum* against *Sal. typhimurium* and this means that *Lb. plantarum* can inhibit the toxic effect of *Sal. typhimurium* in liver cell .

These results come in accordance with previous studies which indicated that probiotics *Lb. plantarum* containing therapeutic effect with multiple mechanisms of action that could disrupt the pathogenesis of liver and may make them superior to conventional treatment and lower portal pressure with a reduction in the risk of bleeding [18,19 and 20]. Similar result was stated by Adwai *et. al*. [21] who mentioned that treatment with *Lb. plantarum* reduced the hepatocellular necrosis and inflammatory cell infiltration, which was reflected by a decrease in the release of the liver enzymes.

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الخلاصة

اجريت الدراسة لمعرفة التأثير الوقائي لبكتريا *Lb plantarum* على كبد الفئران المصابة ببكتريا السالمونيلا *Sal. typhimurium*. درس التأثير المعزز الحيوي الناتج من بكتريا *Lb plantarum* داخل الجسم الحي على 12 فأرة بيضاء تم تقسيمها عشوائيا الى اربعة مجاميع PC, CC, SC و PS وقد تضمنت كل مجموعة على ثلاثة فأرات متساوية بالاعمار والاوزان. عدت المجموعة CC (مجموعة سيطرة)، بينما تم تغذية مجموعة PC ب0.1 مل من بكتريا *Lb plantarum* بعدد خلايا (1×10^{9} cfu/ml).اما المجموعة SC ثم اصابتها 0.1 مل من بكتريا *Sal.*

typhimurium بعدد خلايا (2.5×10^{7} cfu/ml).
المجموعة الاخيرة جرعت بـ 0.1 مل ببكتريا *Lb. plantarum* بعدد خلايا (1×10^{9} cfu/ml) و ثم اصابتها بـ 0.1 مل من بكتريا *Sal. typhimurium* بعدد خلايا (2.5×10^{7} cfu/ml).
اظهرت النتائج ان تجريع فئران المجموعة PC ببكتريا *Lb plantarum* ادى الى انخفاض معنوي($P\leq0.05$) في مستوى انزيم GPT (43.25 وحدة / لتر) و GOT (95.5 وحدة/ لتر) مقارنة بمستواهما في مصل الدم لمجموعة السيطرة. المجموعة SCتم اصابة فئران هذه المجموعة ببكتريا *Sal. typhimurium* مما تسبب بارتفاع معنوي ($P\leq0.05$) في مستوى انزيم GPT (87.3 وحدة/ لتر) وارتفاع عالي المعنوية ($P\leq0.05$) في مستوى انزيم GOT (227.0 وحدة/ لتر) في مصل الدم مقارنة بمستواهما في مصل الدم لمجموعة السيطرة تجريع حيوانات المجموعة PS ببكتريا *Lb plantarum* ثم اصابتها ببكتريا ا*Sal. typhimurium* تسبب بانخفاض معنوي ($P\leq0.05$) في مستوى انزيم GPT (47.5 وحدة/ لتر) وانخفاض عالي المعنوية ($P<0.01$) في مستوى انزيم وGOT (121.3 وحدة/ لتر) في مصل الدم مقارنة بمستواهما في مجموعة SC ، بينما لم يكن هناك أي فرق معنوي عند المقارنة مع مستواهما في مصل مجموعة السيطرة.

اظهرت نتائج الدراسة التشريحية ان الفئران المصابة ببكتريا *Sal. typhimurium*تسبب بتنخرات وتغيرات الخلايا وارتشاح خلايا التهابية في انسجة الكبد مقارنة بالمقاطع الطبيعية المأخوذة من الفئران غير المصابة. كما اشارت النتائج الى ان تجريع الفئران ببكتريا *Lb. plantarum* منع التأثير السام لبكتريا *Sal. typhimurium* على خلايا كبد الفئران.