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Original Article

The Effect of Extra Virgin Olive Oil on Reducing Malondialdehyde Levels and Liver Histopathology in Male Rattus Norvegicus with Hypercholesterolemia

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ABSTRACT

All living things age physiologically. Free radicals attack cells as they age, causing damage, disruption, and death. Free radicals oxidize and damage cell membrane lipids. The body needs optimum antioxidants during oxidative stress. This study hypothesizes EVOO can lower MDA and total cholesterol and repair liver histopathology in hypercholesterolemic Wistar male rats—this research with a pre-post-test control group experiment. The study found that the control group's MDA level was 0.14 ± 0.03 nmol/ml before treatment (pretest), while the group given standard meal for mice and distilled water/day/head for 14 days had a drop to 0.13 ± 0.10 nmol/ml. In treatment group 1, the average MDA level was 0.73 ± 0.03 nmol/ml before treatment, and after 14 days of high-fat, high-cholesterol meal without EVOO, the average MDA level was 0.73 ± 0.03 nmol/ml and dropped to 0.48 ± 0.10 nmol/ml. Treatment group 2 had an average MDA level of 0.22 ± 0.02 nmol/ml before treatment, but after receiving a high-fat, high-cholesterol diet and a 1cc dose of EVOO for 14 days, it decreased to 0.17 ± 0.02 nmol/ml. Before therapy, the mean MDA level in group 3 was 0.17 ± 0.02 nmol/mL. Giving mice a lot of fat and cholesterol and a two cc dose of EVOO for 14 days lowered their MDA levels to 0.08 ± 0.01 nmol/ml, ranging from 0.10 to 0.05. The research concluded that average MDA decreased significantly in treatment group 3 after mice were fed high-fat, high-cholesterol feed and given two ccs of EVOO.

Keywords: *EVOO, MDA Levels, Hypercholesterolemia, Liver*

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INTRODUCTION

Research on cholesterol has increased in recent years due to its association with coronary heart disease (CHD) and other dangerous disorders [1]. Although there are likely other factors at play, research has shown elevated oxidation of LDL cholesterol as a significant contributor to coronary heart disease (CHD). In contrast to high levels of atherogenic low-density lipoprotein (LDL) cholesterol, high levels of protective high-density lipoprotein (HDL) cholesterol helps transport cholesterol away from tissues and back to the liver [1-4].

Atherosclerotic cardiovascular disease (ASCVD) is still the primary cause of death globally. Dyslipidemia is a contributing factor to the development of ASCVD, making it crucial to identify, diagnose, and manage dyslipidemia to avoid both initial and subsequent ASCVD events [5].

The free-form body fat cholesterol is an ester of fatty acids [6,7]. Consumed fat is saturated and unsaturated. The body produces acetyl coenzyme A from carbohydrates and lipids. This molecule forms fatty acids, triglycerides, phospholipids, and cholesterol; therefore, if the body overheats, triglycerides and cholesterol rise [8].

Diets high in cholesterol can cause hypercholesterolemia. A high-cholesterol diet disrupts fat metabolism, raising LDL and HDL levels and risking cardiovascular disease. A high-cholesterol diet also increases adipose

tissue fat. About 25% of accumulated fat's pro-inflammatory cytokines are 1L-6 [8]. These disorders generate low-grade systemic inflammation due to elevated IL-6. IL-6 inhibits lipoprotein lipase (LPL), boosting endothelial lipase lipolysis and lowering HDL [9]. Pharmacological medication can create adverse effects; hence, natural hypercholesterolemia remedies are needed.

Lipid peroxidation is the oldest and best-studied free radical damaging process [10,11]. Cell membranes peroxidize most lipids, mainly unsaturated fatty acids. Malondialdehyde (MDA), a cell-toxic oxidation product of unsaturated fatty acids, is used to evaluate lipid peroxidation [13-14]. MDA levels indicate oxidative stress by indirectly measuring free radical activity.

The current treatment of cardiovascular disease and hypercholesterolemia involves preventing risk factors and using drugs to lower blood lipid levels and antioxidants. However, this is expensive, so conservative efforts are needed to utilize natural ingredients and adopt a healthy lifestyle. Natural extract treatments are being researched since they are cheaper and have fewer adverse effects. Extra Virgin Olive Oil (EVOO) has been used in several trials to reduce hypercholesterolemia and oxidation, which can lead to blood vessel atherosclerosis.

Research on Extra Virgin Olive Oil, as conducted by Jimenez-Lopez et al., shows that consuming EVOO daily provides health benefits such as cardioprotective, antioxidant, anti-inflammatory, anti-tumor properties or acts as a regulator of the gut microbiota, etc [14]. Also, Oliveras-López et al.'s research on daily consumption of EVOO, which contains high levels of bioavailable polyphenols in healthy people, increased antioxidant levels and antioxidant status in plasma and blood cells, and modified PBMC antioxidant gene expression levels [15]. EVOO also helps heal pressure sores [16], reduces blood pressure and urine protein levels in preeclampsia [17], and helps reduce cholesterol levels in hypercholesterolemic older adults [18].

Olive fruit yields EVOO. The Middle East, Italy, Spain, Greece, and other Mediterranean countries have olive trees (*Olea europaea*). Ancient people knew their health benefits[19,20]. Other studies indicate that EVOO contains antioxidant substances such as phenolic compounds, tocopherol, squalene, chlorophyll, and β-carotene [21-22]. Most of the triacylglycerol in EVOO is MUFA-type oleic acid [21]. EVOO typically consumes 25-40 ml or 8-70 grams daily, causing skin reactions and diarrhea above 30 ml. The typical laxative dose is 30 ml of EVOO [22].

Other research suggests EVOO can break down obesity-causing adipocyte cells. According to this research, EVOO olive oil is recommended to replace our high-fat oil diet [23]. EVOO can be ingested in liquid form without cooking because it does not harm the stomach and digestive tract and helps prevent ulcers and gastritis [21]. "Liquid gold" is the nickname given to olive oil. A lower risk of cardiovascular and neurological illnesses is associated with its ingestion, which enhances health. Olive oil has naturally occurring compounds with anti-inflammatory and antioxidant properties, making it helpful in treating xerosis, pruritis, and wrinkles [24].

Meilina (2017) found that EVOO reduces MDA levels in male Wistar mice exposed to cigarette smoke. The study also suggests studying other health impacts of EVOO (medical science) and whether chemical content reduces MDA in experimental samples [25]. Thus, more research is needed to investigate how EVOO affects MDA status in male Wistar rats (Rattus norvegicus) and liver histology. These mice were chosen because multiple studies show they are vulnerable to hypercholesterolemia, resistant to therapy, omnivorous, and physiologically comparable to humans compared to rabbits [28-29].

Based on the background described above, this researcher aimed to conduct laboratory experimental research on the effect of extra virgin olive oil (EVOO) in reducing MDA (*malondialdehyde*) levels and liver histopathological features in hypercholesterolemic male Wistar rats (*Rattus norvegicus*).

METHOD

This is a quantitative laboratory experiment with a pre-test and post-test control group design, a control group, and a treatment group with simple randomization [28]. The statistical method of determining the nature and extent of correlations between independent variables is known as true experimental design. When it comes to research designs, this one is among the most reliable and provides strong evidence for the presence of a link. The most

distinguishing feature is the random sampling of a specific population for use in experimental and control groups. Beginning with the Acclamation of Experimental Animals, next comes Dose Calculation, Treatment Process, Phytochemical Tests, and Histopathology Preparations. Additionally, SPSS is utilized to process all data.

Four Wistar rats (*Rattus norvegicus*) groups were randomly assigned to the control and treatment groups. High-fat, high-cholesterol meal made rats hypercholesterolemic, followed by extra virgin olive oil treatment. After treatment, rat liver MDA and histopathology are examined.

October 2023–December 2023 was the research period. The Medanese Herbarium Laboratory, Plant Taxonomy Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, provided male Wistar rats (*Rattus norvegicus*) for experimentation. The Federer formula calculated the sample size: (n-1) (t-1) > 15 [29].

Following World Health Organization guidelines, this study's experimental animal sample will consist of five randomly selected animals for each group [27]. According to calculations, each treatment group had at least six animals. The minimal sample size is 24 people. A total of 24 animals were sampled from 4 treatment groups of 6 animals each.

The criteria for this research animal experiment consisted of inclusion criteria and exclusion criteria. The inclusion criteria for experimental animals in this study were: Healthy condition, no anatomical abnormalities, average rat body weight of 180-200 grams at 12 weeks of age, and cholesterol level < 120 mg/dl. The exclusion criteria included mice who experienced weight loss, mice who experienced diarrhea, and mice who died during the research [30].

Oral administration of 1 and 2 milliliters of Extra Virgin Olive Oil (EVOO) constitutes the independent variable in this study. The dependent variables are male rats' (*Rattus novergicus*) histological pictures and malondialdehyde levels. A high-cholesterol diet, a precondition variable, can cause hypercholesterolemia.

The standard feed for experimental mice is Brailler-II pellets (BR-II), which contain corn, soybean meal, wheat pollard, coconut meal, fish meal, etc. Experimental animals with hypercholesterolemia are fed 4 mL of quail egg yolks and 10% used cooking oil high in fat and cholesterol. Quail egg yolk has 2,139.17mg/100g of cholesterol, more than other foods. Gastric sonde feeds high-fat, high-cholesterol. EVOO and a high-fat, high-cholesterol diet were provided for 14 days to the treatment group.

The tools used in this research, namely the manufacture and observation of the chemical content of EVOO, include a set of maceration tools, filters, rotary evaporators, evaporator cups, and water baths. Equipment for in vitro testing contains 10 ml, 25 ml, 100 ml measuring flasks, test tubes, test tube racks, BioHit 1000µL micropipettes, measuring pipettes, spatulas, vials, incubators, pH meters, cuvettes, centrifuges, centrifuge tubes, UV-spectrophotometers Vis, beaker glass. The material used in this research is EVOO, purchased and obtained on the market under a particular brand. The chemicals used include trichloroacetic acid (TCA), Ethanol, and Aquades.

The experimental animals were prepared by adapting 28 12-week-old male Wistar rats (*Rattus norvegicus*) to a standard diet in daily-cleaned cages for seven days. Airflow and light are sufficient at 28-32^{o}C. Weight and cholesterol were basic facts. Then randomly divided into four 6-animal groups. Next, they received high-fat, high-cholesterol chow for 14 days. After weighing and measuring 24 mice's cholesterol (> 120 mg/dl), they were fed EVOO and a high-fat, high-cholesterol diet for 14 days. Post-treatment weight, cholesterol, and MDA were assessed. EVOO and high-fat, high-cholesterol feed are administered via gastric probe or orally, while regular feed and water are ad libitum.

Sanchez et al. proposed that the average daily intake of EVOO is 25-40 ml or 8-70 grams; overconsumption can induce skin allergies, and consumption above 30 ml causes diarrhea (22). You are comparing the body surface area of experimental animals to convert EVOO dosages for mice—rat dose=0.018 human. One gram equals one cc. Thus, EVOO dosage: K- (negative control) = no treatment; P1 = high fat feed without EVOO; P2 = 1 cc/head/day; P3 = 2 cc/head/day, 14 days.

Wistar strains acclimated and fed a high-fat diet were randomly separated into four groups of six animals each and given therapy for 14 days. Negative control group (K-) mice were given standard food for mice and distilled

water/day/head. Treatment Group-1 (P1) mice were given high-fat, high-cholesterol food but no EVOO. Treatment Group-2 (P2) mice were given a high-fat, high-cholesterol diet and 1cc of EVOO, and Treatment Group-3 (P3) mice were given a high-fat, high-cholesterol diet and two cc of EVOO.

The Lee index was used to determine the obesity status of the mice. A Lee index value greater than 300 indicates that the mouse is fat. The rats were measured for height using calipers and weight using scales Ohauss [31].

Then, a phytochemical test was carried out to determine whether compounds could affect MDA levels in the blood of hypercholesterolemic mice. Next, one cc of plasma was placed in a test tube to measure MDA levels. Then, add one cc cold with 20% TCA. Vortexed and centrifuged for 10 minutes at 3000 rpm. Another test tube with two cc of 0.67% TBA received the supernatant. All tubes were placed in a tube rack and put in a 100ºC water bath for 10 minutes. Remove it and chill it in ice water. 1 ml of the reaction product was placed in a cuvette and measured at 530 nm with a spectrophotometer. The screen will display numbers used to calculate MDA levels (nmol/ml).

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Histopathology of Tissue A 5-minute hematoxylin stain and 10-minute running water rinse were performed. After staining with eosin for 2 minutes, the sample was placed in a graded alcohol solution, cleared with sill, and covered with an adhesive-coated cover glass. The livers of mice were stained with red Haematoxylin-Eosin and viewed under a light microscope coupled to a digital camera and OptiLab Viewer 2.2 software. 400-times magnification was used to view the preparations in 5 fields. For rat liver histopathology, one slice of tissue will be prepared from each rat. The tissue will be examined under a 400x microscope with five perspectives. In each field of view, 20 liver cells will be randomly selected and scored using the Roenigk Classification histopathology scoring model. Using the criteria of the Roenigk Histopathological Scoring Model, the average score of liver histopathological alterations in mice locations was calculated. Score +1: Normal, Score +2: Parenchymatous, Score +3: Hydropic, Score +4: Necrosis [32].

Quantitative data analysis, encompassing both descriptive and inferential statistics, was employed in the study. Editing, tabulating, cleaning, and analysis of the existing data follows. Visual aids such as tables and figures illustrate the descriptive analysis. Next, carry out the Shapiro-Wilk normalcy test as the sample size is less than fifty [33]. Data is considered non-normal when the significance level is 0.05 for both the Shapiro-Wilk and Kolmogorov-Smirnov tests. The amount of samples that will be utilized is where the two differ in their use. Parametric statistical tests are used because the data distribution is normal.

When researching whether there are significant variations in group means, ANOVA is a useful analytical tool to test hypotheses. The F-test, often known as the F-count value, is the end product of an analysis of variance (ANOVA). Use one-way ANOVA to compare treatment groups within each, and then LSD to compare groups across treatments; use a 5% confidence level for all calculations. The data processing and display facilities of SPSS are utilized in the analysis.

RESULTS

After the test animals had adjusted to their new environment, the results of the measurements taken of their weight and length are displayed in Table 1. The findings indicate that the mice became obese after ingesting high-fat chow, achieving a Lee index value of 0.300 (31). The Lee index readings and the mice's reduced body weight after treatment with EVOO at various concentrations are consistent.

Table 1. Data Body Average Weight

Group	Body Weight (gr)		Naso-anal Length (mm)		Lee index	
	B	A	B	A	B	A
K-	251	240	205	202	0.30	0.30
P1	250	210	204	202	0.30	0.29

P2	252	201	203	201	0.30	0.28
P3	251	189	202	201	0.30	0.29

Note: B = After a high-fat feed, A= After 14 days of giving EVOO, K- = Negative control group (standard feed + distilled water), P1 = Treatment Group-1 (high-fat high cholesterol feed), P2 = Treatment Group-2 (high-fat high cholesterol feed + EVOO 1cc), P3 = Treatment Group-3 ((high-fat high cholesterol feed + EVOO 2cc).

The mean pre-test MDA level in the control group was 0.14 ± 0.03 nmol/ml, ranging from 0.18 to 0.10 (Table 2). In a 14-day control group fed regular mice chow and distilled water, the mean MDA level reduced to 0.13 ± 0.10 nmol/ml, ranging from 0.10 to 0.17. In treatment group 1, the mean pre-treatment MDA level was 0.73 ± 0.03 nmol/ml, ranging from 0.78 to 0.69. In group 1, MDA levels declined to 0.48 ± 0.10 nmol/ml after 14 days of high-fat, high-cholesterol meal without EVOO, with a maximum value of 0.38 and a low value of 0.67. In treatment group 2, the mean pre-treatment MDA level was 0.22 ± 0.02 nmol/ml, ranging from 0.27 to 0.19. After 14 days of a high-fat, high-cholesterol diet and 1cc EVOO dosage, the mean MDA level in treatment group 2 was reduced to 0.17 ± 0.02 nmol/ml, with a maximum of 0.20 and a low of 0.14. In treatment group 3, the mean pre-treatment MDA level was 0.17 ± 0.02 nmol/ml, ranging from 0.14 to 0.20. After receiving a high-fat, high-cholesterol diet and a 2cc dose of EVOO for 14 days, the mean MDA level in treatment group 3 dropped to 0.08 ± 0.01 nmol/ml, with a maximum of 0.10 and a minimum of 0.05. The average MDA decreased significantly in treatment group 3 after mice were fed high-fat, high-cholesterol chow and given a two-cc dosage of EVOO.

Table 2. MDA Descriptive Analysis Results

Group	Treatment	n	Mean	SD	Max	Min
K -	Before	6	0,14	0,03	0,18	0,10
	After	6	0,13	0,02	0,10	0,17
P-1	Before	6	0,73	0,03	0,78	0,69
	After	6	0,48	0,10	0,38	0,67
P-2	Before	6	0,22	0,02	0,27	0,19
	After	6	0,10	0,01	0,12	0,09
P-3	Before	6	0,17	0,02	0,20	0,14
	After	6	0,08	0,01	0,10	0,05

Unit: nmol/ml

These results prove that EVOO is indeed good for use in food preparation, cosmetics and the pharmaceutical industry. The effect of EVOO consumption on health has long been thought to be due to the glycerol fraction component which is rich in MUFA, especially oleic acid. Oleic acid is claimed to be able to increase plasma HDL levels and can reduce LDL. For this reason, oleic acid is thought to be able to prevent cardiovascular disease, which is the main cause of death in industrialized countries [20]. The control and treatment groups' MDA level data, both pre-and post-treatment, demonstrate statistically significant results with a normal distribution, as shown in Table 3, where the p-value is more than 0.05 [34].

Table 4 displays the outcomes of the homogeneity test conducted using the Levene test. In the column for significance, the probability values are 6.114 and 0.399. It can be inferred that the negative control group, treatment group-1, treatment group-2, and treatment group-3 are either wholly homogeneous or their respective populations have the same variance, as the resulting significant probability value is greater than 0.05 (34).

Table 4. Data Homogeneity Test Results

MDA Result	Levene Statistic	df1	df2	Sig.
Before	.399	3	20	.755
After	6.114	3	20	.004

Note: Before= a high-fat feed, After= 14 days of giving EVOO

We used a one-way analysis of variance (ANOVA) to see whether there was a statistically significant difference in performance between the groups after ensuring the data was normal and homogeneous. The substantial value obtained from the One-Way ANOVA test is 0.000, less than 0.05, as shown in Table 5. From these data, we can infer that the treatment group differs significantly from the control group.

To determine whether groups differ significantly from one another, the findings of The LSD Post Hoc Test are utilized in Table 6. This study's Post Hoc LSD test analysis revealed significance values of 0.000, 0005, and 0.006, all less than 0.05, indicating that the group differs significantly from the others.

Then, flavonoids, such as virgin olive oil extract, were tested. We added 1 gram to a test tube, added strong HCl, and heated for 15 minutes in a water bath. Red/orange indicates flavonoids (flavones, chalcone, aurone). If flavonoids are found, the extract turns red. Third, for the saponin test, 1 gram of virgin

olive oil extract is placed in a test tube with 10 ml of hot water cooled and shaken violently for 10 seconds. Saponin is present if the foam is 1-10 cm high in 10 minutes and does not dissolve after adding one drop of 2 N HCl. This study revealed froth in virgin olive oil extract, indicating saponin.

The fourth tannin test involved 1 gram of virgin olive oil extract in a test tube, 10 mL of hot water, 5 minutes of boiling, and 3-4 drops of FeCl3 added to the filtrate. Catechol tannin is present in blue-green (green-black). At the same time, blue and black indicate tannin. The tannin test findings show a blue-black liquid, indicating tannin. Fifth, the steroid test employed 2 grams of virgin olive oil extract (EVOO). Shake it in a test tube with 2 mL ethyl acetate. The ethyl acetate layer was dropped onto a drop plate to dry. After drying, two drops of acetic acid and one drop of concentrated sulfuric acid were added. Terpenoids are present if they become red or yellow. Steroids are present if they turn green. If the steroid/triterpenoid test turns red, triterpenoids are present.

In the glycoside test, 2 grams of virgin olive oil extract was placed in chloroform, heated for 5 minutes with a water heater while shaking, and added a few drops of Lieberman Burchard. A brown-purple ring indicated positive glycosides. This study's glycoside test findings were not brown-purple, indicating no glycosides.

In phytochemical testing, flavonoids, alkaloids, saponins, steroids, and tannins were found in Extra Virgin Olive Oil (EVOO).

Olive oil has a lot of fat, but it's good fat—monounsaturated, polyunsaturated, oleic acid, and omega-3—so it's not all bad. In addition, polyphenols and flavonoids are antioxidants found in olive oil.

Table 3. Normality Test Results

	Groups	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Result	Control (K-)	.172	6	.200*	.957	6	.798
	Treatment P-1	.171	6	.200*	.967	6	.869
	Treatment P-2	.163	6	.200*	.965	6	.856
	Treatment P-3	.207	6	.200*	.918	6	.492

**. This is a lower bound of the true significance.*
a. Lilliefors Significance Correction

Table 5. One-Way ANOVA Test Results

Result		Groups	Sum of Squares	Df	Mean Squares	F	Sig.
MDA Level	Before	Between Groups	1.235	3	.412	336.396	.000
		Within Groups	.024	20	.001		
		Total	1.260	23			
	After	Between Groups	.665	3	.222	70.236	.000
		Within Groups	.063	20	.003		
		Total	.728	23			

Note: Before = a high-fat feed, After = 14 days of giving EVOO

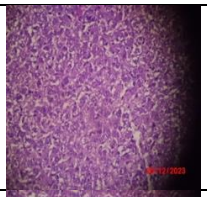
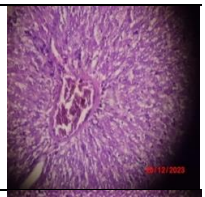
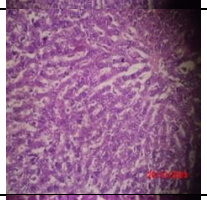
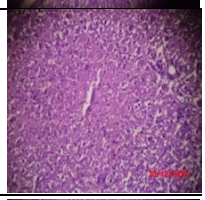
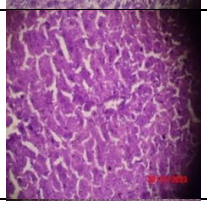
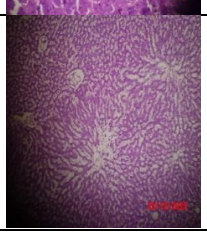
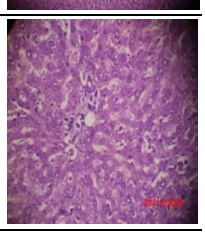
Table 6. LSD Post-Hoc Test Results

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
MDA Results - Before	Control (K-)	P 1	-.59500*	.02020	.000
		P 2	-.14000*	.02020	.000
		P 3	-.11667*	.02020	.000
	Treatment 1 (P1)	K -	.59500*	.02020	.000

		P 2	.45500*	.02020	.000
		P 3	.47833*	.02020	.000
	Treatment 2 (P2)	K -	.14000*	.02020	.000
		P 1	-.45500*	.02020	.000
		P 3	.02333	.02020	.000
	Treatment 3 (P3)	K -	.11667*	.02020	.000
		P 1	-.47833*	.02020	.000
		P 2	-.02333	.02020	.000
MDA Results - After	Control (K-)	K -	-.35333*	.03243	.000
		P 2	.03000	.03243	.006
		P 3	.05500	.03243	.005
	Treatment 1 (P1)	K -	.35333*	.03243	.000
		P 2	.38333*	.03243	.000
		P 3	.40833*	.03243	.000
	Treatment 2 (P2)	K -	-.03000	.03243	.366
		P 1	-.38333*	.03243	.000
		P 3	.02500	.03243	.000
	Treatment 3 (P3)	K -	-.05500	.03243	.005
		P 1	-.40833*	.03243	.000
		P 2	-.02500	.03243	.000

Note: K- = Negative control group (standard feed + distilled water), P1 = Treatment Group-1 (high-fat high cholesterol feed), P2 = Treatment Group-2 (high-fat high cholesterol feed + EVOO 1cc), P3 = Treatment Group-3 ((high-fat high cholesterol feed + EVOO 2cc).

Table 7. Histopathological Results

Groups	Histopathological Image of Fibroblasts	
	Before	After
Control (K-		
Treatment I (P1)		
Treatment II (P2)		
Treatment III (P3)		

Note: K- = Negative control group (standard feed + distilled water), P1 = Treatment Group-1 (high-fat high cholesterol feed), P2 = Treatment Group-2 (high-fat high cholesterol feed + EVOO 1cc), P3 = Treatment Group-3 ((high-fat high cholesterol feed + EVOO 2cc).

Table 7 shows the histopathological examination results under a 400x light microscope. These results found that The presence of fibroblasts might indicate a decrease in MDA. Fibroblast cells were less common and less numerous in the pellet feed control group that did not receive virgin olive extract. Group 1 (P1), which received pelleted feed but did not get virgin olive oil extract, rose in fibroblast count. Fibroblast cell numbers and clustering improved in treatment group 2 (P2), which received pellet feed in addition to 1 cc mg/BW of extra virgin olive oil.

The third treatment group (P3) had the highest density and quantity of animals compared to the other groups. They were given pelleted feed and two cc mg/BW of extra virgin olive. Histopathological examinations revealed that those administered two cc mg/BW of extra virgin olive extract pellets had the densest and most numerous fibroblasts compared to the different groups.

This study's findings corroborate the widespread belief that extra virgin olive oil (EVOO) has numerous positive health effects, including but not limited to: promoting cardiovascular health, lowering cancer risk, alleviating pain and inflammation, strengthening bones, decreasing the likelihood of cardiovascular disease and stroke, and

regulating blood sugar levels and warding off diabetes (14,18,19,24). The histology results show that EVOO aids in the healing of mice liver injury.

DISCUSSION

MDA Levels Before Treatment (Pretest)

Treatment groups 1 (P1), 2 (P2), and 3 (P3) had mean MDA levels ranging from 0.14 ± 0.03 nmol/ml, 0.73 ± 0.03 nmol/ml, and 0.22 ± 0.02 nmol/ml, respectively, in the control group (K-) with hypercholesterolemia. The control and treatment groups' data demonstrated that pretest MDA level data distribution was homogenous and customarily distributed, according to Levene's and Shapiro-Wilk test results, respectively ($p > 0.05$).

Before treatment, the Independent T-test was used to compare the two groups and found no significant change in MDA levels ($p < 0.05$). The mice were subjected to a 14-day diet rich in fat and cholesterol before receiving a therapy designed to enhance free radical production. This would induce oxidative stress, as indicated by elevated blood malondialdehyde (MDA) levels.

During the oxidative stress phase, malondialdehyde (MDA) is produced when cell membranes are damaged by reactive oxygen species (ROS) [37-38].

One byproduct of free radical lipid peroxidation is monohydroxyacetone, or MDA [11-12]. The MDA can characterize free radical activity in cells, making it a biomarker of oxidative stress caused by free radicals [10].

As a biological indicator of lipid peroxidation and a determinant of oxidative stress, monohydroxyacetone (MDA) mediates the formation of this end product [36].

Lipid peroxidation levels affect MDA levels; this, in turn, suggests a high number of free radicals and the presence of an oxidation process in the cell membrane [39-40]. According to Yaman (2021), MDA levels decrease when antioxidant status is high [10].

MDA Levels After Treatment (Posttest)

The control group (K-) had a mean MDA level of 0.13 ± 0.02 nmol/ml after 14 days of distilled water and a high-fat, high-cholesterol diet. The K- K-control group's mean posttest MDA level dropped 0.1 nmol/ml from the pretest. Virgin olive oil extract reduces cell oxidative stress, as shown by lower blood MDA levels over time. In treatment group-1 (P1), MDA levels decreased by 0.25 nmol/ml after receiving extra virgin olive oil and a high-fat, high-cholesterol diet (0.48 ± 0.10 nmol/ml).

This suggests that long-term hypercholesterolemic mice will continue to lower cell oxidative stress, as seen by their more considerable blood MDA reduction than other treatment groups. Treatment groups 2 (P2) and 3 (P3) decreased 0.12 and 0.09 nmol/ml, respectively. The reduction in MDA levels is more significant in mice with hypercholesterolemia without EVOO. Reducing MDA levels reduces oxidative stress, which damages liver cells and membranes and protects SOD enzyme activity [41-42].

For pretest MDA level data in the control and treatment groups, the Shapiro-Wilk and Levene's tests demonstrated that the distribution was normally distributed and homogeneous ($p > 0.05$). The pretest and posttest findings showed that male mice given one cc (1g/kgBW) oral extra virgin olive oil (EVOO) lowered MDA levels but not to normal levels. A 2 cc (1g/kgBW) oral dosage can reach normal MDA levels. The typical MDA level is 0.11 ± 0.09 nmol/ml.

From phytochemical testing, extra virgin olive oil (EVOO) has a high polyphenol concentration. Polyphenols are antioxidant, anti-inflammatory, and anticoagulant [15]. Additionally, EVOO contains antioxidants such as phenolic compounds, tocopherol, squalene, chlorophyll, and β-carotene. EVOO usually consumes 25-40 ml or 8-70 grams daily, causing skin reactions and diarrhea above 30 ml (22). The typical laxative dose is 30 cc of EVOO. EVOO contains antioxidants such as phenolic compounds, tocopherol, squalene, pigment, and β-carotene [19]. This antioxidant prevents free radical-induced cell damage among the non-enzymatic antioxidants that suppress reactive oxygen production [41].

The lack of knowledge about which antioxidant reduces MDA levels weakens this study. This research shows that mice with high cholesterol are more susceptible to free radicals than those with cholesterol but consume EVOO, an exceptionally healthy mouse, as demonstrated by the MDA level test. MDA is a modulator of lipid peroxidation's end product and a biological indicator of oxidative stress [36]. Lipid peroxidation affects MDA levels, indicating many free radicals [25].

CONCLUSION

Consistent with earlier studies, the current investigation shows that Virgin Olive Oil Extract effectively lowers MDA levels in male Wistar white rats (Rattus norvegicus) given a high-fat diet and hypercholesterolemia. According to the results of the phytochemical studies, Extra Virgin Olive Oil (EVOO) contains secondary metabolite chemicals such as tannins, steroids, flavonoids, alkaloids, and saponins.

Hypercholesterolemia is a condition in which Wistar white rats (Rattus norvegicus) consume a high-fat diet; these chemicals are known to lower MDA levels and body weight in the blood serum of these rats.

In light of the findings of previous studies, this article proposes many human subjects for future research into the effects of extra virgin olive oil on lowering malondialdehyde levels and the amounts of healthy compounds found in this oil—oils like Vitamins, Calories, Fat, and Sodium.

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