Effect of Dietary Fat Cholesterol on Blood Characteristics of Experimental Animals

Falah Hadi Jaber[1], Riyadh Shamkhi Ali[2*]
[1] Depertmet of Food Sciences, Faculty of Agriculture, University of Kufa, Najaf, Iraq
[2] Department of Anesthesia and Intensive Care Techniques, Al Taff University College, Karbala, Iraq
*Email: riyadh.alhabeeb@uokufa.edu.iq

Abstract
In this study, cholesterol levels in a number of dietary oils including sesame oil, and animal fats: sheep fat, sheep belly fat, beef fat, free fat, and commercial hydrogenated cooking oil were determined. The oils under study were used with the diet in feeding 42 male rats, which were divided into seven groups. Six groups were fed on diets with 10% added oils or fats, and a control group (standard diet). Treatments were, 6 rats of each group fed on a standard diet supplemented with 10% of; sheep tail fat T1, Sheep belly fat T2, Sesame oil T3, beef fat T4, commercial hydrogenated oil T5, pure cow ghee T6, or the control fed on standard diet only T7. The feeding continued for a period of 42 days. Then, rats were killed, dissected where blood samples were taken from the heart directly for performing lipid profile tests including TC-TG-HDL-LDL and VLDL. In testing cholesterol levels in the oils or fats samples under study, the results showed that beef fat had the highest cholesterol level, followed by ghee fat, sheep tail fat, and sheep belly fat, where the lowest levels of cholesterol were recorded in sesame oil and cooking oil, respectively. Treatments T4-T3-T2-T1 did not differ in blood HDL values in treated rats from the control treatment which was significantly different from the lowest values of HDL in the treatments T5 and T6. The LDL value of T6 treatment decreased with a significant difference from the control, while the other treatments did not differ among each other or compared to the control. However, VLDL values did not differ among treatments. The T6 treatment recorded less blood glucose (GLOU), TC, and TG which significantly differed from the control, as the other treatments did not differ from the control or among each other. The normal levels of blood cholesterol, triglycerides, and blood proteins in rats fed on 10% fat or vegetable oil remove concern and anxiety against these fats and support their nutritional health benefits.
Keywords: essential oil, cholesterol, fats, blood tests, nutrition

1. Introduction

Cholesterol is one of the most important sterols found in animal fats. It is linked to long-chain fatty acids forming esters. It is transported by lipoproteins through the blood in the body. Cholesterol is found in the cell walls and in the internal organs of the body (liver, kidneys and brain). It is also produced internally in the liver, skin, and cells lining the intestines. It is also a source of bile salts, sex hormones, and an external source of cholesterol from animal fats, egg yolks, and brain [1]. Cholesterol is produced in the liver, adrenal glands, intestines, and gonads internally, while 20-25% of cholesterol comes from food [2].The process of transporting cholesterol in the body is conducted by lipoprotein, and it is a complex composition of cholesterol, ester salt, phospholipids, triglycerides, and various proteins such as (LDL, VLDL, and HDL).The latter is considered beneficial and does not pose a threat to heart disease and atherosclerosis, because it transfers cholesterol from the body to the liver. As for low-density lipoprotein, it poses a great risk of transferring cholesterol from the liver to the body [1]. It was also reported [3] that cholesterol is present in red meat (cows - sheep), and cholesterol levels can be reduced by controlling animal feeding. Animal blood samples, the results showed that shrimp waste decreased the level of (LDL) with an increase of (HDL) in the blood [4]. indicated that the percentage of cholesterol in olive oil is lower than that in pork belly fat. Olive oil is considered low-fat compared to lard. The percentage of fat affects the content of fatty acids. Olive oil contains a lower percentage of saturated fatty acids and a higher percentage of fatty acids. Polyunsaturated. While, goat meat pies contain a high percentage of polyunsaturated fats, the total unsaturated fatty acids in sheep and goat pies showed that they are almost balanced products [5]. Those recovering from the (COVID, 19) epidemic, where there are no mechanisms for the consequences of the epidemic, as the study determined serum protein at the beginning of the disease and recovery stages. Where Coved is associated with long-term disorders of cholesterol metabolism and heart muscle even in the recovery phase to identify potential biomarkers for these disorders, severely infected people showed more serious disorders in these pathways, such as disruption of multiple biological pathways such as the immune response [6]. Concluding that family history is associated with the risk of cardiovascular disease as a result of eating fats, and blood type has a significant effect on the rise of TC, TG, LDL, VLDL, and HDL.After conducting tests on a hundred blood samples of men and women using the enzymatic method, blood group (B) recorded an increase in

doi.org/10.31838/hiv23.03.297 Received: 24.11.22, Revised: 19.12.22, Accepted: 11.01.23.

(TC, LDL, HDL) for women, with no significance for these values in males, and blood group (A) recorded an increase in (TC, TG, LDL, (VLDL) In females, they are the most susceptible to infection, and blood group (AB) recorded the lowest rate of coronary artery disease in terms of values (TC, TG, LDL, HDL), so blood group A was the most susceptible to infection. this study aimed to investigate the effect of dietary oils, namely sesame oil, and animal fats: sheep fat, sheep belly fat, beef fat, free fat, and commercial hydrogenated cooking oil on blood characteristics and general health parameters where added at 10% to rat diet.

2. Materials and Methods

Raw Materials for the study were obtained including; Milk fat from a local farm, Special beef fat purchased from a certified butchery, sheep tail fat, Sheep fat (belly fat), Turkish-Altunsa hydrogenated vegetable cooking oil (pure vegetable oils – sunflower oil – palm oil – cotton-seed oil – rapeseed oil – soybean oil), Sesame seeds Helianthus annuus obtained from local market. sesame oil was extracted with a net percentage of (36.70%) by the cold pressing method [7], using Iranian-made device (BEKBDANEH MINI PRESS). As for Animal fat extraction, beef fat at purity of 75%, sheep belly fat at purity of 77%, sheep tail fat at purity of 61, and Butterfat in milk at purity of 52.97% were obtained using following the extraction method by [8].

Determination of cholesterol for oil and fat samples.

The cholesterol level was estimated using the colorimetric enzymatic test method (CHOD-PAP) mentioned by [2] and modified by [9] according to the proposal of (BIOLABO). Where, Cholesterol is calculated using the following equation:
Cholesterol: mg/dl = sample absorbance/control cholesterol x 2.

Experiment general procedure

The adult male white Norway rats (*Rattus norvegicus domestica*) of 5- 6 weeks old were used in the animal house of the College of Science, University of Babylon. Rats were maintained in an air-conditioned room and vacuumed at 23- 24 °C, and a normal lighting period of (12-12) hours/day, light and dark. Male rats were divided into seven groups with tail marking in special cages and water pipettes. Their water was changed daily. The animals were given the first three days of a regular diet without any addition. Adding oil or fat to the diet began at a rate of 10%, while monitoring the activity and safety of healthy animals, and adhering to a specific weight of the diet daily, calculating the remains, weighing the droppings, monitoring the daily weight gain or decrease, and cleaning throughout the experiment period of 42 days. Treatments were feeding 42 rats in total divided into 7 groups, 6 rats of each group on a standard diet supplemented with 10% of; sheep tail fat T1, Sheep belly fat T2, Sesame oil T3, beef fat T4, commercial hydrogenated oil T5, pure cow ghee T6, or the control fed on standard diet only T7. The feeding continued for a period of 42 days,

Ending the experiment and data collection

After the end of the 42-day trial period, feeding was stopped 12 before the measurements. The rats were weighed before being killed. Each rat was anesthetized with chloroform, fixed, dissected, and drawing 5 ml blood samples from the heart directly, and separating the organs (liver, spleen and kidneys), which then were immersed in formalin and dried using filter paper before being weighted.

Collection of blood samples

Blood samples (2 ml) were placed in tubes containing anticoagulant (EDTA) to conduct a complete blood test (CBC). The remaining (3) ml were taken in tubes free of (EDTA) and placed in a centrifuge at a speed of 3000 rpm for 10 minutes for separation and conducting biochemical analyzes tests including TC [10], TG, GLUO [11], HDL, LDL, and VLDL. Results were read at wavelength 500 Nanometer by the enzymatic method [6].

Statistical analysis

The experiment was completely randomized design (CRD) with three replications. The GenStat V.12.1 program was used for statistical analysis. The comparison between the means was done using Duncan's multiple range test where the least significant difference L.S.D. was performed ($P<0.05$).

3. Results and Discussion

Cholesterol levels in different fat types

Table1. Cholesterol values for oil and fat samples

	oil or fat	cholesterol value mg/dl
1	tail fat	13.506
2	Sheep belly fat	8.6402
3	Sesame oil	1.43122
4	cow fat	49.8018
5	Hydrogenated oil	3.5485
6	ghee fat	26.0434

The results of the cholesterol test for the oils and fats under the current study showed values that were 49.801, 26.034, 13.06, 8.640, 3.5488, and 1.430 resulted from beef fat, ghee fat, sheep tail fat, sheep belly fat, hydrogenated oil and sesame oil, respectively (Table1). So, beef fat showed the highest content of cholesterol. This is close to cholesterol value of (52-55) mg/100g found in a previous study by [12]. Similarly, in case of cholesterol level in belly fat [4] and ghee fat [12]. Cholesterol values depend on the living conditions of the animal and the nature of nutrition, and oils and fats are heterogeneous compounds, so cholesterol values vary [12]. As for the cholesterol of sesame and hydrogenated oils, the lowest value of cholesterol was in sesame oil then hydrogenated oil. This is consistent with the findings of [13]. in the research entitled Estimating the Level of Cholesterol in Types of Vegetable Oils. Cholesterol was measured for twenty-one samples in Nigeria with three measurement

methods as the cholesterol values were determined in seventeen samples [14]. Confirming the results of estimating the cholesterol content in 14 samples of edible oils in Iraq [15, 16].

Table2. Cholesterol levels in vegetable oils by three measurement methods				
N	Sample	Ojiako and Akubugwo (mg/ml)	Liebermann-Burchard mg/ml	HBLC MGML
1	Sesame oil	1.20±0	3.996 ±0.404	0.5750± 0.200
2	Palm oil	NO	0.5661±0.012	0.149 ±0.01
3	olive oil	0.421 ±0.029	0.702±0.097	0.58±0.02

Effect of adding 10% fat or oil to rat diet on blood characteristics (RBS-TC-TG)

The results of glucose examination of blood samples of male experimental rats for the treatments under the current study during the 42-day feeding period (Table 2) showed that there were slight differences ($p \leq 0.05$) between the treatments. The highest blood glucose value was recorded for the T2 treatment with a mean of 125.6 mg/dL, and the lowest value was in the T6 treatment (90.9 mg/dL), with a significant difference from the control (113.4 mg/dL). It was indicated by Xu, Y., Zhu et al. (2022) [17] that non-hydrogenated vegetable oils recorded a decrease in the level of blood glucose (CLO).

On the other hand, the TC cholesterol test showed that there were significant differences between the standard control group, which recorded 83.7 mg/dL compared to the lowest value in the T6 treatment with an average of 55.2 mg/dL. This is consistent with the findings of Sahargahi et.al. (2020) [18] stating that clarified butter has been the healthiest source of edible fat for thousands of years. It was also reported [19] the beneficial effects of free fat in the process of atherosclerosis and the reduction of LDL, the atherosclerotic index, and lipid levels. The research recommends the importance of butter and animal fats more than vegetable oils, since the first works to precipitate calcium, magnesium, and phosphorus to increase bone density and protect it from fragility. Salih (2021) [20] also referred to the great role of cholesterol in the body's lipid balance, and as Ngala et al. (2016) [21] concluded, the ability of 10% vegetable oil when fed to experimental rats with type 2 diabetes reduces total cholesterol (TC), as well as reporting that the plant sterols in sesame oil reduce the absorption of cholesterol to lower its level in the blood [22].

Table3. Effect of adding 10% fat or oil to rat diet on blood tests (TG-TC-RBS)						
Treatments	CLOU		TC		TG	
control	113.4	a	83.7	a	68.3	a
T1	119.5	ab	72.8	b	64.1	cb
T2	125.6	abc	74.6	bc	77.5	abc
T3	107.5	abs	61.87	bc	73	abc
T4	112.9	abc	78.5	bc	72.2	bc
T5	93.7	bc	81.6	cb	71.3	bc
T6	90.9	c	55.2	d	61.5	c

Treatments are sheep tail fat T1, Sheep belly fat T2, Sesame oil T3, beef fat T4, commercial hydrogenated oil T5 and pure dairy ghee T6. Values within a column followed with the same letter(s) are not different according Duncan's multiple range tests ($P \leq 0.05$)

Effect of adding 10% fat or oil to rat diet on blood proteins (VLDL-LDL-HDL)

The results showed that the different treatments did not differ significantly ($P \leq 0.05$) among them in the value of high-density lipoprotein (HDL) for blood samples of experimental male rats, which ranged between 33-37 compared to the control 41.47 mg/dL. In general, the lowest values were recorded in the treatments T5 (26.87), and T6 (26.43) mg / dL. However, the values of low-density lipoprotein (LDL). LDL differed significantly between some treatments with control, while the treatments (T5, T2, T4, T3, T1) did not differ between them or compared to control. T6 recorded a value of 16.8 mg / dL to be the lowest value compared to the highest value of LDL for treatment T5 [23] to be agreed with hydrogenated oil risks potentials [24]. The results of the very low-density lipoprotein (VLDL) examination showed that the highest average was recorded in the T5 treatment with a value of 13.43 mg / dL, and the lowest rate was in the T6 treatment (12.27 mg / dL compared to the control, which recorded 12.27 mg/dl.

This is consistent with the findings of Liu, M., Wang et al., (2020) [25] regarding total cholesterol and blood proteins when feeding experimental animals with 10% bovine fat for four weeks. It is also consistent with previous results [21] with the ability of 10% vegetable oil when fed to diabetic rats to lower LDL and increase HDL levels. Also, the ability of plant sterols in sesame oil to reduce the absorption of cholesterol in the diet and thus reduce harmful cholesterol in the blood. Generally, fatty acids are considered dietary supplements that maintain levels of harmful LDL cholesterol [26]. The examinations of 36 male volunteers, aged (55-80) years, without any previous history of cardiovascular disease, and subjected to three diets, the first was rich in virgin olive oil, the second low in beef fats, and the third was standard diet regimen. The three diets did not show differences in the examinations. Biochemistry except for high HDL and low LDL.

Table4. Effect of adding 10% fat or oil to rat diet on blood tests (HDL, LDL, VLDL)						
Treatments	HDL mg/dl		LDL mg/dl		VLDL mg/dl	
Control	41.47	a	33.5	a	13.27	a
T1	37.13	ab	24.8	ab	12.8	a
T2	33.43	b	27.8	a	12.87	a
T3	36.77	ab	25.3	ab	12.55	a
T4	33.67	b	27.2	a	12.7	a
T5	26.43	c	33.6	a	13.43	a
T6	26.87	c	16.8	b	12.27	a

* Treatments are sheep tail fat T1, Sheep belly fat T2, Sesame oil T3, beef fat T4, commercial hydrogenated oil T5 and pure dairy ghee T6. Values within a column followed with the same letter(s) are not different according Duncan's multiple range tests (P≤0.05)

Hydrogenated oils greatly raise the levels of (TG, TC, (LDL) and reduce the values of (HDL) as an explanation for containing a high percentage of SFA [27], but the saturated fatty acids resulting from hydrogenated vegetable oils (ghee) produce unwanted lipoproteins. In it, mice fed on butter and animal fats had lower serum TC, TG, and LDL values compared to those fed margarine .This is explained by the fact that butter and animal fats contain linoleic acid, and it is considered a natural source [19]. Indicating the beneficial effects of ghee fat in the process of atherosclerosis and the reduction of LDL, an index of atherosclerosis. Milk fat was known as high cholesterol fat containing saturated fatty acids, but these acids do not have equivalent effects on the level of cholesterol in plasma. Research indicates that hypercholesterolemia of saturated fats in the human diet is largely due to the carbon chain of fatty acids (16,14,12) Evidence also indicates that C:18 oleic fatty acid is effective in lowering plasma cholesterol levels when it replaces C:16 palmitic fatty acid. Milk fat contains 10% short and medium chain fatty acids, and oleic 35% of the total fatty acids [28].

4. Conclusion

Findings showed that oils and fats maintained acceptable blood glucose, cholesterol, and triglycerides. Still, the highest value of glucose and triglycerides was in the blood of rat male due to feeding on 10% sheep belly fat while the highest value for cholesterol was in those fed on 10% commercial hydrogenated vegetable oil. On the other hand, the lowest value for glucose, cholesterol, and triglycerides were detected in rat's blood-fed on 10% dairy ghee. It was confirmed the healthy indicator of oils and fats by examining blood proteins compared to the control treatment. The highest HDL was in the blood of male rats treated with sheep tail fat followed by sesame oil treatment, while the lowest HDL was for the hydrogenated oil treatment. It was found that 10% of oil and fat maintains the integrity of the heart and blood vessels. Likewise, 10% of animal fats did not increase the harmful high cholesterol.

References

Genaro-Mattos, T. C., Anderson, A., Allen, L. B., Korade, Z., & Mirnics, K. Cholesterol biosynthesis and uptake in developing neurons. ACS Chemical Neuroscience 2019; 10(8): 3671-3681

Li LH, Dutkiewicz, EP, Huang YC, Zhou HB, Hsu CC. Analytical methods for cholesterol quantification. Journal of food and drug analysis 2019: 1(2):375-86

Hatta, M., Priyanto, R., Mas, M. S., & Prahesti, K. I. (2019, March). Chemical characteristic and cholesterol level of local sheep with intensive fattening. In IOP Conference Series: Earth and Environmental Science (Vol. 247, No. 1, p. 012025). IOP Publishing

Teixeira, A., Almeida, S., Pereira, E., Mangachaia, F., & Rodrigues, S. Physicochemical characteristics of sheep and goat pâtés. differences between fat sources and proportions. Heliyon 2019; 5(7): 223-229.

Chen, H. J., Hu, Y., Yao, P., Ning, D., Zhang, Y. P., Wang, Z. G., ... & Pang, D. W. Accurate and Efficient Lipoprotein Detection Based on the HCR–DNAzyme Platform. Analytical Chemistry 2021; 93(15): 6128-6134.

Ataimish, S. H., & Ahmed, H. F. (2022). Evaluation of lipid profile with different ABO blood groups among Iraqi healthy people. Al-Nisour Journal for Medical Sciences, 4(2

Chakraborty, D., Das, J., Das, P. K., Bhattacharjee, S. C., & Das, S.. Evaluation of the parameters affecting the extraction of sesame oil from sesame (Sesamum indicum L.) seed using soxhlet apparatus. International Food Research Journal 2017; 24(2)

Soares Dias, A. P., Ramos, M., & Rijo, B. Rendering of Beef Tallow for Biodiesel Production: Microwave versus Boiling Water and Acetone Fat Extraction. Processes 2022; 10(4): 666

Al-Lahaibi, A. H. M., & Al-Habib, F. M. K. ADDING DIFFERENT FATTY TISSUES TO CHICKEN BURGER AND THE EFFECT OF THIS ON THEIR QUALITATIVE AND STORAGE QUALITIES. Academicia Globe: Inderscience Research 2022; 3(10): 1-18

Sharma, V., & Mobin, S. M. Cytocompatible peroxidase mimic CuO: graphene nanosphere composite as colorimetric dual sensor for hydrogen peroxide and cholesterol with its logic gate implementation. Sensors and 2017

Bondar, R. J., & Mead, D. C. Evaluation of glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides in the hexokinase method for determining glucose in serum. Clinical chemistry 1974; 20(5): 586-590

Keklik, N. M., BOZKURT, H., & TEKİN, A. R. (2018). Effect of different cooking procedures on cholesterol and fat contents of selected meat products. Food Science and Technology, 38, 683-690

Okpuzor, J., Okochi, V. I., Ogbunugafor, H. A., Ogbonnia, S., Fagbayi, T., & Obidiegwu, C. Estimation of cholesterol level in different brands of vegetable oils. Pakistan Journal of Nutrition 2009; 8(1): 57-62.

Almajidi, I. M. Estimation of cholesterol content and ghee fatty acids in edible oils in Iraq. Int J Chem Phys 2015; 4(2): 3-11.

Hasan, M., Hossain, M., Dhar, P. K., & Haque, M. Comparative Study on Cholesterol Content and Physicochemical Properties of Some Branded and Unbranded Commercial Edible Oils in Khulna, Bangladesh. Journal of Chemical Health Risks 2019; 9 (4): 321-329

Zio Souleymane. Université Joseph KI-ZERBO&

Zongo Oumarou University homas SANKARA& Ouattara Ismaël Labia& Bazié Raoul Sylvain Bazoin Laboratoire National de Santé Publique,2020
Xu, Y., Zhu, W., Ge, Q., & Zhou, X. Effect of different types of oil intake on the blood index and the intestinal flora of rats. AMB Express 2022; 12(1): 1
Sahargahi, B., Pasdar, Y., Moradinazar, M., Najafi, F., Darbandi, M., Moludi, J., ... & Shakiba, E. (2020). The Effect of Edible Lipids on Atherogenic Index of Plasma: Results from RaNCD Cohort Study
Chin, S.F., W. Liu and J.M. Storkson. Advances in conjugated linoleic acid research. J. Food Comp. Anal. 1992; 5: 185
Salih, K. J. The major pathways of lipids (triglyceride and cholesterol) and lipoprotein metabolism. Zanco Journal of Pure and Applied Sciences 2021; 33(4):61-72.
Ngala, R. A., Ampong, I., Sakyi, S. A., & Anto, E. O. Effect of dietary vegetable oil consumption on blood glucose levels, lipid profile and weight in diabetic mice: an experimental case—control study. BMC Nutrition 2016; 2(1): 1-8
El-Beltagi, H. S., Maraei, R. W., El-Ansary, A. E., Rezk, A. A., Mansour, A. T., & Aly, A. A. Characterizing the Bioactive Ingredients in Sesame Oil Affected by Multiple Roasting Methods. Foods 2022; 11(15): 2261
Arpón, A., Milagro, F. I., Razquin, C., Corella, D., Estruch, R., Fitó, M., ... & Martínez, J. A. Impact of consuming extra-virgin olive oil or nuts within a Mediterranean diet on DNA methylation in peripheral white blood cells within the PREDIMED-Navarra randomized controlled trial: a role for dietary lipids. Nutrients 2017; 10(1): 15
Bhandari, S. D., Delmonte, P., Honigfort, M., Yan, W., Dionisi, F., Fleith, M., ... & Bergeson, L. L. Regulatory changes affecting the production and use of fats and oils: Focus on partially hydrogenated oils. Journal of the American Oil Chemists' Society 2020; 97(8): 797-815
Liu, M., Wang, D. Q. H., Black, D. D., & Tso, P. Differential effect of four-week feeding of different dietary fats on the accumulation of fat and the cholesterol and triglyceride contents in the different fat depots. Nutrients 2020; 12(11): 3241
26-Kruse, M., von Loeffelholz, C., Hoffmann, D., Pohlmann, A., Seltmann, A. C., Osterhoff, M., ... & Pfeiffer, A. F. Dietary rapeseed/canola-oil supplementation reduces serum lipids and liver enzymes and alters postprandial inflammatory responses in adipose tissue compared to olive-oil supplementation in obese men. Molecular Nutrition & Food Research 2015; 59(3): 507-519
Rezq, A. A., Labib, F. A., & Attia, A. E. M. Effect of some dietary oils and fats on serum lipid profile, calcium absorption and bone mineralization in mice. Pak. J. Nutr. 2010; 9(7): 643-650
Ney, D. M. Potential for enhancing the nutritional properties of milk fat. Journal of dairy science 1991; 74(11): 4002-4012.