Protective Effects of Probiotic Bacteria on Experimental Animals Liver Dysfunction

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التأثيرات الوقائية لبكتيريا البروبيوتيك على خلل وظائف الكبد في حيوانات التجارب

تم تصميم هذا البحث لدراسة تأثير بكتريا البروبيوتيك على الحالة التغذوية وبعض التحاليل الكيميائية على مصل الدم والكبد لدى الجرذان المصابه بالتسمم الكبدى باستعمال رابع كلوريد الكربون. استخدم فى هذا البحث خمس وعشرين من جرذان الألبينو يترواح وزن كل منها 200±10 جرام، وتم تقسيمها إلى مجموعات تحت ظروف درجة حرارة 22–24 مئوية، ودورةالإضاءة ضوء 12 ساعة تبدأ من الساعة السادسة صباحاً لمدة ستة أيام على الأقل قبل بدء التجربة. وبالتالى تقسيم الجرذان إلى ثلاث مجموعات رئيسية (خمسة جرذان للمجموعة الواحدة) على النحو التالي: المجموعة الأولى وهى المجموعة الضابطة السالبة (–Ve): وتم تغذيتها على الوجبة الأساسية فقط، أما المجموعة الثانية وهى المجموعة الضابطة الموجبة (+Ve): وتم تغذيتها على الوجبة الأساسية بعد الحقن برابع كلوريد الكربون. في حين كانت المجموعة الثالثة وهى المجموعة المعالجة لاضطرابات الكبد: تم تقسيم هذه المجموعة بعد حقن حيواناتها برابع كلوريد الكربون إلى ثلاث مجموعات فرعية (خمسة جرذان لكل مجموعة) على النحو التالي: المجموعة الثالثة: وتم تغذيتها على الوجبة الأساسية بالإضافة إلى 10٪ من الحليب الحامض. المجموعة الرابعة: وتم تغذيتها على الوجبة الأساسية بالإضافة إلى 10٪ من اللبن. المجموعة الخامسة: وتم تغذيتها على الوجبة الأساسية بالإضافة إلى 10٪ من الكشك. وقد أظهرت النتائج أن حقن رابع كلوريد الكربون كان مرتبطاً بزيادات معنوية في مستويات مصل الدم من البروتين الكلي، الألبيومين، كذلك نشاط إنزيمات AST و ALT بالمقارنة مع المجموعة الضابطة السالبة والوجبات الغذائية الأخرى. أما بالنسبة للكوليستيرول الكلى والجليسريدات الثلاثية الكلية والليبوبروتين منخفض الكثافة والليبوبروتين فائق منخفض الكثافة (TC، TG، LDL–c و VLDL–c) فقد أظهرت النتائج أن المجموعة التى تغذت على الكشك 7٪ أظهرت فروق معنوية عند ($P < 0.01$) مقارنة بالمجموعة الضابطة الموجبة. وأظهرت هذه الدراسة أن استخدام اللبن، الحليب الحامض، والكشك يمكن أن يحمي من سمية الكبد الناجم عن الحقن برابع كلوريد الكربون

الكلمات المفتاحية: بكتيريا البروبيوتيك، خلل وظائف الكبد، الفئران، اللبن، الحليب الحامض، الكشك.

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Abstract

The present study was designed to investigate the effects of probiotics on the nutritional status and some biochemical parameters of plasma and liver of carbon tetrachloride-induced hepatotoxicity in male albino rats. Twenty-five (25) Sprague-Dawley white male albino rats, each weighing 200±10 g. The rats were divided into five main groups (5 rats each) as follows: group (1) negative or normal group (-Ve): fed on basal diet only, as a control negative. Group (2) positive group (+Ve): fed on a basal diet after injection with CCl□ as a control positive. Treated groups: liver disorders group by CCl4, these groups were further divided into three groups as follows: group (3): fed on a basal diet plus 10% of sour milk. Group (4): fed on a basal diet plus 10% yogurt. Group (5): fed on a basal diet plus 10% of kishk. The results indicated that CCl4 injection was associated with significant increases in the serum levels of total protein and albumin as well as AST and ALT activity as compared with negative control and other diets. As for the TC, TG, LDL-c and VLDL-c; the results found that the group fed on 10% Kishk showed a high significant difference at (P <0.01) compared to the positive control group. This study showed that using yogurt, sour milk, and kishk can protect against induced hepatotoxicity.

Key Words: Probiotic bacteria, liver dysfunction, rats, milk, sour milk, kishk.

Introduction

The liver is the body's biggest organ. Weighing between 3.2 and 3.7 lb., a human liver typically measures 1.44 to 1.66 kg. Many different things can trigger liver diseases: agents of infection, agents of toxicity, agents in food, illnesses of storage, and birth defects **(ADA, 2000; and Zakim *et al.*, 2002)**. There are components in many traditional foods that may offer health benefits. These include fruits, vegetables, soy, whole grains, and milk. Furthermore, novel foods are being created to augment or integrate these advantageous elements for their positive physiological effects or health advantages **(Danik and Martirosyan, 2011)**.

It has long been recognized that probiotics are important. These live microbes have shown promise in preventing and treating gastrointestinal problems and maintaining a healthy digestive system. In boosting your immune system, they are also crucial. The cultures in yogurts and other dairy products contain probiotics. While more and more people are becoming aware of probiotics, their close relatives, prebiotics, are still relatively unknown **(Frank, 2015)**.

Traditional medicine has long advocated the use of probiotic bacteria in the treatment of liver problems; this study used sour milk, yogurt, and kishk as sources of these bacteria. The following research will typically seek to establish the scientific rationale for the usage of these milk products once they have been utilized to produce phytogenic diets.

Materials and methods

Materials:

Milk products, and kishk were procured from the nearby market and grocer in Elmenoufia governorate, Egypt. The EL-Gomhoria pharmaceutical company in Cairo, Egypt, supplied. The basal diet ingredients, the carbon tetrachloride (CCl_4) and biochemical analysis chemical kits. In this study, twenty-five (25) male albino Sprague-Dawley rats, each weighing 200±10 g, were procured from the Serum and Vaccine Center in Cairo.

Experimental design:

For a minimum of six days prior to the studies, twenty-five (25) male albino Sprague-Dawley rats, with a weight of 200±10 g, were kept in group cages under regulated lighting conditions (a 12-hour light cycle beginning at 6 AM) and temperature (22-24°C). Here are the five primary groups into which the rats were split, with five rats in each: Group (1), the control group, was fed a basal diet only in accordance with **Reeves et al., (1993)**. Group (2), which served as a control, was given a basal diet following an injection of CCl4. Groups treated with CCl4 for liver problems were further subdivided into the following three categories: Group 3: Received a basal diet supplemented with 10% sour milk. Feeding group 4: a basal diet supplemented with 10% yogurt. Group 5: given a basal diet in addition to 10% kishk.

Induction of liver disorders:

Subcutaneous injections of a carbon tetrachloride (CCl_4) and paraffin oil mixture were administered to twenty (20) rats twice per week at a dose of 2 mL/kg to induce liver injury. Based on pilot study findings, this dosage was selected as it consistently induced moderate and reproducible liver damage in rats that had fasted for 24 hours . Rats were injected for two weeks **(Kanter *et al.*, 2003)**.

Biological evaluation:

Every three days, participants' weights were monitored during the 28-day investigation. In order to conduct biological evaluations of the various diets, we measured each one for body weight gain (BWG) and BWG% **(Chapman *et al.*, 1959)**, using the following formulas:

BWG (g) = Final weight - Initial weight

$$\text{Body weight gain (BWG \%)} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Biochemical analysis:

After the trial was over, the rats were given diethyl ether in a serial fashion and fasted overnight. The plasma was prepared by drawing blood from the hepatic portal vein and placing it in centrifuge tubes that were clean and dry. The tubes already had a 3.1% sodium citrate solution, which was mixed 1:10 v/v. In order to isolate the plasma, blood samples were spun at 3000 rpm for 15 minutes. Following a meticulous separation process, the plasma was transported to clean, dry Ebendorf tubes and stored at -20° C until analysis **(Turkdogan *et al.*, 2003)**. The following parameters were measured in plasma samples using commercially available (BioMerieux) kits: plasma aspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT) activity according to **(Reitman and**

Frankel 1957), total protein **(Gomal *et al.*, 1949)**, plasma albumin **(Doumas, 1971)**, plasma globulin and albumin, globulin ratio: A/G ratio **(Chary and Sharma, 2004)**, total cholesterol (TC) **(Allain, 1974)**, triglycerides (TG) (**Wahlefeld, 1974)** and very low density lipo-protein cholesterol (VLDL-c): was calculated as mg/dl **(Fassati and Prencipe, 1982)**, high density lipoprotein cholesterol (HDL-c) **(Lopez, 1977)**, low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl **(Castelli, 1977)**, and alpha-fetoprotein (AFP), **(Crandall , 1981).**

Organs weight:

Each rat was swiftly dissected after blood samples were taken from the retro orbital veins, and its liver was removed, rinsed in saline, dried, then weight the liver and finally placed in a 10% v/v formalin solution, all in accordance with the procedures outlined by **(Drury and Wallington, 1980)**.

Statistical analysis:

A statistical package for the social sciences was utilized to conduct the statistical analysis **(SPSS, 1998)** to see how they stacked up against one another in the appropriate tests. It is shown that the mean±SE. To compare the target sample to the control group, a paired t-test was employed. A multiple measures ANOVA was used to statistically analyze the time course studies. It was deemed statistically significant when $P \leq 0.05$, very significant when $P \leq 0.01$, and very highly significant when $P \leq 0.001$.

Results and discussion

Plenty of helpful microbes live in the GI tract (GIT). Some microbes, like Bifidobacteria, are essential to maintaining a healthy gastrointestinal tract. A higher concentration of beneficial bacteria called Bifidobacteria in the gastrointestinal tract may be achieved by eating foods that are high in probiotics. Over time, glucose metabolism is impaired due to stress hormones released by the liver due to insufficient glycogen stores. Fatty liver disease is mostly caused by insulin resistance, which is itself caused by impaired glucose metabolism **(Erejuwa *et al.*, 2010)**.

The obtained results (Table 1) indicated that the mean values of initial weight, final weight, body weight gain and percentage of body weight gain (BWG%) showed significant variations. The results showed that an decreased in weight gain (WG) in all treated groups with sour milk, yogurt, and kishk significantly, as compared to the positive control group.

The present findings summarized in (Table 2) indicated that AST and ALT activity were increased in the control positive group compared to the control negative group. However, inclusion of salbumin, our milk, yogurt, and Kishk in diets reduced plasma AST and ALT activity as compared to the control positive group and toward the normal control (control negative), also, data in the same table showed the values of total proteins, albumin, globulin and plasma albumin-globulin ratio (A/G ratio) remained comparable to that of control groups. There were no statistically significant changes in plasma globulins, plasma albumin, or the albumin-globulin ratio (A/G ratio) among the several experimental groups of rats, but total protein and albumin levels were higher in the positive control group. When prebiotics are present, the growth and biological activity of these bacteria are even more accelerated **(Abeshu and Geleta *et al.*, 2016)**.

The intestinal microbiota benefited from prebiotic components such inulin, oligosaccharides, and ligofructose, which increased the populations of Lactobacillus acidophilus and L. plantarum by 10-100 times in vitro **(Cardarelli *et al.*, 2008).** Prebiotics are nondigestible food ingredients that improve host health by selectively stimulating the growth and activity of bacteria in the colon, collectively known as the gut microbiome **(Dhingra et al., 2012)**. In comparison to the control group, rats with the experimental treatment showed improvements in TC, TG, HDL-c, LDL-c, and VLDL-c, as shown in Table 3. These results are in line with those showing that prebiotic nutrients enhance endothelial activities, reduce LDL-c oxidation, boost HDL-c, decrease platelet clotting ability, and widen coronary blood vessels **(Khalil *et al.*, 2010)**. The use of natural prebiotic components has therapeutic and cardioprotective effects against vasomotor dysfunctions and cardiac diseases caused by epinephrine. It has been noted that the prebiotic components have a balanced connection with radical scavenging action **(Rakha *et al.*, 2008)**.

Table 4 displays the data that showed how the experimental diet affected tumor markers. All groups, but notably the Kishk-fed group, showed a decline in mean AFP values as compared to the control positive group.**.** Prebiotics' role in modulating lipid metabolism, producing short chain fatty acids, balancing intestinal pH, lymphocytes and leukocytes in the lymphoid tissues, enhancing nutrient absorption, and shortening fecal transit time may reduce exposure to carcinogens and tumor promoters (***Yao Y et al ., 2017)***. In vitro and animal studies have investigated the anti-cancer benefits of prebiotic components **(Erejuwa *et al.*, 2014)**.

The data presented in Table 5 illustrate the effect of different diets on liver weight. The mean liver weights for the experimental groups were as follows: 5.5±0.1 g (-Ve), 6.9±0.5 g (+Ve), 4.8±0.4 g (Sour milk), 4.0±0.5 g (Yogurt), and 4.2±0.6 g (Kishk). The results indicate that all dietary treatments had a significant effect on liver weight compared to the positive control group. However, no significant differences were observed among the treatment groups.

Table (1): Effect of experimental diet feeding on biological evaluation in albino rat's body weight

Groups	Initial weight	Final weight	BWG	BWG %
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
-Ve	140.4±3.3[a]	185.2±4.5[b]	44.8±4.3[abc]	32.1±3.6[abc]
+Ve	146.8±3.5[a]	203.8±4.5[a]	57.0±3.9[a]	38.99±3.1[a]
Sour milk	142.8±3.4[a]	179.0±2.3[b]	36.2±4.97[c]	25.7±4.1[bc]
Yogurt	145.4±3.5[a]	178.6±2.7[b]	33.2±5.5[c]	23.2±4.2[c]
Kishk	147.4±3.4[a]	184.0±2.2[b]	36.6±3.5[bc]	25.04±2.8[bc]

Mean with different letters (a, b, c, d and e) in the same column differ significantly at (P< 0.05). Using one way ANOVA test, while those with similar letters are non-significant, *n* (5).
[a] Differences are significant at 5% (P < 0.05), [b] Differences are high significant at 1% (P < 0.01), [c] Differences are very high significant at 0.1% (P < 0.001).

Table (2): Effect of experimental diet feeding on liver functions in albino rats

Groups	AST	ALT	TP	ALB	GLB	A/G Ratio
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
-Ve	25.2±1.6[d]	22.4±2.9[d]	6.8±0.2[a]	4.3±3.2[a]	2.5±0.3[a]	1.8±0.3[a]
+Ve	116.8±3.4[a]	126.6±4.2[a]	5.2±0.3[b]	3.4±0.3[b]	2.1±0.04[a]	1.5±0.1[a]
Sour milk	83.6±3.1[bc]	80.8±2.4[c]	6.2±0.3[ab]	3.9±0.3[ab]	2.3±0.2[a]	1.7±0.2[a]
Yogurt	85.0±4.3[bc]	87.4±3.4[bc]	6.5±0.4[a]	4.2±0.3[a]	2.4±0.4[a]	2.1±0.5[a]
Kishk	92.8±3.4[b]	95.0±5.97[b]	5.8±0.4[ab]	3.7±0.2[ab]	2.1±0.5[a]	2.1±0.4[a]

Mean with different letters (a, b, c, d and e) in the same column differ significantly at (P< 0.05). Using one way ANOVA test, while those with similar letters are non-significant, *n* (5).
[a] Differences are significant at 5% (P < 0.05), [b] Differences are high significant at 1% (P < 0.01), [c] Differences are very high significant at 0.1% (P < 0.001).

Table (3): Effect of experimental diet feeding on lipids profile in albino rats

Groups	TC	TG	HDL-c	LDL-c	VLDL-c
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
-Ve	116.4±4.3[e]	92.0±3.2[d]	42.6±2.9[a]	64.8±2.7[e]	18.4±0.6[d]
+Ve	215.8±5.7[a]	152.2±9.0[a]	30.2±2.4[b]	137.6±2.1[a]	30.4±1.8[a]
Sour milk	136.6±3.4[d]	114.8±6.1[bc]	37.6±1.9[ab]	75.2±2.8[d]	22.96±1.2[bc]
Yogurt	178.4±5.2[b]	117.0±6.1[bc]	36.0±3.04[ab]	114.8±2.7[b]	23.4±1.2[bc]
Kishk	185.6±4.8[b]	126.2±5.2[b]	35.6±3.5[ab]	115.5±3.3[b]	25.2±1.04[b]

Mean with different letters (a, b, c, d and e) in the same column differ significantly at (P< 0.05). Using one way ANOVA test, while those with similar letters are non-significant, *n* (5).

[a] Differences are significant at 5% (P < 0.05), [b] Differences are high significant at 1% (P < 0.01), [c] Differences are very high significant at 0.1% (P < 0.001).

Table (4): Effect of experimental diet feeding on tumor marker in albino rats

Groups	AFP
	Mean±SE
-Ve	2.4±0.2[e]
+Ve	8.5±0.4[a]
Sour milk	5.8±0.5[b]
Yogurt	4.5±0.5[bcd]
Kishk	5.0±0.6[bc]

Mean with different letters (a, b, c, d and e) in the same column differ significantly at (P< 0.05). Using one way ANOVA test, while those with similar letters are non-significant, *n* (5).

[a] Differences are significant at 5% (P < 0.05), [b] Differences are high significant at 1% (P < 0.01), [c] Differences are very high significant at 0.1% (P < 0.001).

Table (5): Effect of experimental diet feeding on liver weight in albino rats

Groups	Liver weight
	Mean±SE
-Ve	5.5±0.1[ab]
+Ve	6.9±0.5[a]
Sour milk	4.8±0.4[b]
Yogurt	4.0±0.5[b]
Kishk	4.2±0.6[b]

Mean with different letters (a, b, c, d and e) in the same column differ significantly at (P< 0.05). Using one way ANOVA test, while those with similar letters are non-significant, *n* (5).

[a] Differences are significant at 5% ($P < 0.05$), [b] Differences are high significant at 1% ($P < 0.01$), [c] Differences are very high significant at 0.1% ($P < 0.001$).

Conclusion

Results show that sour milk, yoghurt, and kishk mitigate CCl_4-induced hepatotoxicity in rats and other liver disease risk factors, including hyperlipidemia and liver indicators. They will be potentially suitable for use for patients infected with the liver disease.

Ethical Approval

All experiments of the study were ethically approved by the Scientific Research Ethics Committee From the University of Alexandria, Animal Ethics Committee, Faculty of Medicine (Approval no. 05- 2025-01, SREC0307052).

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