Effects of probiotics (Lactobacillus acidophilus) on liver functions in experimental colitis in rats

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Summary
The ameliorative effects of probiotics (Lactobacillus acidophilus) on liver functions in experimentally induced colitis in adult male rats were studied. Thirty six male rats were divided randomly into 4 groups, 9 animals per group. First group was considered as control (C), 2nd group (T1) and 3rd group (T2) received (5 × 10^8 CFU) of Lactobacillus acidophilus as probiotics for 2 weeks by oral gavages needle, 4th group was considered as colitis group (T3). Experimentally acetic acid colitis was induced for rat of groups T2 and T3. After 7 days of colitis, at the end of the experiment. Blood sample, 4-5 ml, was collected via cardiac puncture for biochemical analysis and liver sections were isolated for histopathological examination. Results revealed that colitis caused significant (p<0.05) decrease in liver function enzymes AST; AST; ALP and FBS. While Lactobacillus acidophilus recipient succeeded in keeping ALP, FBS, and plasma total protein values within normal, but decreased ALT and AST in coparasim with control group. Histopathological liver section examination showed presence of focal necrosis in acetic acid colitis groups (T2 and T3). Also these changes were prevented in liver sections of rats which received Lactobacillus acidophilus and confirmed the ameliorative effects of probiotics on hepatocellular, preventing hepatocellular damage in experimentally induced colitis.

Keywords: Liver functions, Probiotics (Lactobacillus acidophilus), Experimental colitis, Liver enzyme.

Introduction
The term “probiotics” was defined as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. Its emphasizes the requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host. Although, this definition has been widely used by the entire scientific world, according to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms confer a health benefit on the host when administered in adequate amounts” (1).

The current use of probiotics as a therapy in gastrointestinal disorders ranges from treating mild conditions like transient diarrhea to severe inflammatory bowel disease (IBD) (2). Various strains of probiotic bacteria have been suggested to have beneficial clinical outcomes, but the most consistent evidence has been reported for lactic acid-producing bacteria (3). Propiotic bacteria as acid producing bacteria have been decreased inflammation in IBD (4). Two randomized clinical trials and an open-labeled trial conducted by (5) have demonstrated an improvement in management of paucities in patients receiving (VSL#3) as probiotics. Since then, others have shown similar clinical improvements in IBD, including the induction of remission in ulcerative colitis patients (2). Nonetheless, there is still speculation regarding how probiotics act within the gut to reduce disease severity. Improved gut epithelial barrier function has been suggested as a possible mechanism for beneficial effects of probiotics, but data from animal models are inconclusive (6). Probiotic improved the liver functions, by decreasing serum AST and ALT enzymes (7). Long-term treatment with probiotics (90 or 120 d) decreased circulating liver enzymes by 50%, illustrating a direct beneficial effect on liver function and probiotic-induced increase in total plasma protein concentrations of alcohol-induced cirrhosis patients and the similarities between liver and liver-derived plasma protein synthesis rates and increases in circulating plasma protein concentrations in the cirrhotic
patients may be related to changes in cytokine profiles, which stimulate the acute phase response (8). Role of probiotics on cytokines studied by many researchers and reported that probiotic treatment decreased circulating levels of tumor necrosis factor-a (TNFa) and IL-6 while concurrently increasing circulating levels of IL-10 (9). Probiotic applications for ulcerative colitis or Crohn’s disease patients, it is recommended that the safety of the probiotic used be verified by assessing its potential to translocate not only in healthy conditions but also in conditions with injured intestinal mucosa (10). Most lactobacilli have a remarkable record of safety and have been consumed by humans for decades; however, the possible involvement of certain LAB strains was described in cases of sepsis, endocarditis, or bacteremia, mostly in association with a severe underlying disease or detrimental condition (11). Intra rectal administration of 4% acetic acid solution exhibited mild colitis, with hyperemia and mucosal hemorrhaging within twenty-four hours after receiving acetic acid, colonic damage consisted of hyperemia, hemorrhage, and regions of ulceration and in some animals, and muscle thickening was also apparent (12).

The objective of present work, using a rat model of acetic acid induced colitis, was to determine the impact of probiotic supplementation on liver functions resembling by liver function enzymes (ALT, AST and ALP), and fasting blood sugar and total serum protein and bilirubin in addition to liver histopathology.

Materials and Methods

Thirty six male rats divided randomly into 4 groups, 9 animals per group, were placed into 3 replicate each of 3 animals and handled as follows; control group (C) received (1ml per animal) of distilled water by oral gavages needle for 2 weeks. T1 group received ($5 \times 10^8$ CFU) of Lactobacillus acidophilus as probiotics for 2 weeks by oral gavages needle (13), and had sham colitis. T2 group received ($5 \times 10^8$ CFU) of Lactobacillus acidophilus as probiotics for 2 weeks by oral gavages needle, and had acetic acid colitis. T3 group received (1ml per animal) of distilled water for 2 weeks by oral gavages needle and had acetic acid. After a 14 days of probiotics in dose ($5 \times 10^8$ CFU) treatment for groups (T1, T3), and distal water for group (C, T2). This period was considered as pre colitis period, acetic acid induced colitis to groups (T2, T3) and sham colitis for group (C, T1) this period, 7days, considered as post colitis period. At The end of the experiment blood sample (4-5 ml) was collected from the rat via cardiac puncture technique from each anesthetized animal using disposable syringe (5 ml) and blood was withdrawn into plastic test tubes (gel tube) for serum isolation for biochemical analysis. Liver sections were isolated for histopathological examination.

Colitis was induced in rats by intra rectal (IR) administration of 1ml of 4% acetic acid (AA). Briefly after general anesthesia, a soft 8F pediatric catheter was introduced into the anus for 6 cm and AA solution was carefully administered before taking the catheter out, 2ml air was applied in order to spread (AA) completely in the colon (12).

ALT activity was determined calorimetrically using commercial kits from RANDOX/ UK. The color absorbance was obtained by coupling of pyruvic acid and L-Glutamic acid with 2,4-Dinitrophenylhydrazine. The corresponding colored hydrazones was measured at wave length of 546 nm.

AST measured by colorimetrically method using a commercial kit from RANDOX/UK. Serum is incubated with ketoglutarate for one hour at 37°C and the reaction was stopped and dinitrophenylhydrazine was added. The color absorbs light at 505 nm.

Measurement of ALP depended on the action of alkaline phosphatase, in alkaline medium, hydrolyzes a colorless substrate of disodium phenyl phosphate giving rise to phenol and phosphate. 4 aminoantipyrine and sodium arsenate are used to stop the enzymatic reaction. The liberated phenol could then be measured colorimetrically by adding potassium ferricyanide as a color developing reagent using a commercial kit from Biomerieux/France.

Total serum protein was measured by biuret reaction, depending on the formation of a violate complex whose absorbance was
measured photo metrically the intensity of color produced is proportional to the concentration of protein in the sample (14). Fasting blood sugar was measured by colorimetric method by using of glucose kit (15). Serum bilirubin concentration was enzymatically measured using standard assay (bilirubin D+T kit (HUMAN/ Germany) according to (16). Liver tissues preserved in 10% neutral formalin buffer solution, after fixation, the tissue was trimmed and the specimen were washed with saline for (1-2 hrs) and transferred to following steps: 1. Dehydration: Specimen was passed through ascending grades of ethanol alcohol (70%, 80%, 90%, and 100%). For 1 hour in each concentration. 2. Clearing: Two solutions of xylol commonly used for clearing. The specimens rested 1 hour in each step. 3. Impregnation with paraffin wax, 4. Blocking, 5. Sectioning, 6. Staining. Then histopathological changes determined according to (17).

Data were expressed as the Mean±SE, when a significant interaction between major factors was identified by one way analysis using the SPSS version 11, LSD test was used to identify significant differences between mean values at probability level of (p<0.05) was taken as significant.

Results and Discussion

Results of liver function tests obtained from the present study are represented in (Table, 1). The results showed that acetic acid which induced colitis (T3) caused significant (p<0.05) decrease (p<0.05) in liver function enzymes AST; ALT; ALP and FBS and increased total serum protein at the same time keep the bilirubin within control values. While Lactobacillus acidophilus recipient decreased ALT and AST, also it was affective in keeping ALP, FBS, and plasma total protein values within normal in acetic acid induced colitis group (T2) but the y were increased in sham colitis group (T1) when compared with control group. The present results revealed that total serum bilirubin decreased significantly (p<0.05) in group received probiotics and non-significant in colitis groups (T2 and T3). Aminotransferase measurements are basic investigations for the diagnosis and monitoring of hepatocellular or muscle damage. The balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood determines the level of activity of the enzyme. There are two crucial factors which determine the rate of entry of enzymes into the circulation from the cells of origin. The first is made by those that affect the rate of leak from the cells which may be due to loose of cellular membrane integrity for any cause and the second are those that actually reflect altered rates of enzyme production, due to either increased synthesis of the enzyme in response to metabolic alterations in the cell or due to increased proliferation of the cell itself (18). In the present study results revealed that the serum aminotransferase of experimental rat’s groups (T1, T2 and T3) either received probiotic and/or insulted with acetic acid colitis significantly (p<0.05) decrease, as compared with control group, indicated ameliorative effects on hepatocellular function. This could be partly explained by decreased hepatocellular damage, as a result to reduced bacterial translocation and by stimulatory effects of intestinal mucosa and prevention of liver injury (7 and 19) and to obtain protective effects in animals treatment with a probiotics agent had to be initiated 10 days before challenge with pathogen. (19 - 21). The serum ALP activities is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine (22). Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gallbladder are responsible for maintaining the proper level of this enzyme in the blood (23). The present study suggested that the significant (p<0.05) decrease in serum ALP in rats with acetic acid colitis was caused
by the damage of the hepatobiliary system epithelial cells producing ALP resulted from inflammation caused by acetic acid. On the other hand, recipient of Lactobacillus acidophilus was effective in maintaining the integrity and activity of the epithelial cells lined the biliary duct indicating a direct beneficial effect of probiotics on liver function. In the present study the significant (p<0.05) increase in serum glucose level in T1 group is attributed to the effects of lactobacillus acidophilus on the number and affinity of glucose transporters in the intestinal epithelial cell and this result agrees with (24) who reported that the absorption rate of the intestinal glucose depends on the SGLT-1 affinity and density in the membrane height affinity. SGLT-1 is a primary transporter for glucose absorption that has been found to increase in small intestines of pigs treated with probiotics. Similarly, sodium coupled glucose absorption has been reported to increase in rats orally applied probiotics (25). The increase total serum protein level denoted in the present study could be explained by loss of protein from the diseased bowel wall as mentioned by researches (8, 26 and 27) and for little circumstances from hepatocellular damage as shown in the present study. Furthermore the similarities between liver and liver-derived plasma protein increased in circulating plasma protein concentrations in the cirrhotic patients may be related to changes in cytokine profiles, which stimulate the acute phase response (8). In the present study serum bilirubin level was significantly (p<0.05) decreased in lactobacilli groups (T1and T2) as compared to C and T3 groups. This result could be explained by same previous fact about increasing the bile flow into the intestines and the role of probiotics in increasing the excretion of bilirubin with feces, (28).

Table-1: The ameliorative effects of probiotic on Serum ALT (U/L), AST (U/L), ALP (U/L), glucose(mg/dl), total protein (g/dl), and total bilirubin(mg/dl) in Rats. Means ±SE, n=6

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST(U/L)</th>
<th>ALP(U/L)</th>
<th>Serum glucose mg/dl</th>
<th>Total serum protein gm/dl</th>
<th>Total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group received distill water and have sham colitis</td>
<td>10.29±2.5</td>
<td>31.83±1.2</td>
<td>32.3±2.7</td>
<td>100.5±2.7</td>
<td>11.67±0.6</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>T1 received probiotics in dose (5 × 10⁸ CFU) and have sham colitis</td>
<td>3.66±0.2</td>
<td>12.67±2.2</td>
<td>53.8±6.7</td>
<td>123.2±4.3</td>
<td>15.53±1.3</td>
<td>0.45±0.01</td>
</tr>
<tr>
<td>T2 received probiotics in dose (5 × 10⁶ CFU) and have acetic acid colitis</td>
<td>6.11±0.6</td>
<td>19.83±1.8</td>
<td>38.02±2.9</td>
<td>105.4±5.3</td>
<td>18.16±0.3</td>
<td>0.98±0.10</td>
</tr>
<tr>
<td>T3 received Distill water and have acetic acid colitis</td>
<td>6.75±1</td>
<td>26.43±2.4</td>
<td>19.1±1.12</td>
<td>87.67±9.5</td>
<td>18.08±0.1</td>
<td>1.34±0.10</td>
</tr>
</tbody>
</table>

The capital letter denote significant differences between groups.

Light microscopic observation revealed that the C, and T1 groups showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in between the hepatic cords with fine arrangement of Kupffer cells (Figures, 1 and 2). Also T1 liver section revealed slight dilution of sinusoids and bile ducts with hyper plasia and monocytes infiltration in portal area correlated with mononuclear cells infiltration around and inside blood vessels .The results of the histopathological analysis confirming the ameliorative effects of probiotics on
hepatocellular, preventing hepatocellular damage in experimentally induced colitis are shown in (Figure, 3). Liver sections of rats had experimentally acetic acid induced colitis revealed a focal necrosis (Figure, 4), less kupffer cells, individual necrosis of hepatocytes resemble by vaculation of cells, and dilation and congestion of blood vessels, causing edema, most of the necrotic lesions and mononuclear vaculation were decreased if it was found in liver sections of rats received probiotics 14 days before acetic acid induced colitis. Furthermore there were, dilation of sinusoids and congestion of blood vessels with cellular aggregation that forming granulomatous lesion. And most of cells are apoptosis feature with condense chromatin. In case of the data obtained from the histopathology examination, they are in agreement with previously mentioned results, and probiotics showed a significant ability to maintain the liver cellular integrity these result were in agreement with (7). These inflammatory changes were reduced in rats received probiotics T1 and T2, reflecting the ameliorative role of Lactobacillus acidophilus in maintaining liver functions. Beneficial effects of probiotics in liver indicated that probiotics are well tolerated, can improve liver function, and may reduce the marker for lipid peroxidation (29). The role of probiotics in liver disease stated that, in the liver health, the main benefits of probiotics might occur through preventing the production and uptake of lipopolysaccharides in the gut, and therefore reducing levels of low grade inflammation (30).

Figure, 1: Photomicrograph of a section of liver of C group, normal appearance portal area, po=portal area, K=kupffer cell. X40, E and H stain.

Figure, 2: Photomicrograph of a section of the liver of rats T1 group, mononuclear cell aggregation around and inside blood vessels .m=mononuclear cell, k=kupffer cell, s= sinusoid. X40, E and H Stain.

Figure, 3: Photomicrograph of a section of the liver of rats T2 group, Lymphoid aggregation around portal area and increase in kupffer cells, Po= portal area, LA=lymphoid aggregation, K= kupffer cell. X40, EandH stain.

Figure, 4: Photomicrograph of a section of liver of rats T3 group showing central vein with focal necrosis and vaculation, n= necrosis, v= vacolation X40, E and H Stain.
References


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تأثير البروبايوتوك (البكتريا المنتجة لحمض اللبنيك) على وظائف الكبد في حالة التهاب القولون المستحدث في الجرذان

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الخلاصة

صممت هذه التجربة لدراسة التأثير الوظيفي للبروبايوتوك (البكتريا المنتجة لحمض اللبنيك) على بعض وظائف الكبد في ذكور الجرذان المستحدث فيها التهاب القولون التقرحي بواسطة تأذيع حمام الخيل (4%) في المستقيم. استعمل 36 جرذ ذكر قسمت إلى 4 مجموعات كل مجموعة ضمت 9 جرذان المجموعة الأولى مجموعة سيطرة. جرعت المجموعة الثانية والثالثة البروبايوتوك بجرعة (0.5%×10^8) وحدة مستعمرة بكتيرية عن طريق الفم و بعد أسبوعين عرضت المجموعة الرابعة والثالثة إلى التهاب القولون التقرحي تجريبياً ذلك بفضل محلول حمام الخيل (4%) في الشرج. وبعد أسبوع تم جمع عينات الدم عن طريق القلب وعزلت مقاطع من الكبد لغرض دراسة التغيرات النسبية. أظهرت النتائج أن اختلافات في الانزيمات الكبدية بين المجموعات أظهرت أن البروبايوتوك كان له تأثيرات وظيفية حماية في الكبد.

الكلمات المفتاحية: بروبايوتوك، التهاب القولون، الكبد، أنزيمات الكبد.