Evaluation of Animal and Plant Milks Against High-Fat Diet-Induced Hypercholesterolemia and Liver Dysfunction in Rats

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Abstract
To compare the effects of cow's milk, on some metabolic and biochemical parameters of rats submitted to hypercholesterolemic diet. Hypercholesterolemia was experimentally induced in rats. Liver function enzymes and blood glucose levels were also monitored to assess the effects of the different milk types on body weight and lipid profile. The hypercholesterolemic control group showed a marked deterioration in the parameters measured; it included increased body weight, total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, liver enzyme activities (ALT, AST, and ALP), and blood glucose concentration when compared with the healthy control group. All milk-treated groups showed significant improvements in these indicators. Oat milk showed the best results in all beneficial effects from all treatments tested, with the highest reductions in body weights and most pronounced improvements in lipid profile, liver enzyme activities, and blood glucose levels. Soy milk, almond milk, and cow's milk also showed favorable effects, though to a lesser degree than oat milk. The results generally show that the types of milk tested could be useful functional foods in managing metabolic disorders related to hypercholesterolemia, and in improving general health. Oat milk, in particular, falls into this category.

Keywords: Cow's milk, oat milk, almond milk, soy milk, cholesterol, rats, liver enzymes, blood lipids

Introduction:

Obesity and insulin resistance Hypercholesterolemia increases the risk of cardiovascular diseases and metabolic disorders. High levels of cholesterol and triglycerides in the blood are associated with increased risk for atherosclerosis, non-alcoholic fatty liver disease, insulin resistance, and diabetes tybe 2. The main dietary factors that directly affect the development of these diseases are diets high in saturated fats and cholesterol since they affect fat and carbohydrate metabolism and the functions of the vital organs, especially that of the liver, as the most important organ in regulating lipid metabolism within the body (1). In recent years, functional foods have become more popular because they provide natural dietary ways to prevent or reduce metabolic diseases. Milk and dairy products and plant-based milk alternatives are attracting increasing interest due to their high content of bioactive compounds with potential health benefits. Cow's milk contains high-biological-value proteins, bioavailable peptides, and calcium, which may support lipid and glucose metabolism. Almond milk is rich in unsaturated fatty acids, phenolic compounds, and vitamin E with antioxidant properties. This text is from references (2; 3). Oat milk is a major source of soluble beta-glucan fiber, which can reduce cholesterol and enhance insulin sensitivity. Soy milk provides plant proteins and isoflavones, which are essential for the regulation of lipid and glucose metabolism ,and for cutting the risk of cardiovascular disease (4; 5). This study was conducted in light of the increased interest in natural food in the quest for alternatives or adjuncts in the management of metabolic disorders associated with hyperlipidemia. The research was to assess the impact of cow's milk, almond milk, oat milk, and soy milk on some biochemical and physiological indicators in hypercholesterolemic rats. The parameters monitored focused on changes in body

ISSN 2072-3857

weight, blood lipid profile, liver function, and blood glucose level.

Materials and Methods

2-3 Methods of Work.

Source of cow's milk: Samples of milk were collected from a herd of cows owned by a breeder in the region of Al-Alam, to the east of Tikrit/Salah AL-Din. The milk was filled in sterile clean glass bottles and transported to the laboratory where it was stored in a refrigerator at 4°C until the time of use. The White side test was used to check the safety of the milk and its freedom from mastitis. According to (6), the presence of sediment, gelatin, or both indicates mastitis.

Preparing almond milk: Almond milk was prepared as described by (7) with some modifications. The almonds were soaked after cleaning in distilled water at a weight/volume ratio of 3:1 almonds:water for 48 hours at room temperature. Then wash the almonds and put them in an electric blender; add distilled water at a ratio of 4:1 almonds:water. Run the blender for 5 minutes. Filter immediately to remove solids from the product; then, the resulting almond milk was cooled. Stored in a refrigerator in tightly sealed containers.

Preparing oat milk: In the conventional method, oat groats are cleaned dry milling to remove impurities. Then, they are allowed to soak in water for a specific period to soften the groats and facilitate the extraction of their nutrients. After the appropriate time of soaking, the groats are ground with the right proportion of water to obtain a uniform suspension. The liquid extract free from undissolved solid particles is then homogenized for better consistency and stability, and lastly pasteurized for microbial safety and extended shelf life. It is then suitably packed in containers and stored under recommended conditions. (6).

Preparation of Soybean Milk: Soybean milk was prepared according to . To prepare 4 liters of soybean milk, 500 g of dried soybeans was used. The beans were thoroughly washed with warm water, placed in a suitable container, and allowed to soak in about four times their volume of water at 27°C for 12 hours. The soaked beans were washed several times with warm water to remove husks and an unpleasant odor. They were then ground — husks included — with 1 liter of water using an electric blender to get a homogenous juice. The remaining 3 liters of water were then added. The extract was heated to 60°C with continuous stirring. The soybean milk was then extracted by filtering through a piece of sieve to remove undissolved materials and stored refrigerated at 4°C until use.

Animal Preparation: Adult white male albino rats, 2-3 months old and weighing between 190-210 g, obtained from the College of Veterinary Medicine, Tikrit University, were used. They were placed in special animal breeding cages and subjected to a photoperiod of 12 hours of light followed by 12 hours of darkness at a temperature of 25°C.

Preparation of the Balanced Feed: The balanced feed was prepared according to the (18) guidelines, containing 50 g/kg of cellulose, 150.0 g/kg of casein, 100 g/kg of oil, and 5 g/kg each of vitamins and minerals, 100 g/kg of glucose, and 536.5 g/kg of starch.

Preparation of the high-fat diet: The high-fat diet was prepared based on the method introduced by Hulbron in 1982, whose work required the addition of 2% purified cholesterol to the standard diet for the induction of hyperlipidemia in rats. This diet was fed to the rats for 21 days, and feeding was continued until the end of the experiment.

Experimental design: Randomly, the experimental animals were assigned into six groups of three animals in each group. All groups were fed the high-fat diet for three weeks, except for the first group, which was fed only the balanced diet. Thereafter, each group of animals was treated with 1 ml of milk, according to the treatment group. The dose was divided into two 0.5 ml doses that

ISSN 2072-3857

were given every 12 hours by gavage. Treatment was given for 28 days.

- **Group T1 (Negative Control):** These animals were kept healthy and fed a standard diet; water was provided ad libitum throughout the experiment.
- **Group T2 (Positive Control):** These animals were fed a high-cholesterol diet over the course of the experiment and also supplied with distilled water.
- **Group T3:** They were on a high-fat diet and received 1 ml of cow's milk per day throughout the study.
- **Group T4:** They were on a high-fat diet and received 1 ml of almond milk per day.
- **Group T5:** was fed a high-fat diet and given 1 ml of oat milk per day.
- **Group T6):** was fed a high-fat diet and given 1 ml of soy milk per day.

Biochemical Tests: After the experimental period, the rats were fasted for about 12 hours and then anesthetized with chloroform. Blood samples were collected directly from the heart into test tubes without EDTA, then were incubated were incubated for about 15 minutes in a water bath maintained at 37°C. The serum was separated by centrifuging at 3000 rpm for 15 minutes and stored at -18°C until analyzed for the different biochemical parameters. These included total cholesterol, triglycerides, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and the antioxidants glutathione peroxidase and malondialdehyde. Aldehyde and AST and ALT assays in animal serums were performed according to the methods of (8).

Statistical analysis: The obtained data were subjected to analysis of variance using the General Linear Model in the statistical package (9) to test the significance of the effects of the factors under a completely randomized design. (10) was also carried out to determine the significance of the differences among the means of the factors under study for the traits at (0.05) level.

Results and Discussion:

Effect of Different Treatments on Average Weights of Experimental Animals: the results in table (1) shows the effect of feeding rats a standard diet (T1), a high-fat diet (T2), and a high-fat diet with daily administration of 1 ml of treatments (T3, T4, T5, T6). The different types of milk were observed to affect the average daily weight gain and final weight gain among the groups of experimental rats after 28 days of feeding and administration. The results showed that the average daily weight gain for the rats fed treatment T1 was (0.160) g/day, with a final weight gain of (4.50) g. The table also shows that the highest daily weight gain was observed in the rats fed treatment T2, with an average daily gain of (0.300) g/day and a final weight gain of (8.41) g. The average daily weight gain for the rats fed treatments (T3, T4, T5, T6) was [missing information]. (-0.091, -0.255, -0.257, -0.322)g/day respectively and the final increase was at (-2.57, -5.16, -7.20, -6.02)g respectively.

Table (1) Effect of different treatments on rat weights (g)

Rats daily body change in weight weight (g)	The amount of increase or decrease in body weight after 28 days (g)	Body weight (g)		treatments
		End of the experiment	Beginning of the experiment	
0.160	4.50	228.73[d] 2.264±	224.23[b] 1.213±	T1

ISSN 2072-3857

0.300	8.41	262.15[a] 2.731±	253.74[a] 2.759±	T2
-0.091	-2.57	248.50[b] 2.482±	251.07[a] 2.311±	T3
-0.255	-5.16	244.63[c] 2.311±	249.79[a] 3.098±	T4
-0.257	-7.20	247.21[c] 3.065±	254.41[a] 2.954±	T5
-0.322	-6.02	246.14[c] 2.273±	252.16[a] 3.078±	T6

- The numbers in the table represent the mean ± standard deviation.

- Different letters in the same column indicate significant differences ($p<0.05$) between the study groups.T1 = Healthy Control T2 = Infected Control T3 = Infected and Treated with Cow's Milk T4 = Infected and Treated with Almond Milk T5 = Infected and Treated with Oat Milk T6 = Infected and Treated with Soy Milk.

The weight gain of rats fed a high-fat diet is due to increased energy intake and increased fat accumulation in body tissues, which is associated with elevated concentrations of cholesterol, triglycerides, and low-density lipoproteins. This resulted in a higher weight gain compared to healthy animals and is a natural response to diets high in fat and calories (11). Results showed that the administration of cow's milk to rats led to a decrease in body weight when compared with the high-cholesterol control group. This can be attributed to the fact that cow's milk contains high-quality bioavailable proteins, especially casein and whey proteins, which help to improve metabolism of fats and energy while reducing accumulation of fats in the body. Also, the calcium in milk may help to reduce absorption of fat from the digestive tract and increase its excretion, thus positively impacting regulation of body weight. This is referenced from (2). The findings also indicated that almond milk significantly facilitated the shedding of weight, which was ascribed to its high levels of phenolic compounds, flavonoids, and vitamin E, which also play the role of antioxidants. These work to cut down on oxidative stress related to disorders of lipid metabolism and enhance the efficiency of metabolic processes. Unsaturated fatty acids together with dietary fiber in almonds may also help in reducing fat and cholesterol absorption and improving energy utilization, hence weight loss (12). Body weight of all the mice in the oat milk group was significantly lower than those in the water and glucose groups. Oat milk has the highest soluble beta-glucan fiber among the three types of milk, hence reducing fat and cholesterol absorption in the intestines. It also increases the viscosity of intestinal contents to limit the utilization of dietary fats. This fiber source can promote a feeling of fullness as well as better insulin response and control over lipid metabolism, all beneficial for managing weight. (12) In the same way, feeding rats soy milk led to a reduction in weight compared to untreated infected animals. This could be due to the fact that soy contains plant proteins and isoflavones, which are important for the regulation of fat metabolism as well as for reducing the synthesis and accumulation of fats in the body. Additionally, the active compounds in soy may help improve insulin sensitivity and raise fatty acid oxidation, thus reducing the weight gain linked with high-fat diets(13) It is possible that the weight loss in the rats that received the various treatments of milk could have been due to their active components having effects on improving fat metabolism and reducing fat accumulation in the body, as well as their anti-oxidative stress effect against hyperlipidemia. This led to improved metabolic efficiency and a resultant reduction in body weight compared to the infected control group.

ISSN 2072-3857

Effect of Different Treatments on Blood Lipid Profile:

Table 2 presents the impact of oral administration of treatments (T6, T5, T4, T3) on the blood lipid profile of rats in relation to healthy (T1) and infected (T2) rat groups. From the table, it is noted that the level of cholesterol in treatment T2 increased significantly to a level of (210.71) mg/dL at a probability level ($P<0.05$) as compared to treatment T1 which was (115.37) mg/dL. The values of treatments (T6, T5, T4, T3) decreased significantly to levels of (164.79), (150.70), (145.92), and (152.58) mg/dL, respectively. Also, the table shows that the level of triglycerides in treatment T2 had a significant increase to (180.35) mg/dL. For treatment T1, it was 97.83 mg/dL, and for the rats infected and treated with treatments T3, T4, T5, and T6, it was 134.72, 131.94, 135.14, and 142.52 mg/dL, respectively. The same results showed that the concentration of high-density lipoproteins (HDL) in rats T2 decreased to 72.87 mg/dL, compared to 42.31 mg/dL in rats T1. Rats infected and treated with treatments T3, T4, T5, and T6 recorded concentrations of 38.18, 40.68, 38.51, and 34.65 mg/dL, respectively.

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 / treatments
53.50^{e} ±1.46	19.56^{d} ±1.33	42.31^{a} ±1.16	97.83^{d} ±1.74	115.37^{e} ±1.43	**T1**
146.77^{a} ±2.85	36.07^{a} ±1.14	27.87^{d} ±0.94	180.35^{a} ±2.88	210.71^{a} ±2.60	**T2**
101.64^{b} ±2.19	28.50^{b} ±1.21	34.65^{c} ±1.28	142.52^{b} ±2.19	164.79^{b} ±2.56	**T3**
87.05^{c} ±1.84	27.02^{c} ±1.87	38.51^{c} ±1.03	135.14^{c} ±2.48	152.58^{c} ±2.29	**T4**
78.86^{d} ±2.11	26.38^{c} ±1.09	40.68^{b} ±1.75	131.94^{c} ±1.67	145.92^{d} ±1.88	**T5**
85.58^{c} ±1.72	26.94^{c} ±1.50	38.18^{b} ±1.40	134.72^{c} ±1.52	150.70^{c} ±1.64	**T6**

- The numbers in the table represent the mean ± standard deviation.

- Different letters in the same column indicate significant differences ($p<0.05$) between the study groups.

- T1 = Healthy Control T2 = Infected Control T3 = Infected and Treated with Cow's Milk T4 = Infected and Treated with Almond Milk T5 = Infected and Treated with Oat Milk T6 = Infected and Treated with Soy Milk.

The results were similar to those of (2) who showed that a high-fat diet leads to health risk by increasing cholesterol levels in the body mainly low-density lipoproteins (LDL) instead of high-density lipoproteins (HDL), and by raising triglyceride levels in blood serum which leads to plaque formation in the lining of the arteries thereby impeding blood flow and giving rise to a myriad of diseases.

It was also observed that cow's milk given to hyperlipidemia rats substantially lowered their blood lipid profile. This is similar to the findings of (14) who showed that feeding 1 ml of yogurt to hypercholesterolemia rats daily significantly reduced their blood lipid profile. He attributed this to the role of fermented dairy products containing probiotic bacteria in reducing blood lipids in hyperlipidemia animals. It is also stated that

ISSN 2072-3857

almond milk and probiotics resulted in a very significant decrease in the blood lipid profile. These results are in agreement with those obtained by (15). The reason is that sweet almond milk contains insoluble dietary fiber. There is evidence to support the relationship between consumption of insoluble dietary fiber and reduced levels of cholesterol in the serum. This fiber functions by enhancing the secretion of bile acids into the feces. This will stop their reabsorption through the hepatic-enteric portal circulation. The low level of bile acids stimulates the liver cells to increase the conversion of cholesterol to bile acids thus the concentration of cholesterol between cells falls (3). Sweet almonds are a rich source of monounsaturated fatty acids and also of polyunsaturated fatty acids. They have high linolenic acid content which helps significantly in lowering cholesterol levels because it stimulates the secretion of cholesterol from the intestines and its oxidation into bile acids. This is according to (4). Sweet almond milk contains significant levels of natural antioxidants such as flavonoids, which play a major role in scavenging free radicals and reducing lipid peroxidation. Vitamin E also helps to stop the destruction of cell membranes by free radicals as it interrupts and neutralizes them, hence reducing their effect. Other antioxidants, such as vitamin C and glutathione, also work synergistically with vitamin E in this process. This is according to (15) .The results show that all the types of milk used contributed to improving the blood lipid profile in hypercholesterolemic rats, although the degree of effect varied based on the chemical composition and bioactive compounds of each type. Oat milk looked like the most effective in lowering total cholesterol and LDL, while the other types showed varying positive effects in improving lipid indicators and reducing metabolic disturbances resulting from high-fat diets (5).

Effect of Different Treatments on Liver Enzyme Activity:

Table 3 shows the effect of different treatments on liver enzyme activity of healthy and hypercholesterolemic rats. It is observed that the ALT enzyme level in hypercholesterolemic rats was significantly higher, that is, 47.03 IU/L as compared to 24.73 IU/L in the healthy treatment (T1). The ALT levels in treatments T3, T4, T5, and T6 were 36.93, 33.32, 37.26, and 39.65 IU/L, respectively. The table also shows that the AST enzyme level in hypercholesterolemic rats was 87.90 IU/L, significantly higher than the healthy rat group, which was 56.82 IU/L. The levels of AST in hypercholesterolemic and treated treatments (T3, T4, T5, and T6) were 71.39 and 67.16 IU/L, respectively. Results presented in the same table disclose that the level of the ALP enzyme in rats with high cholesterol increased markedly to reach (94.46) IU/L compared to healthy rats, which was (84.57) IU/L. The infected groups and those treated with T3, T4, T5, and T6 recorded (86.55, 86.06, 86.33, and 88.78) IU/L respectively.

Table (3) Effect of different treatments on the activity of liver enzymes (international unit/liter) in the blood serum of healthy male rats and those with hypercholesterolemia

ALP (IU/L)	**AST (IU/L)**	**ALT (IU/L)**	**Variables / Coordinates**
84.57^{d} ±2.51	56.82^{e} ±1.38	24.73^{e} ±0.87	**T1**
94.46^{a} ±2.12	87.90^{a} ±2.92	47.03^{a} ±1.35	**T2**
88.78^{b} ±1.87	72.33^{c} ±1.39	39.65^{b} ±1.57	**T3**
86.33^{c}	72.12^{c}	37.26^{c}	**T4**

ISSN 2072-3857

±1.35	±1.74	±1.22	
86.06^{c} ±1.20	67.16^{d} ±1.08	33.32^{d} ±1.04	**T5**
86.55^{c} ±1.76	71.39^{c} ±1.54	36.93^{c} ±1.70	**T6**

- The numbers in the table represent the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between the study groups.
- T1 = Healthy Control T2 = Infected Control T3 = Infected and Treated with Cow's Milk T4 = Infected and Treated with Almond Milk T5 = Infected and Treated with Oat Milk T6 = Infected and Treated with Soy Milk.

The results indicated a significant increase in the liver enzymes ALT, AST, and ALP activities in rats with hypercholesterolemia in relation to the healthy control group. Findings in the present study are in agreement with those of (16) and (17) who revealed that fat-rich diets cause fat accumulation in the liver and enhance oxidative stress; hence, damage to liver cells with subsequent release of enzymes into the blood. Results were also indicative that treatment with cow's milk, almond milk, oat milk, and soy milk led to a significant reduction in liver enzyme activities compared to the hypercholesterolemic group. Components of milk were in agreement with those of (2) regarding metabolic indicators and liver function. Milk components were also in agreement with (3) in support of phenolic compounds and antioxidants present in almonds. (4) found beta-glucan fibers in oats helpful in reducing fat accumulation and improving liver function. (5) demonstrated that isoflavones and plant proteins in soy play a significant role in reducing oxidative stress and improving liver function. Oat milk showed the best response in decreasing liver enzymes; then, almond milk; and then, soy milk; and cow's milk had a relatively smaller effect. However, all treatments resulted in a significant improvement compared to the control group. This variation may be due to the different content of bioactive compounds, antioxidants, and dietary fibers in each treatment, influencing fat metabolism and protecting liver tissue.

Effect of different treatments on blood glucose levels in experimental animals:

Table (4) depicts the efficacy of different therapies on blood sugar levels in normal rats and those with hyperlipidemia. From the figure, it is evident that glucose levels in treatment T2 were significantly higher at (149.80) mg/dL as compared to (114.47) mg/dL in rats of treatment T1. On the other hand, glucose concentrations dropped in infected and treated treatments T3, T4, T5, and T6 to reach (124.35, 120.51, 126.72, and 134.03) mg/dL, respectively.

Table (4) effect of different treatments on the concentration of glucose in the blood serum of healthy and hypercholesterolemic rats.

Blood sugar (mg/dL)	**Variables / Coordinates**
114.47^{e} ±2.04	**T1**
149.80^{a} ±2.68	**T2**
134.03^{b} ±2.45	**T3**
126.72^{c}	**T4**

ISSN 2072-3857

±1.76	
120.51^{d} ±1.13	**T5**
124.35^{c} ±2.54	**T6**

The numbers in the table represent the mean ± standard deviation.
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- T1 = Healthy Control
T2 = Infected Control
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T5 = Infected and Treated with Oat Milk
T6 = Infected and Treated with Soy Milk

Results in almond milk were in line with (3) who proposed that phenolic compounds and flavonoids in almonds may contribute to better glucose metabolism and reduced oxidative stress related to metabolic disorders. Results for oat milk were also consistent with (4) who indicated that beta-glucan fiber helps improve insulin sensitivity and regulate blood glucose levels. Results in soy milk also agreed with (5) who suggested that isoflavones and plant proteins in soy play a major role in improving blood glucose regulation and reducing insulin resistance. The decline in the cow's milk group may be due to milk proteins and specific bioactive peptides that help improve insulin response and regulate glucose metabolism (2).

Conclusions:

Results of the present study revealed that the tested milk types ameliorated metabolic and biochemical parameters in hypercholesterolemic rats. This was manifested by the improvement of lipid profiles, optimization of liver function, and reduction of blood glucose levels. Oat milk exhibited the most pronounced beneficial effects among the treatments; thus, it can be considered a potential functional food for the management of hypercholesterolemia and associated metabolic disturbances. These results imply that the consumption of plant-based milk alternatives, particularly that of oat milk, can improve cardiovascular and metabolic health.

Conclusion:

Oat milk can be suggested as a dietary component for the enhancement of lipid metabolism and metabolic health in general. More experimental and clinical studies are needed to understand the mechanisms of the positive effects to establish the levels of intake that would be considered optimal and to determine its long-term efficacy and safety in different populations.

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ISSN 2072-3857