Original Article

# The Effect of Different Levels of Flaxseed Oil on Biochemical Changes in Hypercholesterolemic Rats

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## Abstract

Flaxseed oil has various benefits over multiple chronic diseases in humans and animals. Besides, flaxseed oil has shown the potential to avert lipid disorders. However, various researches have indicated that lower serum cholesterol in animals and human beings can be attributed to whole flaxseed oil. Therefore, this study aimed at determining the effect of different levels of flaxseed oil on biochemical changes in hypercholesterolemic rats. In this study, (24) albino mice were used and divided into two main groups. The first was a group of mice infected with hypercholesterolemia, the second negative control group was a group of non-infected mice. They were then divided into four groups with two of them given different concentrations of flaxseed oil (20% and 25%). Of the other two groups, one positive infected and another uninfected negative control group were not kept on the experimental diet meaning that all mice were divided into 4 groups consisting of 6 mice in each group. The result showed that non-significant differences in HDL levels between 20%, 25% flaxseed oil and control negative when compared with control positive Also, there were significant decreases in ALP between the groups replaced with different percentage of flaxseed oil compared with control (+) group. The results suggest to use different levels of flaxseed oil for hypercholesterolmic patients, also suggests future studies may be to evaluate the efficacy and advantage of using flaxseed oil as extracts.

**Keywords:** Flaxseed oil, Biochemical changes, Hypercholesterolemic, Therapeutic value

## Introduction

Nowadays, the demand for people and animals to use flaxseeds in food and beverages has increased and gained dramatically attention. Flaxseed oil is one of the most important vegetable oils because of its rich content of polyunsaturated fatty acids (PUFA), especially omega-6 and omega-3 fatty acids, lignans (phytoestrogens), soluble antioxidants, and lignin (fiber) that are known to promote cardiovascular health [1, 2]. Flaxseed oil was known to increase the blood lipid and tissue sensitivity to insulin and aid to suppress allergic and inflammatory reactions [3]. Moreover, its highly health-promoting effect is mainly due to the presence of carotenoids and phenolic acids having an antioxidant role and phytosterols with their anti-sclerotic effect [4]. Other studies have indicated that flaxseed can modestly lower scrum total and low-density lipoprotein cholesterol concentrations, lower postprandial glucose absorption, reduce some markers of inflammation, and increase serum levels of the omega-3 [5]. It has been demonstrated that substitution of shortening with flaxseed oil in the level 0-50 % induces an increase in spread ratio, thickness, diameter, weights, and breaking strength of cookies [6]. Also, in comparison to soybean, flaxseed oil was found to improve the blood hematological parameters and lipid profile inducing an effective effect by lowering the severity of oxidative stress and stimulating antioxidant defense mechanisms. The abject toxicity and curative potential of flaxseed oil have been studied. [7] demonstrates the protective potent effect of flaxseed oil against renal toxicity inducing adjustment of histopathological and biochemical parameters It has also been reported that consumption of 1,000 mg/kg of flaxseed oil in rats limits the renal cytotoxicity and does not affect the toxicity [8]. Mohamed M. Aly-Aldin *et al*. (2015) showed that the effects of replacing corn oil content (10%) in the standard diet of hypercholesterolemic rats with flaxseed oil at different levels resulted in significantly improved lipid profiles, liver and kidney functions, and glucose levels in hypercholesterolemic rats.

Based on the above facts, this study aims to look into the influence of different flaxseed oil levels on biochemical changes in hypercholesterolemic rats.

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**How to cite this article:** Alzahrani MSH. The Effect of Different Levels of Flaxseed Oil on Biochemical Changes in Hypercholesterolemic Rats. Arch Pharm Pract. 2022;13(2):88-93. https://doi.org/10.51847/G9077KTvQM

## Materials and Methods

### Chemicals and Reagents

Cholesterol was acquired as a pure white crystalline powder from a Biogenetic company in Cairo, Egypt.

### Preparation of Flaxseed Oil

(Seeds were ground and packed in cheesecloth from National Research Center then pressed using a laboratory (carver) press). The Agriculture Research Center, Oil Crops Department, Giza, Egypt provided the Flaxseeds (*Linum usitatissimum)* (2019 year production).

### Basal Diet Composition of Tested Rats

The basal diet in the experiment consisted of methionine (0.3 %), vitamin mixture (1 %), casein (10 %), salt mixture (4 %), com oil (10 %), choline chloride (0.2 %), cellulose (5%) and the remainder was corn starch (69.5%) according to Campbell (1963) [9].

### Preparation of Hypercholesterolemic Rats

Normal rats were fed a special diet for inducing hypercholesterolemia, the diet was prepared from fine ingredients per 100 g [10]. Diet had the following composition: salt mixture 4%; Fat 10% (corn oil 10%); sucrose 10%; choline chloride 0.2%; vitamin mixture 1%; cholesterol powder 1.5% (obtained from Morgan Co. Cairo, Egypt) and neutral casein (obtained from Morgan Co. Cairo, Egypt) 16.28g (protein content 12%), corn starch up to 100.

### Animals

In this study, 24 white male (adult) albino rats weighing approximately 150 ± 10 g were used. The mice were housed in wire cages under standard laboratory conditions and as an adjustment period, they were fed a basal diet for one week. To prevent food loss or contamination, rats were given water through a glass tube protruding from the wire cage from an inverted bottle on one side of the cage, which was placed in a non-spilling feeding cup.

### Experimental Groups

A random division of the rats into 4 groups each of 6 rats with the groups being labeled as:

- Group (1): Control negative group, in which the normal rats fed on a basal diet (control "-").
- Group (2): Hypercholesterolemic, control positive group, in which injected rats fed on a basal diet (control "+").
- Group (3): Hypercholesterolemic group fed on basal diet + 20% flaxseed oil.
- Group (4): Hypercholesterolemic group fed on basal diet + 25% flaxseed oil

### Biological Evaluation

During the 28-day test period, food intake was recorded daily and body weight was recorded weekly. Weight gain (B.W.G.), feed efficiency ratio (F.E.R.), and visceral weight were all determined by: [11].

### Blood Sampling

Blood samples were collected after fasting for 12 hours at the end of the experiment. Blood was collected in a dry, clean centrifuge tube using a reverse orbital method using a microcapillary glass tube and coagulated in a room temperature water bath (37°C) for 30 minutes. Blood was centrifuged at 3000 rpm for 10 minutes. Some of it was subjected to glucose measurement to separate the serum while the rest was aspirated carefully, transferred to a clean custom-fit plastic tube, and cryopreserved until analysis (-20°C). Organs (liver, spleen, heart, and kidney) were removed, washed with saline, weighed, and stored in formalin solution (10%) as per the procedures.

### Biological Evaluation

% Body weight gain (BWG %), food intake (consumption), and feed efficiency ratio (FER) were assessed as per [11]. This equation was employed.

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100 \quad (1)$$

$$FER = \frac{Gain\ in\ body\ weight\ (g/day)}{Food\ Intake\ (g/day)} \quad (2)$$

$$\text{Relative organ weight} = \frac{\text{Organs weight}}{\text{Animal body weight}} \times 100 \quad (3)$$

### Organs Weight

Each rat was rapidly opened, the organs (spleen, lungs, kidney, heart, liver, and brain) were removed, washed in saline solution, weighed, and stored in formalin solution (10% V/V) immediately after taking retro-orbital blood samples as per the stipulations, and compared to control group.

### Biochemical Analysis

- chemical kits as per [12] were used to evaluate serum glucose.
- A calorimetric enzyme determination of Triglycerides was performed concurring with Fassati and Prencipe (1982) [13] while the total levels of cholesterol were determined according to Allain, (1974) [14].
- HDL-cholesterol: All lipoproteins were selectively precipitated by the magnesium ions and phosphotungstic acid except the HDL fraction-cholesterol present in the supernatant analyzed by a similar procedure as the total cholesterol, ascribed to Lopez, (1977) [15].
- V-LDL and LDL- cholesterol: The evaluation of LDL and VLDL (very low-density lipoproteins) was carried out according to the method of Lee *et al*., (2008).
- The procedures of Tietz, (1976) were employed in the assessment of Alanine transferase (ALT) [16].

- The method of Henry (1974) [17] and Yound (1975) was employed in the assessment of Aspartate Transferase (AST).
- Henry's, (1974) kinetic method was used to determine creatinine [17].
- Patton and Crouch's, (1977) enzymatic method was used to evaluate urea [18].

### *Statistical Analysis*

The Analysis of variance (ANOVA), one-way classification, and the least significant difference (LSD) methods were employed in the calculation of statistics.

## Results and Discussion

This study aims to look into the influence of different flaxseed oil levels on biochemical changes in hypercholesterolemic rats.

1. Effect of different flaxseed oil levels on body weight gain, feed-efficiency ratio, and feed intake.

Data in **Table 1** indicates body weight gain in both normal and hypercholesterolemic treated rats after 4 weeks of feeding. Results showed flaxseed oil at 20% was not significantly different ($P \geq 0.05$) from flaxseed oil at 25% in reducing body weight gain. It has been reported that rats fed high cholesterol diet exhibited a significant increase in body weight inducing clinical secondary complications, also the result of food intake showed non-significant variation ($P \geq 0.05$) with all groups of flaxseed oil and positive control when compared with negative control. while results on feed efficiency ratio (FER) showed no significant ($P>0.05$) difference between groups (20% and 25%) flaxseed oil when compared with control negative.

**Table 1.** Effect of different flaxseed oil levels on body weight gain, feed-efficiency ratio, and feed intake

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | Sig | LSD |
|---|---|---|---|---|---|---|
| Body weight gain | 52.2 ± 11.43 [a] | 21 ± 8 [b] | 55.6 ± 10.64 [a] | 52.2 ± 7.05 [a] | * | 12.674 |
| Food intake | 478.8 ± 31.86 [b] | 544.2 ± 32.19 [a] | 516.6 ± 17.1 [ab] | 562.8 ± 38.9 [a] | * | 41.635 |
| Feed efficiency ratio | 0.194 ± 0.03 [a] | 0.09 ± 0.04 [b] | 0.198 ± 0.05 [a] | 0.21 ± 0.04 [a] | * | 0.055 |

Values denote arithmetic averages ± Standard error of the average.
Averages with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

2. Effect of various flaxseed oil levels on Relative Weight of some organs.

After 4 weeks of feeding, the summarized results in **Table 2** showed the effect of level flaxseed oil on organ weight/body weight in both normal and hypercholesterolemic treated rats.

Results outlined no significant ($P>0.05$) difference between all groups in relative lung, heart, kidney, and spleen weight. On the other hand, the liver weight variation in the normal rats' group and hypercholesterolemic treated rats groups fed a diet (positive control) significantly reduced ($P<0.05$) as compared to flaxseed oil at 20% and 25%, respectively.

**Table 2.** Effect of different flaxseed oil levels on Relative Weight of some organs

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | Sig | LSD |
|---|---|---|---|---|---|---|
| LUNG | 0.55 + 0.08 a | 0.54 a+ 0.05 | 0.58 a + 0.11 | 0. 56 a + 0.11 | NS | 0.092 |
| LIVER | 2.75 + 0.37 b | 2.48 b+ 0.2 | 3.008 a + 0.23 | 3.12 a + 0.46 | * | 0.288 |
| HEART | 0.32 + 0.01 b | 0.29 b+ 0.03 | 0.29 b + 0.01 | 0.286 b + 0.03 | * | 0.031 |
| KIDNEY | 0.65 + 0.05 a | 0.59 a+ 0.05 | 0.56 a + 0. 02 | 0.52 a+ 0.07 | NS | 0.065 |
| SPLEEN | 0.45 + 0.01 a | 0.48 a+ 0.16 | 0.55 a+ 0.04 | 0.47 a+ 0.12 | NS | 0.134 |

Values denote arithmetic averages ± Standard error of the average. averages with different letters (a, b, c, d) in the same column differ significantly at $p \leq 0.05$ using one way ANOVA test, while those with similar letters are non-significant.

3. Effect of different flaxseed oil levels on Serum Lipids profile and atherogenic index

**Table 3** represents the effect of feeding different levels of flaxseed oil on T. Lipids, PH. Lipids, and T-Cholesterol, in both Hypercholesterolemic and normal treated rats after 4 weeks of feeding.

- Total Lipids and Ph. Lipids in **Table 3** concerning serum total Lipids the results showed that all groups were significantly more ($P<0.05$) when compared with the control negative. Also, Ph. Lipids showed that rats fed on 25% flaxseed oil were non-significant ($P<0.05$) when contrasted to control negative.
- Cholesterol values in the normal rate group were 81.6±5.13mmol/L, while in Hypercholesterolemic treated rats groups kept on the diet with various flaxseed oil levels were 119.6±2.19 mg/dl for positive control, 90.4±6.99 mg/dl for 20%, and 84.8±30.3 mg/dl for 25% of flaxseed oil, respectively. These results are in

agreement with Yari *et al*., (2021) indicating that a reduction in serum triglyceride, a risk factor for cardiovascular disease in hemodialysis patients, concentration can be realized through daily consumption of 6 g of flaxseed oil without affecting other lipid parameters, especially lipoprotein (a) [19].

**Table 3.** Effect of different flaxseed oil levels on Serum Lipids profile and Atherogenic index

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | sig | LSD |
|---|---|---|---|---|---|---|
| T. LIPIDS | 237.6 +10.5 c | 318.8 + 3.19 a | 260.2 + 1.79 b | 243.8 + 2.88 c | * | 7.669 |
| PH.LIPIDS | 102.2+ 2.17 b | 111 + 2.83 a | 102.2 + 2.49 b | 101 + 1.41 b | * | 3.065 |
| CHOLESTEROL | 81.6 + 5.13 c | 119.6 + 2.19 a | 90.4 + 6.99 b | 84.8 + 3.03 bc | * | 6.327 |

Values denote arithmetic averages ± Standard error of the average. Averages with different letters (a, b, c, d) in the same column differ significantly at p≤0.05 using one way ANOVA test, while those with similar letters are non-significant.

4. Effect of different flaxseed oil levels on T.G, HDL, LDL, and VLDL

In both normal and liver disorder rats, the effect of 4 weeks of feeding different levels of flaxseed oil on T.G, HDL, LDL, and VLDL is shown in **Table 4**.

T.G in the normal rat group was (53.2±5.85) mg/dl. While Hypercholesterolemic treated rats groups fed supplement diet at different levels (positive control, 20%, and 25% flaxseed oil) showed T.G values (91.4±5.13, 60.4± 3.58, and 56.2±2.68) mg/dl respectively.

- The HDL value in the normal rat group was (46±1.41) mmol/L. While in Hypercholesterolemic treated rats groups fed a diet with different levels of flaxseed oil were (41.6±0.89, 44.6±0.89, and 44.4±.55) mg/dl at the respective levels (positive control, 20%, and 25%)mg/dl. the result showed nonsignificant differences between 20%,25% flaxseed oil, and control negative when compared with control positive.
- The LDL value in the normal rat group was (25.56±3.24) mg/dl. While in Hypercholesterolemic treated rats groups fed a diet with different levels of flaxseed oil were (61.4±3.88, 36.28±3.8, and 25.56±2.27) mg/dl at levels (positive control, 20%, and 25%)mg/dl, respectively. groups of 20% and 25% flaxseed oil high significantly (P<0.05) when compared with control negative.
- VLDL values in the normal rate group were (10.84±1.17) mmol/L. While in Hypercholesterolemic treated rats groups fed a diet with different levels of flaxseed oil were (18.28±1.03,12.08±0.72, and 11.24±0.54) mg/dl at levels (positive control, 20%, and 25%)mg/dl, respectively. The outcome indicated insignificant differences between 20%, 25%, and control negative when compared with control positive

The current results are in good agreement with Vijaimohan *et al.*, (2016) [20]. Concerning the LDL-C / HDL-C ratio, the value of the LDL-C / HDL-C ratio of the hyperlipidemic group (2.58 mg/dl) was significantly higher than all groups. In addition to that, the control (-) group was no significantly ($p > 0.05$) compared with rats fed a diet containing 75% flaxseed oil. There were significant ($p < 0.05$) differences between all groups replaced with flaxseed oil (25, 50, and 75%) groups. Our results are in good agreement with those obtained by [21] reported that LDL/HDL ratio can be used as a predictive parameter of in vivo LDL oxidation. A significant increase in TC / DHL-C and LDL-C / HDL-C ratios was observed in high fat-fed rats which affect cardiovascular diseases. Flaxseed oil may have inhibited the apolipoprotein, (3-synthesis or increased its catabolism which explains the reduction of these ratios in rats. The diet rich in ALA caused a substantial reduction in several proatherogenic factors, such as TC, LDL-C, and LDL-C / HDL-C.

**Table 4.** Effect of different flaxseed oil level on T.G, HDL, LDL and VLDL

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | Sig | LSD |
|---|---|---|---|---|---|---|
| T.G | 53.2 + 5.85 b | 91.4 + 5.13 a | 60.4 + 3.58 b | 56.2 + 2.68 b | * | 6.015 |
| HDL | 46 + 1.41 a | 41.6 + 0.89 b | 44.6 + 0.89 a | 44.4 + 0.55 a | * | 1.323 |
| LDL | 25.56 + 3.24 c | 61.4 + 3.88 a | 36.28 + 3.8 b | 25.56 + 2.27 c | * | 4.501 |
| VLDL | 10.84 + 1.17 b | 18.28 + 1.03 a | 12.08 + 0.72 b | 11.24 + 0.54 b | * | 1.203 |

Values denote arithmetic means ± Standard error of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05 using one way ANOVA test, while those with similar letters are non-significant.

5. Effect of different flaxseed oil levels on Some renal Functions

**Table 5** reflects the effect of different levels of flaxseed oil on creatinine, urea and uric acid values in both normal and Hypercholesterolemic treated rats fed diet with different

levels of flaxseed oil. urea the results revealed that insignificant between (20% and 25% flaxseed oil when compared with control negative. Accorded to the same table normal rats group recorded serum while creatinine level showed that significantly higher (P<0.05) 25% flaxseed oil when compared with control negative.

- Also in the same table normal rats group recorded U.acid levels were insignificant among all groups. The current results in a good agreement with Abdulkader and Shaikh, (2018) [22]. They aimed to evaluate the effects of flaxseed oil on thioacetamide-induced nephrotoxicity in male rats. The animals were divided into 4 groups. The first group of mice served as a control. The second group of mice was exposed to thioacetamide. The third group of mice was given flaxseed oil and thioacetamide. The fourth group of mice was given flaxseed oil. After 3 weeks, a significant increase in serum creatinine and uric acid was observed in the TAA-treated rats. In the thioacetamide group, serum creatinine, blood urea nitrogen, and uric acid levels increased significantly after 6 weeks. Histopathologically, severe structural changes in renal corpuscles were seen in the thioacetamide-treated rats' renal sections, including the degeneration of the glomeruli and Bowman's cyst. Administration of flaxseed oil protected the biochemical and histopathological changes observed by thioacetamide exposure. The results of this study, therefore, suggest that flaxseed oil protects against thioacetamide-induced kidney damage and that the protective effect of flaxseed oil may be due to its antioxidant activity.

**Table 5.** Effect of different flaxseed oil level on Some renal Functions

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | Sig | LSD |
|---|---|---|---|---|---|---|
| UREA | 27.6 + 4.77 a | 28.8 + 0.45 a | 23 + 1.41 b | 22.4 + 0.55 b | * | 3.372 |
| CREATININ | 0.598 + 0.07 b | 0.69 + 0.03 a | 0.672 + 0.02 a | 0.64 + 0.05 ab | * | 0.060 |
| U.ACID | 1.68 + 0.39 a | 2.26 +.14 a | 1.56 + 0.09 a | 1.44 + 0.09 a | NS | 0.316 |

Values denote arithmetic averages ± Standard error of the average. Averages with different letters (a, b, c, d) in the same column differ significantly at p≤0.05 using one way ANOVA test, while those with similar letters are non-significant.

6. Effect of different flaxseed oil levels on Some Liver Functions

**Table 6** revealed the effect of flaxseed oil on enzyme activity (GOT, GPT, and ALP) in both normal and Hypercholesterolemic treated rats groups. The GOT level was insignificant between 20% and 25% olive oil when compared with the control negative, while the normal rats' group represented ALP level (indicate insignificant differences between all groups. These results are on the same line as those obtained by [23]. Concerning ALT there were significant increases between the control (+) group by mean (31.75 u/L) compared with the control (-) group (19.00 u/L). Also, there were significant decreases between the groups replaced with different percentages of flaxseed oil compared with the control (+) group.

**Table 6.** Effect of different flaxseed oil levels on Some Liver Functions

| Parameters | Control (-) | Control (+) | 20% Flaxseed oil | 25% Flaxseed oil | sig | LSD |
|---|---|---|---|---|---|---|
| S.GOT | 94.8 + 6.61 b | 102.8 + 1.79 a | 95.4 + 3.55 b | 92.6 + 3.97 b | * | 5.825 |
| S.GPT | 42.8 + 3.89 bc | 48.2 + 1.3 a | 44.4 + 1.52 b | 40.4 + 0.89 c | * | 2.998 |
| ALP | 104.6 + 7.96 a | 111.4 + 7.4 a | 113.8 + 3.0 a | 106.2 + 6.26 a | NS | 8.650 |

Values denote arithmetic means ± Standard error of the mean. Means with different letters (a, b, c, d) in the same column differ significantly at p≤0.05 using one way ANOVA test, while those with similar letters are non-significant.

## Conclusion

The results showed that flaxseed oil has a strong effect in decreasing the cholesterol of hypercholesterolemic rats and the improvement rate increased in the group containing 20% flaxseed oil, because it contains sterol and phenolic acids which consider antioxidant that might increase antioxidant enzyme activities such as superoxide dismutase, catalase, and glutathione peroxidase which improves heart function very clearly.

### *Recommendations*

1. Different levels of flaxseed oil should be utilized in the remedy for hypercholesterolemic patients.
2. To lower LDL and atherogenic index levels various levels of flaxseed oil may be suggested.
3. More research should be carried out to determine the efficacy and advantage of using flaxseed oil as extracts.

ACKNOWLEDGMENTS: None
CONFLICT OF INTEREST: None
FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

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