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EVALUATION OF THE EFFICACY OF ALMOND MILK FORTIFIED WITH PROBIOTICS IN REDUCING BLOOD LIPIDS AND LIVER ENZYMES IN EXPERIMENTAL HYPERLIPIDEMIC RATS.

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Abstract:

The effect of nutrition on almond milk on some physiological activities such as glucose, cholesterol, total cholesterol, triglycerides, high-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol (HDL-C), lipoprotein-w-cholesterol, low-density lipoprotein-cholesterol, was studied. (LDL-C), cholesterol in the blood. (190-210) grams and randomly divided into (6) groups, each group included (6) rats, rats group, rats group, infected group, infected and treated group. Almond milk, infection and treatment of Yogurt Bilbenrt. Among the most important results obtained was an overall decrease in the concentrations of glucose, cholesterol, glucose, glucose, glucose, glucose, glucose, glucose, local oil, LDL-C, triglycerides, LDL-C, compared with the control group, while a decrease Significant ($p \leq 0.05$) in HDL-C concentrations. The list of results showed a significant decrease ($p \leq 0.05$) in the level of blood glucose, cholesterol, triglycerides, LDL-C, AST, ALT and a significant increase in HDL concentrations for central groups, almonds and yogurt compared with the control group.

Keywords: Probiotics, Almond Milk, Blood Lipids, Liver Enzymes

INTRODUCTION

Cholesterol acts as a precursor for hormones and vitamins, and is a component of the nerve cell, but high levels of total blood cholesterol or other blood fats are a risk factor in the development of coronary heart disease. Diet contributes to lowering cholesterol levels, and despite the wide variation in cholesterol levels from one person to another. However, probiotics have a role in influencing cholesterol levels (Parvez et al., 2019), and there are alternative natural therapeutic methods for lowering cholesterol, including probiotics (Alok et al., 2020). The level of cholesterol in the body can be reduced by avoiding eating foods with a high content of cholesterol such as red meat and foods with a high content of saturated fats, egg yolks, etc., and consuming foods rich in omega-3 amino acids such as walnuts, almonds, flax seeds and fish (Jun, 2009). Studies have shown that many foods have the possibility of lowering cholesterol, such as almond milk. The mechanism of its reduction in the body is due to the water-soluble fiber, which increases the viscosity of the contents of the intestines, as it creates a thick, immobile layer, which in turn acts as a physical barrier that prevents the absorption of glucose, fats, cholesterol and bile acids. (2016). Consumption of fermented milk has a positive effect on reducing serum cholesterol in humans significantly, as Ooi and Liong, (2020) mentioned the possibility of avoiding the use of cholesterol-lowering drugs in cases of high cholesterol by using probiotics.

MATERIALS AND WORKING METHODS

Milk source: Milk samples were collected from a herd of cows belonging to one of the breeders in the Al-Alam area, east of the city of Tikrit / Salah al-Din. Clean, sterile glass bottles were used for packing and transporting the milk to the laboratory and placing it in the refrigerator at a temperature of (4)°C. The White Side Test was used to ensure that the milk was safe and free of mastitis, and as in the narrator (2003) the formation of a precipitate or jelly or both was evidence of an infection of the udder.

Preparation of almond milk: Almond milk was prepared according to what was mentioned by Al Tamimi (2016) with some modifications. After cleaning the almonds were soaked in distilled water weight/volume in a ratio of 3:1 almonds: water for 48 hours at room temperature, then the almonds were washed and put In the electric mixer, add the distilled water, the proportions of almonds: Water 4:1. The mixing process was conducted for 5 minutes, then the product was filtered to separate the solids, and then the resulting almond milk was sterilized at a temperature of 121 °C for 5 minutes, cooled and kept in the refrigerator in tightly closed containers; To carry out the examinations and subsequent transactions.

Manufacture of almond milk: After preparing almond milk, it was pasteurized at a temperature of (63) C for a period of (30) minutes, then the milk was cooled to a degree of (42-45) C, then bio-enhancers were added to it at a rate of (3%) according to the following species, the starter L. bulgaricus and Str. thermophilus, the initiator of L. bulgaricus and L. acidophilus., and the initiator of Bifidobacterium longum and L. acidophilus. The milk was mixed with the starter for two minutes, then filled with special containers, the containers were covered, and transferred to the incubator at a temperature of 42-45 °C until the completion of coagulation, which takes about 4 hours, then the samples were cooled at a temperature of (5±2) °C until the tests were performed (Al-Aqiqi, 2017).

Manufacture of yogurt: The method used by Tamime and Robinson (1999) was followed, which consisted of filtering the milk with a clean piece of gauze and gradually raising the temperature to 90oC for half an hour. thermophiles) at 3% and mix the milk with the initiator for two minutes, then fill in special containers, cover the containers, and transfer to the incubator at a temperature of 42-45oC until the completion of coagulation, which takes up to 4 hours, then cool the samples at a temperature of (5±2) C until the tests are performed.

Preparing the animals: Adult white male Albino rats were used, 2-3 months old, with weights ranging between 190-210 g, obtained from the College of Veterinary Medicine / University of Tikrit and placed in special cages for animal husbandry. The animals were subjected to a photocycle (12) One hour of light and (12) hours of darkness, and the temperature at 25° C.

Weighted food preparation: The weighted food has been prepared as mentioned in the NRC (2002) to contain cellulose 50g/kg, casein 150.0g/kg, oil 100g/kg, vitamins 5g/kg, minerals 50g/kg, glucose 100 g/kg starch 536.5 g/kg.

Preparation of the high-fat diet: The high-fat diet was prepared based on what Hulbron (1982) mentioned, where 2% of pure cholesterol was used with the standard diet to infect rats with hyperlipidemia. The rats were fed this diet for 21 days and the feeding continued until the completion of the experiment.

Experiment design: The experimental animals were randomly distributed into six groups, each group consisting of six animals, and they were fed a high-fat diet for three weeks, except for the first group that was fed only the weighted food. After that, each group was dosed daily with 1 ml of milk, according to the type of treatment. The dose was divided twice, 0.5 ml every 12 hours by oral dosing by tube feeding. The dosing was continued for 28 days.

1- The first group T1 (negative control): these animals were left intact and fed on a standard diet only, with continuous water being given throughout the duration of the experiment.

2- The second group T2 (positive control): was fed a diet high in cholesterol for the duration of the experiment, given distilled water.

3- The third group T3: They were fed a diet high in fat and daily dosed with (1) ml of yogurt for the duration of the experiment.

4- Fourth group T4: They were fed on a high-fat diet, with daily 1 ml of almond milk and buttermilk.

5- The fifth group T5 was fed on a high fat diet with daily doses of 1 ml of almond milk, L. bulgaricus and Lactobacillus acidophilus.

6- The sixth group T6: was fed on a high-fat diet and was dosed daily with 1 ml of almond milk, Bifidobacterium longum and Lactobacillus acidophilus.

Biochemical tests: After the end of the experiment period, the rats were prevented from food for about 12 hours, then the animals were anesthetized by chloroform, then blood was drawn from the heart directly and placed in test tubes that did not contain (EDTA) and left for about a quarter of an hour in a water bath at 37° C. Then the serum was obtained by centrifuge machine at 3000 rpm for 15 minutes and kept at a temperature of (-18) degrees Celsius until conducting special biochemical tests that include measuring the level of total cholesterol, triglycerides, and high-density lipoproteins (HDL). -C), Low-density lipoproteins (LDL-C), Estimation of the antioxidants glutathione peroxidase and Malonedidehyde, Estimation of aspartate aminotransferase (AST), Allanine aminotransferase (ALT) in animal sera according to methods used by Tietz (2005) .

Statistical analysis: The results of the experiments were analyzed using the Linear Model General within the ready-made statistical program SAS (2004) to study the effect of factors on according to the complete random design (CRD). studied at the level (0.05).

RESULTS AND DISCUSSION

The effect of different treatments on the average weights of experimental animals: It is noted from Table (1) the effect of dosing with a standard diet (T1), a high-fat diet (T2) and a high-fat diet with the rats being fed daily with 1 ml of the treatments (T3, T4, T5, T6), It is noted that the effect of different therapeutic milks on the rate of daily increase and final weight gain among groups of experimental rats after 28 days of feeding and dosing. (5.33) g, as the table shows that the highest daily increase appeared in the rats fed the T2 treatment, where the daily increase rate was (0.901) g / day and the final weight gain is (25.24 g). fed on treatments (T3, T4, T5, T6) it amounted to (-0.169, -0.226, -0.292, -0.406) g/day, respectively, and the final increase was at (-4.74, -6.33, -8.19, -11.37) g Straight.

Table (1) Effect of different treatments on the weight of rats (gm)

Average daily changes in body weight (gm)	Amount of increase or decrease in body weight after 28 days (gm)	body weight (g)		Transactions
		end of the experiment	The beginning of the experience	
0.190	5.33	222.67d ±3.124	217.34b ±1.223	T1
0.901	25.24	275.09a ±2.547	249.85a ±2.759	T2
-0.169	-4.74	240.44b ±2.683	245.18a ±2.321	T3
-0.226	-6.33	236.57c ±2.387	242.90a ±3.118	T4
-0.292	-8.19	237.08c ±2.122	245.27a ±3.098	T5
-0.406	-11.37	236.15c ±3.452	247.52a ±2.975	T6

The numbers in the table represent the mean values ± standard deviation.
- Different letters in the same column indicate significant differences (p<0.05) between study groups.
T1 = healthy control T2 = infected control T3 = infected and treated with yogurt T4 = infected and treated with almond milk and yogurt T5 = infected and treated with almond milk and L. bulgaricus and Lactobacillus acidophilus T6 = infected and treated with almond milk and Bifidobacterium longum and Lactobacillus acidophilus

The weight increase that occurred in the average weight of cholesterol-treated rats is the result of a high concentration of triglycerides and cholesterol, as well as an increase in low-density lipoproteins, and this increase is normal as a result of feeding the animals on a high-calorie diet (Kobyliak, 2016). It is noted from the results that feeding rats with yogurt led to a decrease in the body weight of rats with high cholesterol, and this is consistent with what was found by Al-Jubouri (2020). To improve bowel movement and thus enhance the efficiency of the digestive process and the digestive system in general. The results show that feeding rats with almond milk fortified with probiotics led to a significant decrease in body weight after 28 days of feeding. Neutralizing free radicals and protecting the cell membranes, thus preventing the effects of free radicals resulting from feeding a diet high in cholesterol; Hence the role of sweet almonds in providing antioxidants (vitamin E, vitamin C and flavonoids) that reduce the oxidative effects caused by triglycerides, which is positively reflected in improving the level of metabolic processes. The reason for the low weight of rats may be attributed to the loss of appetite to eat, which in turn causes a decrease in the amount of nutrients absorbed by the body and thus a decrease in body building and then improving body weight, and that tannins inhibit the process of fat synthesis stimulated by the hormone insulin, and thus decrease weight and energy (Mbatha et al. , 2005)

Effect of different treatments on the average blood lipid profile: Table (2) shows the effect of oral administration of treatments (T6, T5, T4, T3) (T3) on the blood lipid profile of rats compared with the group of healthy (T1) and infected (T2) rats, as It is noted from the table that the value of cholesterol in treatment T2 increased significantly at the probability level (P<0.05) to reach (215.65) mg/dL compared to treatment T1 which was at (120.31) mg/dL, and it is clear that there was a significant decrease in the values of the treatments (T6, T5). , T4, T3) to have their values (153.84, 138.04,142.27,145.39) mg/dl, respectively. It is noted from the table that the value of triglycerides in treatment T2 recorded a significant increase, reaching (184.12) mg/dl compared to treatment T1 which was at (100.90) mg/dl, while its value decreased in infected cows treated with treatments T4, T5 and T6, to reach (131.06, 131.20, 133.19) mg/dl, respectively. The same results showed that the concentration of high-density lipoproteins in T2 rats decreased to (29.13) mg/dl compared to T1 which was (45.68) mg/dl. straight.

Table (2) Effect of different treatments on blood lipid profile (mg/dL)

LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)	TC (mg/dL)	Variables / Transactions
54.45f ±0.83	20.18d ±1.33	45.68a ±0.51	100.90d ±1.54	120.31e ±1.11	T1
149.70a ±2.77	36.82a ±1.14	29.13d ±0.87	184.12a ±2.88	215.65a ±2.54	T2
90.23b ±2.16	29.40b ±1.21	34.21c ±1.15	147.02b ±1.46	153.84b ±2.35	T3
81.29c ±1.80	26.63c ±1.87	37.47c ±1.58	133.19c ±1.38	145.39c ±2.03	T4

75.84d ±1.47	26.24c ±1.50	40.19b ±1.09	131.20c ±1.77	142.27c ±1.87	T5
69.86e ±2.05	26.21c ±1.09	41.97b ±1.64	131.06c ±2.12	138.04d ±1.33	T6

The numbers in the table represent the mean values ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between study groups.
T1 = healthy control T2 = infected control T3 = infected and treated with yogurt T4 = infected and treated with almond milk and yogurt T5 = infected and treated with almond milk and L. bulgaricus and Lactobacillus acidophilus T6 = infected and treated with almond milk and Bifidobacterium longum and Lactobacillus acidophilus

The results agreed with what was stated in Maja, (2013), which showed that a fatty diet will have a significant impact on health in terms of cholesterol formation and an increase in the proportion of low-density lipoproteins from it at the expense of high-density lipoproteins, high levels of triglycerides in the blood serum and plaque formation within the endothelium. Arteries, which leads to obstruction of the flow of blood within the vessels, which results in many diseases. It was found from the results that feeding high-fat rats with yogurt led to a significant decrease in the blood lipid profile. This is consistent with what Al-Jubouri (2018) found, which indicated that feeding high cholesterol rats with 1 ml per day of yogurt led to a significant decrease in the lipid profile. The reason for this was attributed to the role of fermented milk containing probiotic bacteria in lowering blood fats in experimental animals suffering from high blood fats. It is also noted that the dose of almond milk and probiotics has led to a significant decrease in the blood lipid profile. These results are consistent with what Hussein et al. (2020) found. The reason for this is that sweet almond milk contains insoluble dietary fiber. There is evidence of the relationship between fiber intake. Insoluble food and low level of cholesterol in the blood serum, and the role of these fibers through their role in improving the secretion of bile acids in the stool, and this will prevent their re-absorption through the hepato-intestinal portal cycle. This lack of bile acids level stimulates liver cells to increase the conversion of cholesterol into acids. bile and thus the concentration of cholesterol between cells decreases (Mohamed et al., 2019). Sweet almonds are one of the rich sources of monounsaturated fatty acids as well as polyunsaturated fatty acids and contain a large amount of linolenic acid, which plays a major role in lowering cholesterol levels by stimulating the secretion of cholesterol from the intestines, and stimulating its oxidation into bile acids (Mary, 2005). Sweet almond milk contains natural antioxidants in good quantities, such as flavonoids, which play a major role in scavenging free radicals and reducing fat oxidation, in addition to the role of vitamin E in preventing the destruction of cell membranes that occurs as a result of the presence of free radicals by interfering with them and neutralizing them. Its effect is in addition to other antioxidants such as vitamin C and glutathion, which have a synergistic role for vitamin E to do this work (Hussein et al., 2020).
Effect of different treatments on the blood glucose rate of experimental animals: Figure (1) shows the effectiveness of dosing with different treatments on blood glucose for healthy and hyperlipidemic rats. It is noted from the figure that the glucose concentration in treatment T2 recorded a significant increase, as it was at (142.61) mg/dL. Compared to the group of T1-treated rats, which recorded (111.25) mg/dL, while we notice a decrease in their concentrations in the infected and treated treatments T3, T4, T5 and T6, which are (121.58,122.46,122.23,129.78) mg/dL, respectively.

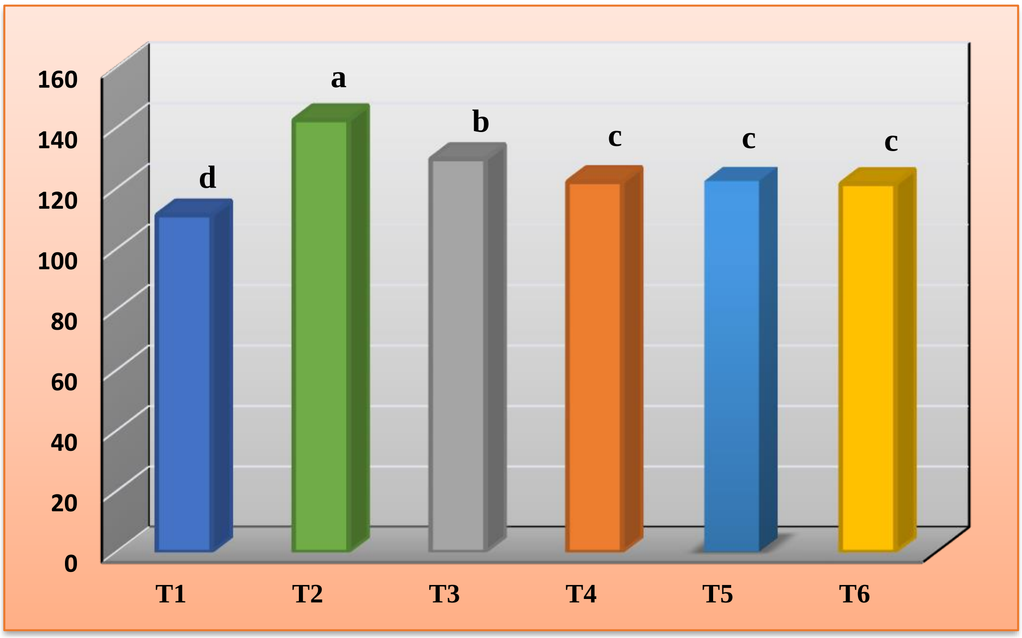

Figure (1) shows the effect of different treatments on the glucose concentration in the blood serum of rats
T1 = healthy control T2 = infected control T3 = infected and treated with yogurt T4 = infected and treated with almond milk and yogurt T5 = infected and treated with almond milk and L. bulgaricus and Lactobacillus acidophilus T6 = infected and treated with almond milk and Bifidobacterium longum and Lactobacillus acidophilus

The results of the study agreed with regard to the affected group, which was fed a high-calorie diet and in which there was a high concentration of blood sugar, with what was mentioned by Lau et al. (2014). May cause insulin resistance. In addition, fatty acids impair muscle glucose uptake by competitive inhibition. These results are also consistent with the findings of Al-Jubouri (2018), which indicated the effectiveness of fermented yogurt containing L. bulgaricus and Str. thermophiles in reducing blood glucose level in hypercholesterolemic rats from 177.64 mg/dL to 135.91 mg/dL. Treatment with almond milk and probiotics for groups affected by hyperlipidemia led to a clear improvement in the level of blood glucose. The reason for this can be attributed to the anti-diabetic action of almond milk as it contains flavonoids that inhibit the enzyme Aldose reductase, thus preventing the conversion of sugar into Sorbitol, therefore, prevents complications caused by sorbitol (Murray et al., 2000). In addition, the vitamins and minerals that make up almonds are one of the reasons that sweet almond milk works to reduce the rate of blood sugar concentration. Sweet almonds are one of the important sources of vitamins and minerals that have a role in lowering blood sugar. It contains biotin, which has an important role. In lowering the glucose level for infected groups, and those treated with sweet almond milk compared to those without treatment through the role of biotin (B-vitamin) in the cellular metabolism of glucose, whereby biotin stimulates the enzyme glucokinase, which is very important in stimulating glucose metabolism (Hussein et al., 2020). In addition to this, sweet almonds contain a large proportion of arginine, which contributes significantly to lowering the level of blood sugar, and arginine stimulates skeletal muscle to enter glucose by increasing the GLUT-4 insulin receptors on its plasma membrane (Higaki et al., 2001).

The effect of different treatments on the activity of liver enzymes: Table (3) shows the effectiveness of dosing with different treatments on the effectiveness of liver enzymes for healthy and hypercholesterolemic rats. international units/liter, while its value in the proper treatment T1 was (22.86) international units/liter, and its value was recorded in treatments T4, T5 and T6 (31.45,35.07,35.49) international units/liter, respectively. Cholesterol was at (81.83) IU/L, and it was noted that it was significantly higher compared to the group of healthy rats who recorded (59.75) IU/L, while its value in the infected and treated treatments T4, T5 and T6 was (70.09,71.32,75.04) IU/L on the straight. The results of the same table showed that the concentration of ALP enzyme in the rats infected with high cholesterol increased significantly to (97.95) IU/L compared to the healthy rats, which was (86.37) IU/L, and the infected groups treated with treatments T3, T4, T5 and T6 were recorded (87.12, 88.85, 89.07, 91.13).) IU/L respectively.

Table (3) The effect of different treatments on the activity of liver enzymes (international units/liter) in the blood serum of male rats with hypercholesterolemia

ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Variables / Transactions
86.37e ±1.48	59.75e ±1.13	22.86e ±1.66	T1
97.95a ±2.13	81.83a ±2.08	45.16a ±1.58	T2
91.13b ±1.05	75.26b ±1.45	37.78b ±0.73	T3
89.07c ±1.65	75.04c ±1.28	35.49c ±1.22	T4
88.85c ±1.29	71.32d ±1.47	35.07c ±1.05	T5
87.12d ±0.76	70.09d ±1.92	31.45d ±1.93	T6

The numbers in the table represent the mean values ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between study groups.
T1 = healthy control T2 = infected control T3 = infected and treated with yogurt T4 = infected and treated with almond milk and yogurt T5 = infected and treated with almond milk and L. bulgaricus and Lactobacillus acidophilus T6 = infected and treated with almond milk and Bifidobacterium longum and Lactobacillus acidophilus

The results regarding the increase in liver enzymes in the group of animals with hyperlipidemia agreed with Emamat et al. (2018), as the high content of the ration of total fat led to damage in liver tissues due to health problems caused by fat in terms of high blood pressure and impeding its smooth flow. Inside the various organs, including the liver tissues, which caused an increase in hepatic enzymes in the blood serum as a result of their excretion from the cells. These results agree with the findings of Al-Jubouri (2018), which found a significant decrease in the values of liver enzymes (ALP, AST, ALT) for rats with high fats treated with yogurt. The reason for this decrease was attributed to the role of lactic acid bacteria in improving metabolic indicators in the liver and from Then improve its functions by improving its metabolic processes (Minelia et al., 2004). In general, the results showed that almond milk fermented using probiotic bacteria gave the best result in terms of its ability to reduce the effectiveness of liver enzymes, which means that they worked to improve the functional performance of the liver, and this result may be attributed to the synergistic action of lactic acid bacteria and the components of almond milk Health-beneficial, Que et al. (2019) reported that almond milk proteins reduce fat accumulation in liver and kidney tissues, as well as reduce the incidence of cell death due to lipotoxicity. Ou et al. (2011) also indicated that lactic acid bacteria such as Lb.acidophilus inhibit some of the compounds responsible for the occurrence of oxidative processes in the body, such as (t-BHP) tert-butyl hydroperoxide, which causes damage to liver cells. Therefore, consumption of food products containing: These bacteria provide protection for the liver cells.

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