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The Effect of Bacillus licheniformis on weight gain, Blood picture and Lipid Profiles in rats feed high Cholesterol diet

ABSTRACT

The study aims to detect the effect of adding of Bacillus licheniformis bacteria (1×10^8 cells/day/animal) to reduce the negative effect of cholesterol added to the food ration by 2% on body weights and the effect on some parameters, blood images and lipid profiles in male laboratory rats after a period of 28 days. In this study, 15 adult male Albino Sprague-Dawleyweanling rats of 8-9 weeks of age and average weight of 142 g are used. The animals are randomly distributed into 3 groups with each group consisting of 5 animals. The results show that the addition of cholesterol causes a significant increase at ($P < 0.05$) in body weight, as the weight gain is 43.18 g compared with 19.13 g in the control group. The results also show a decrease in total number of red blood cells, hemoglobin levels, HCT and a significant increase in the number of white blood cells and platelets for groups of rats feeding cholesterol at (6.12 , 10.44 , 35.32 , 11.94 , 850.60) compared with the control group (7.09 , 12.30 , 38.54 , 9.22 , 597.40) respectively. The results also show an increase in the level of cholesterol, LDL and triglycerides, and a significant decrease in the level of HDL compared to the control group.

The addition of Bacillus licheniformis with cholesterol has a significant effect in reducing the negative effect of cholesterol on all the above-measured parameters.

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INTRODUCTION

Bacillus bacteria are used as health food supplements to prevent gastrointestinal disorders, and as therapeutic agents to treat urinary tract infections and improve gut barrier function and gut flora (Hill *et al.*, 2014). Bacteria potentially used as probiotics include *Bacillus subtilis* and *B. licheniformis* that are ubiquitous and widely distributed in most places, including the gastrointestinal tract. These bacteria are very suitable for use as they do not produce toxic substances, easy to grow, able to function at high temperatures and do not produce any metabolic side product (Elshagabee *et al.*, 2017).

Bacillus licheniformis generally known to be safe has been widely used as an antiviral and immune-regulating agent in clinical treatment (Kan *et al.*, 2021). The use of these bacteria can relieve dextran sodium sulfate (DSS)-induced colitis and modify dysbiosis during inflammatory bowel disease. It has been shown that administration of the bacteria can counteract colitis and intestinal dysbiosis caused by colitis in mice and improve growth performance in broiler chickens (Xu *et al.*, 2018).

Studies have shown that *Bacillus licheniformis* has an effect on blood lipid levels, especially lowering cholesterol, and that it contributes to partial prevention of weight gain and an increase in the level of growth hormone caused by HFD after treatment. These results indicate that the potential positive effect of bacteria on obesity caused by HFD in mice is not dependent on modifying the gut

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flora, and these bacteria can maintain the gut barrier function, modify the gut flora, counteract the inflammation caused by intestinal problems and even improve growth. Moreover, these bacteria can tolerate harsh environments and colonize the intestine easily (Zhou *et al.*, 2016).

Cholesterol is one of the most common circulating lipids discovered in the eighteenth century as a major component of gallstones. The French chemist Cheverul described it in a molecular form in 1816 and named it cholesterin. Although steroids are present in most tissues of the body of mammals, the ratio of bound cholesterol to free cholesterol varies significantly. In human blood plasma there is 60-80 % of cholesterol in a related way, as for the brain and nervous tissue, cholesterol enters as its main component, as it forms the myelin sheath of nerve cells (Aguilar-Ballester *et al.*, 2020).

Most of those who develop hypercholesterolemia suffer over time from many diseases, most notably coronary heart disease, medically abbreviated as CHD (Coronary heart diseases), atherosclerosis, stroke, and heart attack, in addition to disabilities, which amount to about 4% (Ko *et al.*, 2020). A study conducted by De Boer *et al.* (2018) has proved that food containing a high percentage of fat eventually leads to the accumulation of cholesterol-rich fatty substances on the cells lining the artery wall, in addition to calcium deposition and plaque formation, which leads to obstruction of the normal flow of blood, eventually leading to the formation of a blood clot (clot), which is known as a stroke. This work was aimed at investigating the effectiveness of *Bacillus licheniformis* on some biological parameters of male rats exposed to the cholesterol.

MATERIALS AND METHODS

Fifteen laboratory animals of male Dawley-Albino Sprague rats are obtained from The Technical Research Center \ Al-Nahrain University. Their ages range from 8 to 9 weeks, with average weights of 142 grams. The animals are divided into three groups with five replicates for each group in stainless steel cages to be reared under the appropriate conditions in terms of ventilation, lighting, temperature of 25 °C and humidity of 45-70 %. During the breeding period, appropriate food and drinking water are provided, as well as attention to the cleanliness of cages. Sawdust is replaced periodically throughout the experiment period, as mentioned in (Messora *et al.*, 2016).

The laboratory rats are randomly divided into three groups with five replicates as follows:

(T1) is the control group which includes the group of rats fed on the standard ration only. (T2) is the group of animals affected by hypercholesterolemia and this group includes the animals fed on the provender plus 2 % cholesterol. (T3) is the group of animals affected with hypercholesterolemia and having treatment, this group includes animals fed on the provender with 2% cholesterol and treated with the probiotic *B. licheniformis*.

The current study was conducted in the laboratories of the College of Agriculture, Tikrit University for the purpose of isolating and diagnosing *Bacillus licheniformis* from different nutritional models that included raw cow's milk, different dried fruits, indomie, leafy vegetables (radish and cauliflower) by used Luria Britari Agar media.

Oral administration of the probiotic *B. licheniformis* at a dose of 1 ml and at 1.5×10^8 cells/day/animal is continued in comparison with MacFarland standard solution throughout the experiment. The starting weight is taken after a day of feeding the animals during the experiment that lasts for 5 weeks. The weight gain is calculated by calculating the difference between the starting weight and the final weight. Weight gain (g) = final weight - Initial weight.

Immediately after the end of the experiment period, the animals are starved it must be not more than 10 hours. The laboratory animals are anesthetized using chloroform. One of the blood tubes contains an anticoagulant substance ethylene diamine tetraacetic acid (EDTA) for blood tests, and the other is free of it, which are centrifuged using a centrifuge at 3000 rpm for 15 minutes to obtain the serum that is kept at temperature -20 until the analysis is performed.

From the blood collection tubes containing the anticoagulant substance EDTA, the total number of red blood cells (RBC) ($10^6/\text{mm}^3$), the total number of white blood cells (WBC) ($10^6/\text{mm}^3$), hemoglobin (Hb) (g/dl), Platelets % and HCT% using a Complete blood picture device.

Total cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, total protein, albumin and globulin are determined as mentioned in Tietz (2005), using a number of standard solutions (kits) supplied by Biolabo (France). The analyzes are performed by a spectrophotometer of Shimadzu type (Japan) and according to the recommended wavelength for each analysis. The concentrations are calculated using equations according to the company's instructions supplied to each crew.

These results analysis was carried out through the experimental system within the ready-made statistical program (SAS, 2001) and the complete random design system (CRD). The averages were selected according to Duncan's multiple-range test (Duncan, 1955) to determine the significance of the conditions among the averages of the factors affecting the studied traits under experiment when Probability level (0.05).

RESULTS AND DISCUSSION

Effect of *B. licheniformis* on weights of laboratory animals

Table (1) shows the effect of adding 2 % cholesterol to the food ration on the weight of rats for 28 days. The results show a significant increase at ($P < 0.05$) in the weights of rats feeding with cholesterol, where the weight gained is at (43.18) g compared to the control group with (19.13) g, respectively. When *Bacillus licheniformis* is added with cholesterol, it leads to a significant decrease in the weight of rats compared to the second group in which cholesterol is added alone.

The result agree with the findings by Wang *et al.*, (2010) in the occurrence of a rise in the weight of rats when fed a provender containing cholesterol. The reason is attributed to the fact that treatment with cholesterol causes an imbalance in the metabolism and absorption of fats through its effect on the mechanism of fat oxidation and thus leads to the accumulation of fats in specific areas of the body and consequently weight gain. The weight gain in the average weight of cholesterol-treated animals is the result of a high concentration of cholesterol and triglycerides, as well as a high level of low-density fats, and this increase is normal as a result of animals feeding on cholesterol.

Table (1): Effect of *B. licheniformis* on growth parameters in cholesterol-exposed rats

Treatments	Initial weight	Final weight	Weight gain
	gm		
T1	139.12 ±4.56	158.25 ± 2.32	19.13c ± 1.28
T2	135.45 ±1.79	178.63 ± 1.42	43.18a ±0.27
T3	135.76 ±1.79	159.27 ± 0.56	23.51b ± 1.22

T1 control group, T2 hypercholesterolemic control group, T3 Hypercholesterolemic control group treated with probiotic bacteria.

Different letters in the same column indicate significant differences at the probability level 0.05 .

These results also agree with what is mentioned by Abu Bakar *et al.* (2021) who indicate the possibility of an increase in the average body weights of experimental animals that are fed a provender high in cholesterol. This leads to the accumulation of fat in different areas of the body, which causes a dramatically huge increase in weight over time. The results also agree with Nazari *et al.* (2016), who show that the biostimulants have a very significant effect in the process of reducing weight and getting rid of fat around the abdominal area if they are taken on a daily basis and within acceptable levels. Moreover, they possess the ability to reduce the average sizes of fat cells in all fatty tissues for experimental animals. Cengiz *et al.* (2015) indicate that probiotics stimulate the secretion of various enzymes such as amylyolytic and proteolytic lipolytic. This aids in the digestion of nutrients in the gut, and in turn improves metabolic processes within the intestine and helps in the process of weight loss.

Effect of *B. licheniformis* on blood picture parameters.

Table (2) shows the effect of adding cholesterol at 2% of the weight of the provender for 28 days in blood pictures. The results show a significant increase at ($P < 0.05$) in the total number of white blood cells and platelets for cholesterol groups, which are at (11.94 and 850.60) compared to

the control group, with (9.22 and 597.40), respectively. This also causes a significant decrease in red blood cells, hemoglobin and the percentage of red blood cells (6.12, 10.44 and 35.32) compared to the control group, which is (7.09, 12.30 and 38.54), respectively. When *Bacillus licheniformis* is added with cholesterol, it leads to a significant decrease in white blood cell concentrations and the percentage of red blood cells, and a significant increase in the values of red blood cells, hemoglobin and platelets compared to the second group to which cholesterol is added alone.

Table (2): Effect of *B. licheniformis* on blood picture parameters in cholesterol-exposed rats

Treatments	RBC (mm ³ /10 ⁶)	Hb (g/dl)	HCT %	WBC (mm ³ /10 ³)	PLT (10 ³ /mm ³)
T1	7.09 a±0.09	12.30a ±0.12	38.54a±0.32	9.22c± 3.08	597.40c ±2.09
T2	6.12c± 0.26	10.44c± 0.45	35.32c±0.14	11. 94a± 1.86	850.60a± 1.43
T3	6.83b± 0.22	11.84b± 0.67	38.12b±1.52	10.42b± 1.72	739.12b± 1.38

T1 control group, T2 hypercholesterolemic control group, T3 Hypercholesterolemic control group treated with probiotic bacteria

Different letters in the same column indicate significant differences at the probability level 0.05 .

Blood cells can be subjected to lysis due to exposure to oxidative stress by treatment with cholesterol, which leads to the formation of deposits called Heinz bodies inside the red blood cells, which helps to degrade them, thus reducing their number in the bloodstream (Ferracini *et al.*, 2021).

The results agree with what Rashid (2013) mentions in that eating foodstuffs with a high content of cholesterol makes the body more susceptible to disease, as well as stimulates fats, especially triglycerides, to produce more white cells. The high numbers of these cells is also an indication that the body has a specific problem, as it is considered as a means of defense for the body against the health problems it faces. The results also agree with Abd El-Hack *et al.* (2021), who state that the addition of *B. licheniformis* at a concentration of 2 ml leads to a positive effect on the values of RBC, Hb, WBC, MCV and PCV.

The results also agree with Beski and Al-Sardary (2015), who explain that the probiotics have the ability to improve the rate of red blood cells and return it to normal ratios through many activities, most notably reducing the pH and production of vitamin B-complex, which has a significant impact on improving the quality of erythrocytes and controlling the concentration of hemoglobin and the average corpuscular volume (Al-Hadidy, 2011). The results also agree with what is mentioned by Abudabos *et al.* (2016) that the probiotics work to enhance the size of the corpuscles and modify the presence of red blood cells within the balanced limits by removing bacterial, viral and fungal pathogens and reducing blood lipids.

Effect of *B. licheniformis* on lipid profile

Table (3) shows the effect of adding cholesterol on the lipid profile at 2% of the weight of the provender for 28 days. The results show a significant increase at ($P < 0.05$) in the concentrations of cholesterol, triglycerides and LDL for rat groups given the cholesterol. They are at (247, 187 and 148) mg/dl compared with the control group, which is (176, 122 and 98) mg/dl, respectively. It also causes a significant decrease in the concentration of HDL (31 mg/dl), compared with the control group. When *Bacillus licheniformis* is added with cholesterol, it leads to a significant decrease in cholesterol, triglycerides and LDL concentrations, and a significant increase in HDL concentration compared with the second group to which cholesterol is added alone.

The reason for the high cholesterol values may be due to the increase in the activity of the cholesterol acyl transferase enzyme, which is responsible for the absorption of cholesterol, which is stimulated by the lack of insulin as a result of oxidative stress that affects the pancreatic beta cells by the effect of active oxygen classes, and thus the level of cholesterol absorption by the intestines increases (Abdullah, 2010). Moreover, it may be attributed to the disturbances in fat metabolism due to stress and the formation of fat peroxide and unsaturated fatty acids, which leads to inhibition of the secretion and excretion of steroid substances and bile salts, as well as the occurrence of some disorders in the processes of digestion and absorption in the intestines (Hassan and Frank, 2001).

Table (3): Effect of *B. licheniformis* on the lipid profile of cholesterol-exposed rats

Treatments	CHOL.	TG	LDL	HDL
	mg/dl			
T1	176c	122c	98c	54a
T2	247a	187a	148a	31c
T3	192b	156b	117b	47b

T1 control group, T2 hypercholesterolemic control group, T3 Hypercholesterolemic control group treated with probiotic bacteria

Different letters in the same column indicate significant differences at the probability level 0.05 .

The reason for the low HDL may be attributed either to liver injury by some diseases, or as a result of high levels of cholesterol, triglycerides and LDL as the function of HDL is the reverse transport of cholesterol from tissues to the liver (Stryer, 1997). If the concentrations of cholesterol and triglycerides increase in tissues and vessels in the bloodstream, this impedes a reduction in the efficiency of HDL in transporting cholesterol. In addition, the oxidation of LDL and the demolition of internal cholesterol in the body due to the active classes of oxygen leads to a reduction in the level of HDL, which is the basis for the process of transferring cholesterol from the cells of the body to the liver, thus reducing its level in the blood vessels (Guyton and Hall, 2006).

These results are consistent with what Hanaa *et al.* (2009) report in that consumption of therapeutic bacteria leads to a decrease in the level of total cholesterol, TG and LDL and an increase in HDL in the blood. The results also agree with what St-Onge *et al.* (2000) state in that consuming a probiotic contributes to reducing cholesterol, as bacteria work to lower cholesterol and thus reduce chronic heart disease. The production of Hydroxy methyl-glutarate by the probiotic bacteria inhibits the enzyme glutaryl-CoA-Hydroxy methyl reductase, which is a requirement for the process of cholesterol formation and reduces heart disease by 2-3% (Shah, 2007), and this is generally consistent with Liang (2006) who states that the probiotic bacteria work to reduce the level of cholesterol in the blood, despite the fact that this decrease may be affected by other factors such as the bacterial strain or the supplements used. The reason for the decrease in cholesterol may be due to the fact that these organisms produce enzymes They analyze fat and some demolish cholesterol to take advantage of it as a carbon source.

Li *et al.* (2020) also report that the effect of therapeutic bacteria on TG and HDL levels depends on the type of bacteria and strain used. The reason for the decrease in triglycerides may be attributed to the activation of the lipoprotein lipase enzyme, which works on the breakdown of these substances into fatty acids that are absorbed by fat cells, or due to the inhibition of the processes of synthesis of fatty acids that enter into the fat-building processes in the liver by inhibiting the activity of key enzymes, especially glycerol-3-phosphate acyltransferase. It may also be due to the breakdown of the lipoproteins which is rich in (Delzenne *et al.*, 2001).

The results also agree with what Kimoto *et al.* (2002) indicate concerning the fact that the bio-enzymes work on the production of enzymes which break down fat to take advantage of it as a carbon source. It may also work by converting cholesterol into bile acids by BSH enzyme (Bile Salt Hydrolase) and the ability of the enhancer to bind cholesterol to the small intestine. The decrease in cholesterol may be attributed to the ability of the enzyme Cholesterol dehydrogenase, which is produced by probiotics, to stimulate the conversion of cholesterol to Cholest-4-en-3-one, which acts as an intermediary and auxiliary factor in the conversion of cholesterol to Coprostanol, which are smaller units that are easy to break down and utilize. It also reduces cholesterol absorption in conjunction with improving excretion (Ahn *et al.*, 2003).

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تأثير بكتريا *Bacillus licheniformis* في الوزن المكتسب وصور الدم ومرتسم الدهون في الجرذان المغذاة على عليقة عالي الكولستيرول

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الخلاصة

هدفت الدراسة الى معرفة تأثير اضافة بكتريا *Bacillus licheniformis* 1) $108 \times$ خلية / يوم / حيوان) للتقليل من التأثير السلبي للكولستيرول المضاف الى العليقة بنسبة 2 % في اوزان الجسم وبعض معايير وصور الدم والدهون في ذكور الجرذان المختبرية بعد مدة 28 يوماً. استخدم في هذه الدراسة 15 من ذكور الجرذان من النوع Albino Sprague- Dawleyweanling بالغة بعمر 8 – 9 أسابيع ومتوسط الوزن 142 غم، وزعت الحيوانات عشوائياً إلى 3 مجاميع وفي كل مجموعة 5 حيوانات. بينت النتائج ان اضافة الكولستيرول سبب في ارتفاع معنوي عند ($P < 0.05$) في اوزان الجسم اذ كان الزيادة الوزنية عند 43.18 غم مقارنة مع مجموعة السيطرة 19.13 غم. وبينت النتائج انخفاض العدد الكلي لكريات الدم الحمراء ومستوى الهيموكلوبين والـ HCT وارتفاع معنوي في عدد خلايا الدم البيضاء والصفائح الدموية لمجاميع الجرذان المغذاة على الكولستيرول (6.12 , 10.44 , 35.32 , 11.94 , 850.60) مقارنة مع مجموعة السيطرة (7.09 , 12.30 , 38.54 , 9.22 , 597.40) على التوالي. وكذلك بينت النتائج ارتفاع مستوى الكولستيرول و LDL و الكليسيريدات الثلاثية وإنخفاض معنوي في مستوى الـ HDL مقارنة مع مجموعة السيطرة. وكان لأضافة بكتريا المعزز الحيوي *Bacillus licheniformis* مع الكولستيرول تأثيراً معنوياً في خفض التأثير السلبي للكولستيرول على جميع المعايير المقاسة اعلاه.

الكلمات المفتاحية:

Bacillus licheniformis ، الكولستيرول ، معايير النمو ، صور الدم ، مرتسم الدهون.