Journal of Medical and Pharmaceutical Sciences
Issue (1), Volume (2)
March 2018
ISSN: 2522-333X

المجلة العربية للعلوم و نشر الأبحاث
Arab Journal of Sciences & Research Publishing
AJSRP

# Modulate hypercholesterolemia as a metabolic disturbance induced oxidative stress injury by functional effects of some mix plant seeds in experimental rats

**Nabila Y. Mahmoud**

Associate professor || Department of Nutrition and Food Science || Faculty of Home Economics || Al Azhar University || Egypt
Associate professor || Department of Food Science and Nutrition || Faculty of Science || Taif University || Taif || KSA

**ABSTRACT**: The moderated effect of flax, sunflower and pumpkin seeds are common used as edible seeds in human nutrition. The study focused on flax/sunflower/pumpkin seeds mix powder on rat suffered from hypercholesterolemia. The feed intake, body weight gain, feed efficiency ratio, relative liver and heart weight, lipid profile, liver enzymes, kidney function, oxidative stress markers, lipid peroxide levels and heart histopathology for normal and hypercholesterolemic rats were examined. Main first group (6 rats) was fed on basal diet for 12 weeks. Main second group (24 rats) fed on diet with hypercholesterolemia for 8 weeks, then diet supplemented with mix seeds powder 3, 6 and 9%, respectively for successive 4 weeks. The results clearly demonstrate that dietary treatment with mix flax /sunflower /pumpkin seeds (30 g; 60 g and 90 g) for 4 weeks has a powerful modulating effect to improve biological evaluation, serum blood levels and heart tissues changes of hypercholesterolemic rats then, this mix seeds have the potential in reducing cardiovascular diseases complications due to hypercholesterolemia.

**Keywords:** hypercholesterolemia, lipid profile, enzymatic antioxidants, male albino rats, heart tissues.

## INTRODUCTION

Cholesterol is an essential part of every cell in human body. It is necessary to form new cells and to repair older cells after injury. Also, Cholesterol is used to form hormones by adrenal glands such as cortisol, to form testosterone by testicles, and to form estrogen and progesterone by the ovaries. Triglycerides supply our bodies' energy. Triglycerides meet immediate energy needs in muscles or stored as fat for future energy requirements. Elevated serum cholesterol levels are a major risk factor for coronary artery disease and elevated triglyceride levels are a milder risk factor. Lipoproteins are the large particles of cholesterol, triglycerides, and proteins found in blood. Cholesterol and triglycerides are non-soluble fats that found in the blood [1]. As part of lipoproteins cholesterol and triglycerides can be carried around in bloodstream throughout the body. The major lipoproteins in blood are low density lipoproteins (LDL), very low density lipoproteins (VLDL), and high density lipoproteins (HDL) [1, 2]. Hypercholesterolemia is a very important problem faced many societies and is a cause of health professionals concern. It constitutes one as the major risk factors for atherosclerosis and its complications, acute infarctation of the myocardium or hypertension as the development of cardiovascular diseases [3]. In addition, there is a

close correlation between these diseases and lipid abnormalities, especially high level of cholesterol, and blood pressure [4]. Plant seeds have gained interest for their health benefits due to their rich content of nutrients and fatty acids. Due to lack of enzymes essential for desaturation at carbon atoms 3 and 6, the n-3 and n-6 families cannot be produced in the body. Thus, the parent fatty acids in these families (linolenic acid [18:3 n-3] and linoleic acid [18:2 n-6], respectively) are essential and can be derived from the diet only. Essential fatty acids (EFA) and their derivatives played the major role in lipid metabolism, platelet functions, immune system, inflammatory response, and epidermal functions [5]. Dietary essential fatty acids (EFA), linoleic acid and alpha-linolenic acid are converted to long-chain polyunsaturated fatty acids (LCPUFAs) by desaturase and chain-elongation enzyme systems [6]. Phospholipids, cholesterols, saturated fatty acids and monounsaturated fatty acids can be synthesized within the human body.

Because mammals cannot introduce a double bond beyond the delta-9 position in the fatty acid chain, linoleic (n-6) and linolenic acid (n-3) must be ingested in foods [7]. Use of plant-based n-3 fatty acid to be alternative to fish consummation may be important for maintaining optimal eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids status in plasma and cell membranes. Flaxseed is an excellent source of EFA, dietary fiber and contains protein and several micronutrients. Flax/sunflower/pumpkin seeds are considerably different in the PUFA content, with a high alphalinolenic acid (ALA) n-3 level and high linoleic acid (LA) n-6 content [8]. The ideal ratio of LA n-6 and ALA n-3 in diet is not known, but ratio of 1 : 1-2 has been considered beneficial to health with effects on cell membrane fluidity and membrane function [9]. The balance required in the diet between n-6 and n-3 fatty acid is important due to their competitive nature and their different biological roles [10].

Sunflower seeds has gained importance due to increased content of nutrients especially, oleic and linoleic acid that may help diminishing the cholesterol leading to reduction in heart diseases [11]. Phytosterols protect the body from inflammation and tumors by neutralizing free radicals and avoiding oxidative stress injury to cells. Vitamin E has a positive effect role on coronary system of the body and hence, reduces stroke and atherosclerosis [12]. Seeds folic acid helps in the formation of blood and nucleic acids. Sunflower seeds can be resolved mental stress and uneasiness by choline and tryptophan. Minerals like magnesium, zinc and selenium have antioxidant actions that improve the immune system and other chronic diseases [13]. Pumpkin seeds are rich natural source of LAn-6, oleic acid, and antioxidant vitamins, such as carotenoids and tocopherols [14]. Hence, this study was designed to find the effect of seeds mixture feeding rats, blood biochemical analysis and heart tissue examination in hypercholesterolemic rats.

## MATERIAL AND METHODS

**Diet composition:**

Basal diet was consists of (g/1000g of diet) casein 120; corn starch 729; soybean oil 80; salt mixture50; vitamin mixture 10; cellulose 10 and total calories (kcal) 4118. Composition of hypercholesterolemic diet (g/1000g of diet) were 240 g/kg of casein (85 % protein); soybean oil (250 g/kg ); salt mixture (100 g/kg); vitamins mixture (20 g/kg ); cellulose (80 g/kg); cholesterol 10; coline 0.4; corn starch (299.6 g/kg) [15] and tap water supply was given ad-libitum daily. Cholesterol and cholic acid has been used as pure chemical powder for hypercholesterolemia. They were obtained from Sigma–Aldrich Company (St. Louis, MO, USA) cholesterol product number C8667 Sigma Grade, ≥99% and choline chloride C1879 ≥98%. All seeds were purchased from local seeds market from Cairo city.

**Animals and experimental designs:**

Male albino rats (Sprague Dawley Strain) was weight 140±10 g, aged 8 weeks. Rats were purchased from the laboratory animal house of faculty of science, Cairo University. They were acclimatized for one week to laboratory condition, kept under temperature 20 - 25°C and humidity 55 - 60 % with a 12 h light/ dark cycle. Rats kept as one rat in each metal cage and classified into two main groups. Main fist group: kept as control group (six rats per group), received basal diet. Main second group: twenty four rats were received cholesterol powder 10 g/kg and choline chloride 0.4 g/kg in their diet for successive eight weeks as hypercholesterolemic agent. After these period rats were divided to subgroups. Subgroup (1): positive control fed on basal diet only a model of hypercholesterolemic rats. Subgroup (2): mix seeds powder 30 g /kg diet. Subgroup (3): mix seeds powder 60 g /kg diet. Subgroup (4): mix seeds powder 90 g /kg diet for successive 28 days. Laboratory principles care was followed in accordance with ethics committee's protocols for experimental animals in research Cairo University, Faculty of Science [16].

**Biological Evaluation:**

The quantities of diet which were consumed (feed intake) and wasted were assessed every day. Body weight was recorded twice / week. On the last day of the experimental protocol, rats were fasted overnight and allowed free access to water. Feed intake, body weight gain (BWG %) and feed efficiency ratio (FER) were calculated [17]. Body weight gain and feed efficiency ratio were calculated using the following equations: Feed intake = Initial Weight of diet (g) - Weight of diet lost (g). Weight Gain (WG) (g) = Final Weight (g) – Initial Weight (g). Feed efficiency ratio = Gain in body weight (g)/ Feed intake (g). At the end of the experimental period heart, kidneys and liver were removed carefully from each rat after an abdominal laparotomy, washed with saline solution, dried with filter paper and weighted [18]. Relative organ weight calculated by the following formula: Relative organ weight (ROW) % = Organ Weight / Final Body Weight× 100.

**Blood samples and Biochemical Analysis:**

After the experimental period, all rats were fasted 12 hours then, anaesthetized by diethyl either 60% for100 s. Blood was collected by orbital sinus/plexus bleeding. Blood serum were separated from collected samples then centrifuged for 10 minutes at 3000 revolutions/minute. Serum was carefully separated into dry clean Wasserman tubes by using a Pasteur pipette. Serum was used freshly for determination of biochemical analysis. Kits used to determine biochemical analysis produced by Egyptian American Company for laboratory service and supplied by Alkan Company. Serum cholesterol and triglycerides was measured using spectrophotometric method [19, 20, 21, 22]. HDL-Cholesterol was measured using a spectrophotometric method [23] and LDL-Cholesterol were calculated by Friedwald formula, VLDL = TG/5, LDL = Total Cholesterol – (VLDL+HDL). Concentration represented in mg/dL [24]. Atherogenic index was calculated by using formula = log (TG/HDL-C) [25]. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were determined according to Reitman and Frankel [26] and alkaline phosphatase enzymes (ALP) [27]. Serum urea was performed measured according to Patton and Crouch [28], serum creatinine was determined according to the method described by Kroll et al. [29]. The previous tests were measured by using auto analyzer (UV-1800VIS Spectrophotometer, Shanghai, China (Mainland). Enzymatic antioxidant activity as super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured by high performance liquid chromatography HPLC according to O'Gara et al. [30], lipid peroxidation products were assayed by measuring malondialdehyde (MDA) according to Ohkawa et al. [31].

**Histopathological examination:**

Heart tissues of sacrificed rats were obtained at different developmental phases and fixed in 4% paraformaldehyde phosphate buffered solution. The fixed tissue blocks were prepared and paraffin sections at 6 microns (μm) thickness. Samples were slide from the paraffin-embedded material and serial sections were stained with hematoxylin eosin (Hand E) for light microscopy at x 400 [32].

**Statistical analysis:**

Tables' data was calculated as mean values with their standard deviation (S.D.) of each group. Values were statistically analyzed according to Armitage and Berry [33] by using (SPSS version 20.0; SPSS, Inc.) one-way analysis of variance (ANOVA), P values (0.05) were considered significant.

## RESULTS

**Biological evaluation:**

Mixed seeds of flax/sunflower/pumpkin showed improvement effect on, body weight gain (BWG %), feed efficiency ratio (FER) of rats suffered from hypercholesterolemia. Feed intake recorded significant increases ($p \leq 0.05$) of daily intake in hypercholesterolemic group as compared to normal rats group

(23.73±0.2and 23.35±0.1g/day, respectively). All treated mix seeds recorded significant decreases ($p \leq 0.05$) as compared to hypercholesterolemic group (23.55±0.3, 23.49±0.2 and 23.46±0.1 g/day, respectively). Body weight gain % and FER illustrated significant decreases of hypercholesterolemic group (1.44±0.4 and 0.09±0.2%) as compared to normal group (1.75±0.3 and 0.11±0.4 %). All supplemented diet with mix seeds group revealed to significant increases in BWG% and FER as compared to hypercholesterolemic group. From these results mix seeds recoded the best values of biological evaluation compared to hypercholesterolemic group, which near to normal values as shown in table 1.

**Table 1. Nutritional effect of mixed flax/sunflower/pumpkin seeds on biological evaluation of hypercholesterolemic rats**

| Groups | Feed intake (g/day) | BWG % | FER |
|---|---|---|---|
| **Normal rats** | **23.35±0.1$^{c}$** | **1.75±0.3$^{a}$** | **0.11±0.4$^{ab}$** |
| **Hypercholesterolemic rats** | **23.73±0.2$^{a}$** | **1.44±0.4$^{c}$** | **0.09±0.2$^{c}$** |
| **Mix seeds 3%** | **23.46±0.1$^{c}$** | **1.45±0.8$^{b}$** | **0.102±0.5$^{b}$** |
| **Mix seeds 6%** | **23.49±0.2$^{c}$** | **2.02±0.8$^{b}$** | **0.126±0.4$^{a}$** |
| **Mix seeds 9%** | 23.55±0.3$^{b}$ | 1.62±0.8$^{b}$ | **0.108±0.3$^{ab}$** |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Results in table 2 recorded relative organs weight % of treated and hypercholesterolemic rats. Heart weight % showed non-significant increases ($p \leq 0.05$) of hypercholesterolemic group as compared to normal rats (0.55±0.06 and 0.52 ±0.23%). Supplemented diet with mix seeds non-significant decreases of heart weights % as compared to control groups. Kidneys weight % recorded non-significant decreases ($p \leq 0.05$) of hypercholesterolemic group as compared to normal rats (1.14±0.10 and 1.28 ±0.23 %). Treated groups with different percent of mix seeds showed non-significant decreases of kidneys weight % as compared to control groups. Livers weight % showed significant increases ($p \leq 0.05$) in hypercholesterolemic group (3.51 ±0.32 and 3.25±0.24%) as compared to normal group. Mix seeds percent recorded significant decreases of liver weight % as compared to hypercholesterolemic group.

**Table 2. Nutritional effect of mixed flax/sunflower/pumpkin seeds on relative organs weight % of hypercholesterolemic rats**

| Groups | Heart | Kidney | Liver |
|---|---|---|---|
| **Normal rats** | **0.52 ±0.23$^{ab}$** | **1.28 ±0.23$^{a}$** | **3.25±0.24$^{b}$** |
| **Hypercholesterolemic rats** | **0.55±0.06$^{a}$** | **1.14±0.10$^{ab}$** | **3.51 ±0.32$^{a}$** |
| **Mix seeds 3%** | **0.54±0.10$^{ab}$** | **0.99±0.06$^{b}$** | **2.89±0.34$^{c}$** |
| **Mix seeds 6%** | **0.50±0.04$^{b}$** | **0.96±0.04$^{b}$** | **2.83±0.26$^{c}$** |
| **Mix seeds 9%** | 0.48±0.07$^{b}$ | 0.93±0.07$^{b}$ | **2.64±0.23$^{d}$** |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

**Biochemical analysis**

**Lipid profile**

Total cholesterol (TC) and triglyceride (TG) serum results data in table 3 illustrated significant increases ($p \leq 0.05$) in hypercholesterolemic rats group as compared to normal rats group (258±5. 5, 123±3.1 and 170±4.3, 95±2.4 mg/dL, respectively). Mixed flax/sunflower/pumpkin seeds in all supplemented diets percent recorded significant decreased in TC and TG as compared to hypercholesterolemic rats group. The best results were found in mix seeds 12%, which near to normal rats of triglyceride (94±0.1 mg/dL).

**Table 3. Nutritional effect of mixed flax/sunflower/pumpkin seeds on serum cholesterol and triglyceride of hypercholesterolemic rats**

| Groups | T. Cholesterol mg/dL | Triglyceride mg/dL |
|---|---|---|
| **Normal rats** | **123±3. 1** $^{d}$ | **95±2.4** $^{c}$ |
| **Hypercholesterolemic rats** | **258±5. 5** $^{a}$ | **170±4.3** $^{a}$ |
| **Mix seeds 3%** | **156±2. 3** $^{b}$ | **110±3.3** $^{b}$ |
| **Mix seeds 6%** | **147±2. 4** $^{b}$ | **100±3.1** $^{b}$ |
| **Mix seeds 9%** | 136±3. 2 $^{c}$ | **94±0.1** $^{c}$ |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

Serum LDL-c, VLDL-c and calculated AI data showed significant increases ($p \leq 0.05$) of hypercholesterolemic rats group as compared to normal rats group (190±3.5, 34±1.3, 0.69±0.03, 60 ±2.1, 19±1.4, and 0.33±0.04 ,respectively). All supplemented diets with mix seeds recorded significant decreases of low density lipoproteins and atherogenic index results as compared to hypercholesterolemic rats group. High percent of mix seeds found as good result group, which closed to normal rats data (74.2±2. 2, 18.8±1.2 and 0.34±0.03, respectively) as in table 4.

**Table 4. Nutritional effect of mixed flax/sunflower/pumpkin seeds on serum HDL-c, LDL-c, VLDL-c and atherogenic index of hypercholesterolemic rats**

| Groups | HDL-c mg/dL | LDL-c mg/dL | VLDL-c mg/dL | Atherogenic index mg/dL |
|---|---|---|---|---|
| **Normal rats** | **44±3. 1** $^{d}$ | **60 ±2.1** $^{d}$ | **19±1.4** $^{b}$ | **0.33±0.04** $^{c}$ |
| **Hypercholesterolemic rats** | **34±3. 5** $^{a}$ | **190±3.5** $^{a}$ | **34±1.3** $^{a}$ | **0.69±0.03** $^{a}$ |
| **Mix seeds 3%** | **38±2. 3** $^{b}$ | **96±2.3** $^{b}$ | **22±1.3** $^{b}$ | **0.46±0.02** $^{b}$ |
| **Mix seeds 6%** | **40±2. 4** $^{b}$ | **87±2.4** $^{c}$ | **20±1.1** $^{b}$ | **0.39±0.01** $^{b}$ |
| **Mix seeds 9%** | 43±3. 2 $^{c}$ | 74.2±2. 2 $^{cd}$ | 18.8±1.2 $^{b}$ | **0.34±0.03** $^{c}$ |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

**Liver enzymes:**

Liver enzymes as serum aminotransferases ALT and AST in table 5 illustrated significant increases ($p \leq 0.05$) in hypercholesterolemic rats group (89.3±2.2, 69.5±2.7, 125.85±2.58, 26.8±1.3, 23. 9±2.3 and 92.98±1.32 U/L) as compared to normal rats. All supplemented diet groups revealed to significant decreases of all enzymes especially in high mix group and Arabic gum followed by purslane and cress seeds groups. The process of supplementation diet of treated groups recorded a significant decrease in liver enzymes results, especially in the highest concentration group (27.29±1.5, 25.5±2.2, 106.26±4.24 U/L, respectively) as shown in table 5.

**Table 5. Nutritional effect of mixed flax/sunflower/pumpkin seeds on ALT, AST and ALP enzymes of hypercholesterolemic rats**

| Groups | ALT U/L | AST U/L | ALP U/L |
|---|---|---|---|
| Normal rats | 26.8±1.3$^{d}$ | 23. 9±2.3$^{d}$ | 92.98±1.32$^{a}$ |
| Hypercholesterolemic rats | 89.3±2.2$^{a}$ | 69.5±2.7$^{a}$ | 125.85±2.58$^{c}$ |
| Mix seeds 3% | 47.2±1.6$^{b}$ | 42.6±2.3$^{b}$ | 111.67±3.87$^{b}$ |
| Mix seeds 6% | 35.96±1.4$^{c}$ | 35.9±2.4$^{c}$ | 109.63±1.16$^{b}$ |
| Mix seeds 9% | 27.29±1.5$^{d}$ | 25.5±2.2$^{d}$ | 106.26±4.24$^{b}$ |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

**Kidney function:**

Table 3 revealed to kidney functions as serum urea nitrogen and creatinine results of hypercholesterolemic rats and supplemented diet groups with concentrations mix of flax/sunflower/pumpkin seeds. Urea and creatinine results showed significant increase ($p \leq 0.05$) in hypercholesterolemic group (37±3.85, and 1.22±0.25 mg/dL) as compared to normal rats group (32 ±2.28 and 0.65±0.14 mg/dL). All treated seeds groups' recorded significant decrease of serum urea and creatinine especially in mix seeds 6% group as compared to hypercholesterolemic rats group, which closed to normal data in urea results (31.5±2.4 mg/dL).

**Table 6. Nutritional effect of mixed flax/sunflower/pumpkin seeds on urea and creatinine of hypercholesterolemic rats**

| Groups | Urea mg/dL | Creatinine mg/dL |
|---|---|---|
| Normal rats | 32 ±2.28$^{c}$ | 0.65±0.14$^{e}$ |
| Hypercholesterolemic rats | 37±3.85$^{a}$ | 1.22±0.25$^{a}$ |
| Mix seeds 3% | 35±1.43$^{b}$ | 0.92±0.09$^{b}$ |
| Mix seeds 6% | 33.9±1. 3$^{b}$ | 0.85±0.15$^{c}$ |
| Mix seeds 9% | 31.5±2.4$^{c}$ | 0.77±0.24$^{d}$ |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

**Antioxidants enzymes and lipid peroxidation:**

Serum antioxidants enzymes were expressed as SOD and GSH-Px of rat groups. Data in table 7 illustrated significant decreases of SOD and GSH-Px of hypercholesterolemic rats (460±7.47 mmol/L and 0.33±0.02 µ/mg) as compared to normal rats (988±2.73 mmol/L and 0.98±0.03 µ/mg). Mix seeds groups showed significant increases ($p \leq 0.05$) as compared to hypercholesterolemic rats group. Treated mix seeds 6% groups recorded high significant increases values as compared to hypercholesterolemic group (898±7.90 mmol/L and 0.72±0.05 µ/mg). Lipid peroxidation as serum MDA showed significant increases ($p \leq 0.05$) in hypercholesterolemic group as compared to normal rats (3.67±0.3 and 1.85±0.2 n mol/L). Supplemented diet groups recorded significant decreases values as compared to hypercholesterolemic group, the best results found in mix seeds 3% group followed mix seeds 6% group (2.39±0.3 and 2.28±0.1n mol/L).

**Table7. Nutritional effect of mixed flax/sunflower/pumpkin seeds on enzymatic antioxidants and lipid peroxidation of hypercholesterolemic rats**

| Groups | SOD mmol/L | GSH-Px µ/mg | MDA ( n mol/L) |
|---|---|---|---|
| **Normal rats** | **988±2.73$^{e}$** | **0.98±0.03$^{a}$** | **1.85±0.2$^{d}$** |
| **Hypercholesterolemic rats** | **460±7.47$^{c}$** | **0.33±0.02$^{c}$** | **3.67±0.3$^{a}$** |
| **Mix seeds 3%** | **590±7.43$^{b}$** | **0.49±0.03$^{b}$** | **2.45±0.2$^{b}$** |
| **Mix seeds 6%** | **735±10.45$^{c}$** | **0.53±0.04$^{b}$** | **2.39±0.3$^{bc}$** |
| **Mix seeds 9%** | 898±7.90$^{d}$ | 0.72±0.05$^{b}$ | **2.28±0.1$^{c}$** |

Mean± SD values, means in the column with different letters are significantly different ($p \leq 0.05$).

**Heart histopathological examination:**

Heart tissues of negative rat group showed the normal cardiac myocytes as found in Fig.1. Heart tissues of hypercholesterolemic rat illustrated congestion of myocardial blood vessels as showing in Fig. 2, intermuscular oedema as showing in Fig.3, and intermuscular haemorrhage as showing in Fig.4. Rat hearts of mixed flax/sunflower/pumpkin seeds 3% group showed moderate congestion of myocardial blood vessels as illustrated in Fig. 5. Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group revealed low congestion of myocardial blood vessels in Fig.6, and few intermuscular oedema and haemorrhage in Fig.7. Heart tissues of mixed flax/sunflower/pumpkin seeds 12% group revealed to no histopathological changes in Fig.8.

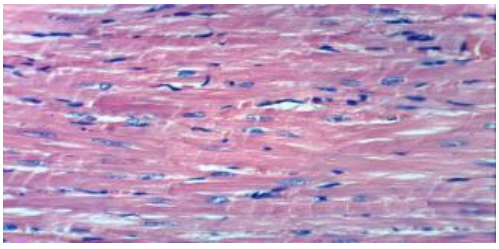


**Fig.1. Cardiac myocytes of negative group.**

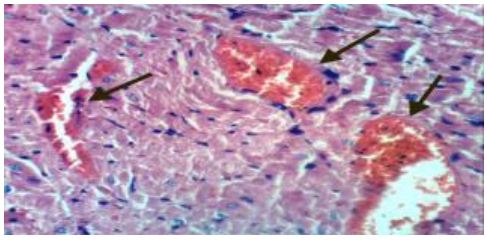
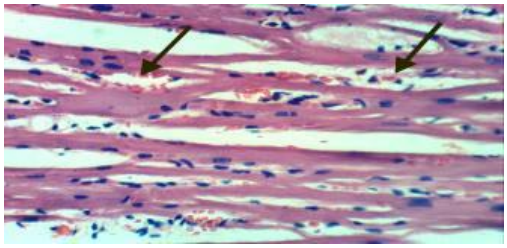
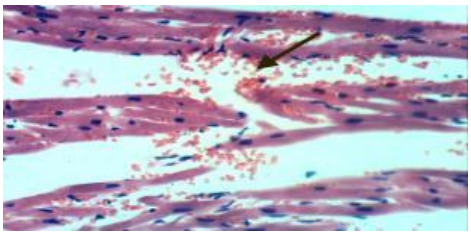
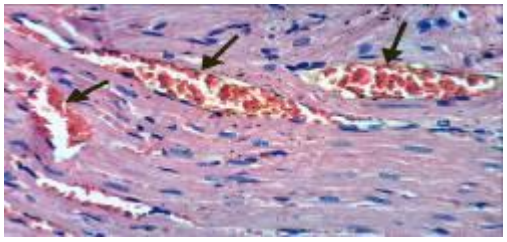
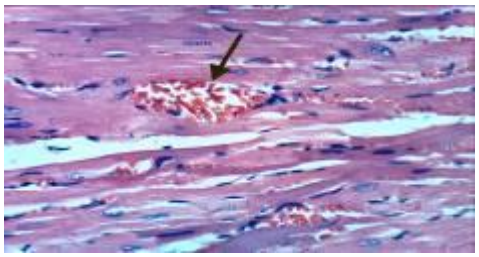
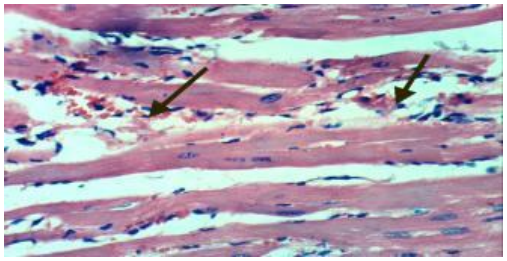
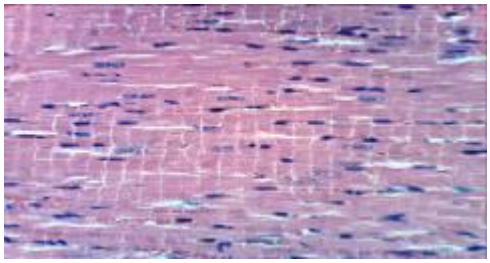


**Fig.2. Heart tissues of hypercholesterolemic**

| | |
|---|---|
| | group. |
|  <br>Fig. 3. Heart tissues of hypercholesterolemic group. |  <br>Fig. 4. Heart tissues of hypercholesterolemic group. |
|  <br>Fig. 5. Heart tissues of mixed flax/sunflower/pumpkin seeds 3% group. |  <br>Fig. 6. Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group. |
|  <br>Fig. 7. Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group. |  <br>Fig. 8. Heart tissues of mixed flax/sunflower/pumpkin seeds 12% group. |

## DISCUSSION

This study was performed to investigate the nutritional effect of flax/sunflower/pumpkin mix seeds on hypercholesterolemia for male albino rats by estimated biological, biochemical and histopathological examinations. Various medicinal plants the play an important role in inhibition of oxidative stress of hypercholesterolemic conditions as a therapeutic approach and efforts have been made to identify the antioxidative functions them [34]. Alteration in oxidative stress induced by reactive oxygen species (ROS) and impairments of the antioxidant system play a critical role in the pathogenesis of many diseases as hypercholesterolemia and subsequent cardiovascular diseases [35]. This results in the research agreement with those who used pure cholesterol to elevate serum or tissue cholesterol levels to examine the etiology of hypercholesterolemia-related metabolic disturbances and antioxidant enzymatic systems [36]. Also, with that results of hypercholesterolemic diet caused significant increases in serum AST activity, liver L-MDA concentration and decreases in liver tissue GSH concentration also, liver SOD activity [37]. In

addition, feeding cholesterol-rich diets forms free radical production (ROS), followed by oxidative stress and hypercholesterolemia [38, 39]. Impairment of liver tissue caused by dyslipidemia may be led to adverse effect by increasing lipid peroxidation which in turn produces damage to liver tissue. After this stage, outflow of liver enzymes from cytosol to the blood stream indicate that inability of liver to metabolize ALT and AST [40]. Administration of 30% flaxseed cake for 90 days improved total plasma antioxidant status and lowered liver thiobarbituric acid reactive substances [41]. Dietary flaxseed may also offer protection against ischemic heart disease by improving vascular relaxation responses and by inhibiting the incidence of ventricular fibrillation [42]. Supplements of 10 g flax seed oil revealed to significant decreases in serum total cholesterol, LDL-cholesterol, HDL-cholesterol [43]. Feeding oils rich in polyunsaturated fatty acids (PUFA) as corn oil and sunflower oil increases lipid peroxidation significantly and thus challenge the antioxidant defense system and may increase the susceptibility of tissues to degradation products of lipid peroxides [44]. Ristic-Medic et al. [7] indicated that dietary milled sesame/pumpkin/flax seed mixture added to a habitual diet lowered triglyceride and improved fatty acid profile and pruritus symptoms in hemodialysis patients.

High phenolic and flavonoid content of sun flower seed (Helianthus annuus L.) revealed to improve cytotoxic and antioxidative potential of sunflower seeds as a chemopreventive agent [45]. Feeding high fat diet increased blood lipids, oxidative stress and lowered the serum antioxidant enzymes activity, liver tissue and uterus ($p < 0.05$). On the other hand, n-3 fatty acids in fish oil, canola oil and sunflower oil prevented the dyslipidemia induced loss of antioxidant enzyme activities in serum, liver and uterus [46]. Many reviews provide the chemists, biologists and researchers on the roles of pumpkin seed oil extracts that possess promising biological activities [47]. Animal's studies found that oxidized oils decreased the whole body weight, which was ameliorated by the co-administration of un-oxidized oils. The levels of serum biochemical parameters were improved by co-administration of pumpkin seed oils [48]. Pumpkin seed protein isolate may be useful for patients suffering from liver diseases due to its hepatoprotective and hypolipidemic activities in animals [49]. Treatment of atherogenic rats with pumpkin seeds significantly decreased serum concentrations of TC and LDL-C [50]. Versari et al. [51] suggests that, hypercholesterolemia and hypertension may favor to development of different functional and structural changes in the early phases of carotid atherosclerosis. Regarding of histopathological examination of heart, our results are agreement with study revealed that a normal histopathological structure in the heart of normal rats group. Whereas, heart of hypercholesterolemic rats group showed sever focal inflammatory cells infiltration in the degenerated myocardial bundles. However, heart of rat from all other groups revealed no histopathological alteration in myocardium [52]. Both preclinical and clinical studies suggested that elevated oxidative and/or nitrative stress plays a key role in cardiac complications induced by hypercholesterolemia. So, modulation of hypercholesterolemia-induced myocardial oxidative/nitrative stress is a practical approach to prevent or treat deleterious cardiac

consequences [36]. In this regard this results can said, the availability of healthy food choices can contribute in enhancing the nutrient profile of foods through reductions in the salt, sugar and saturated fat content; and increasing the content of ω-3fatty acids and other bioactive compounds [53].

## CONCLUSIONS

All previous data can recommended that, flax/sunflower/pumpkin mix seeds have modulation effect of biochemical analysis levels and improve heart tissues changes of rats. Hens, human beings can use flax/sunflower/pumpkin mix seeds to increment high levels of lipid blood levels, liver enzymes, kidney function and enhance heart tissues by their high nutritional content values and functional effects.

## ACKNOWLEDGMENTS

More sincere thanks and gratitude to Immunology and Allergy Unit, Faculty of Medicine, Al Azher University Cairo for their efforts on biochemical analysis and histopathological examination of this research.

## REFERENCES

1. Drummond, K. E. and Brefere, L. M. Nutrition for Foodservice and Culinary Professionals (8 th Ed.). John Wiley & Sons. 2014. ISBN 978-0-470-05242-6.
2. National Heart Foundation of Australia. Cholesterol. ABN 98 008 419 761, 2013. copyright@heartfoundation.org.au. INF-084-C.
3. Gomes, M. R.; Tomaso, H.; Nazarian, G. K.; Bjarnason, H.; Dietz Jr., C. A. and Hunter, D. W. Upper extremity deep vein thrombosis and chronic pulmonary embolism resulting in pulmonary artery hypertension. AJR Am. J. Roentgenol. 1998; 170, 1532-1534.
4. Mahan, L. K. and Scott-Stump. Krause Alimentos, Nutrição e Dietoterapia. São Paulo: ROCA, 1998.
5. Sardesai, V.M. Nutritional role of polyunsaturated fatty acids. Journal of nutrition and biochemistry. 1992; 3:154–166.
6. Wayne, S., Fenton, J.H., and Michael K. Essential Fatty Acids, Lipid Membrane Abnormalities, and the Diagnosis and Treatment of Schizophrenia. Biological Psychiatry journal. 2000; 47: 8–21.
7. Ward, O. P. and Singh, A. Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry Journal. 2005; 40: 3627-3652.
8. Ristic-Medic D., Perunicic-Pekovic G., Rasic-Milutinovic Z., Takic M., Popovic T., Arsic A., and Glibetic M. Effects of dietary milled seed mixture on fatty acid status and inflammatory markers in patients on hemodialysis. The Scientific World Journal. 2014; Article ID 563576, 9 pages. http://dx.doi.org/10.1155/2014/563576.

9. Simopoulos, A. P. Essential fatty acids in health and chronic disease. American Journal of Clinical Nutrition. 1999; 70 (3):560-569.
10. Ristic-Medic, D., G. Ristic, and V. Tepsic. Alfa linolenic acid and cardiovascular disease. Medicinski Pregled 2007; 60(1) 43-47.
11. Chowdhury, L., Banu A., Khan S. and Latif A. Studies on the Fatty Acid Composition of Edible Oil. Bangladesh Journal of Science and Industrial Research. 2007; 42: 311-316.
12. Singh, S. Devaraj and Jialal I. Vitamin E, oxidative stress, and inflammation. Annual Review of Nutrition. 2005; 25:151-174.
13. Nandha, H. Singh, K. Garg and S. Rani. Therapeutic potential of Sunflower seeds. International Journal of Research and Development of Pharmacy. 2014; 3: 967-972.
14. Xanthopoulou, M.N., Nomikos T., Fragopoulou E. and Antonopoulou S. Antioxidant and lipoxygenase inhibitory activities of pumpkin seed extracts. Food Research International. 2009; 42(5-6):641-646.
15. Matos S.L., de Paula H., Pedrosa M.L., dos Santos R.C., Luiz de Oliveira E., Júnior Deoclécio A.C. and Silva M.E. Dietary Models for Inducing Hypercholesterolemia in Rats. Brazilian archives of biology and technology. 2005; 48(2): 203-209.
16. Fahmy S. R. and K Gaafar. Establishing the first institutional animal care and use committee in Egypt. Philosophy, Ethics and Humanities in Medicine. 2016; 11:2, 1-6.
17. Chapman, D.G., R. Castilla and J.A. Campbell. Evaluation of protein in food. I. A method for the determination of protein efficiency ratio. Can J Biochem Physiol. 1959; 37, 679-689.
18. Drury, R.A. and E.A. Wallington. Carton's histological technique. 5th Ed.Oxford University Press, New York, U.S.A 1980.
19. Ellefson, R.D., Caraway, W.T. and Tietz N.W. Fundamentals of clinical chemistry, Ed; P506, 1976.
20. Richmond, W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay for total cholesterol in serum. Clinical Chemistry. 1973; 19:1350-1356.
21. Roesclau P., Bernt E. and Gruber W. Enzymatic determination of total cholesterol in serum. Z kiln Chem Klin Biochem journal. 1974; 12:226-229.
22. Bucolo, G., and David, H. Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry. 1973; 19:476-482.
23. Lopez-Virella, M.F. High density lipoprotein cholesterol by selective precipitin. Clinical chemistry. 1977; 23: 882.
24. Friedewald, W.T. and Levy, R.I. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clinical Chemistry. 1972; 18: 6.
25. Nwagha U.I. Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. African Health Sciences. 2010; 10(3): 248-252.

26. Reitman S. and Frankel S.A. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol., 1957; 28(1), 56-63.
27. King, J. The phosphohydrolases acid and alkaline phosphatases. In: Practical and Clinical Enzymology, Eds., King, J. Van Nostrand Co. Ltd., London 1965; pp. 191-208.
28. Patton C.J. and Crouch S.R. Enzymatic colorimetric method for determination of urea in serum. Anal Chem. 1977; 49: 464-469.
29. Kroll M.H., Roach N.A., Poe B. and Elin R.J. Mechanism of interference with the jaffe reaction for creatinine. Clin Chem. 1987; 33:1129-1132.
30. O'Gara C.Y., Maddipati K.R. and Marnett L. J. A sensitive electrochemical method for quantitative hydroperoxide determination. Chem Res Toxicol. 1989; 2:295-300.
31. Okhawa, H.; Ohishi, N. and Yagi, K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry. 1979; 95: 351–358.
32. Lan Wei Ke-Mei Peng, Huazhen Liu, Hui Song, Yan Wang and Lia Tang. Histological examination of testicular cell development and apoptosis in the ostrich chick. Turk. J. Vet. Anim. Sci. 2011; 35(1): 7-14.
33. Armitage P., Berry G. and J.N.S., Matthews. Statistical methods in medical research, survival analysis. 4th Ed,Wiley-Blackwell Science Inc. 2008; 568-90.
34. Adaramoye, O.A., Akintayo, O., Achem, J. et al. Lipid lowering effects of methanolic extract of vernonia amygdalina leaves in rats fed on high cholesterol diet. Vascular health and Risk Management. 2008; 4(1). 235--241.
35. Hamelet, J., Demluth, K., Paul, J.L. Delabar J.M. and Janel N. Hyperhomocysteinemia due to cystathionine beta synthase deficiency induces dysregulation of genes involved in hepatic lipid homeostasis in mice. J Hepatol. 2007; 46(1). 151-159.
36. Bocan, T.M. Animal models of atherosclerosis and interpretation of drug intervention studies. Curr. Pharm. 1998; 4: 37–52.
37. Csonka C., Sárközy M., Pipicz M., Dux L. and Csont T. Modulation of Hypercholesterolemia-Induced Oxidative/Nitrative Stress in the Heart. Oxidative Medicine and Cellular Longevity. 2016; Article. ID 3863726, 23 pages. http://dx.doi.org/10.1155/2016/3863726.
38. Hussein S.A., El Senosi Y.A.F., Hassanien M.R.R. and Hammad M.M.F. Ameliorative Effect of Curcumin on Hepatic Oxidative Stress, Antioxidant Status, Cardiac Markers Enzymes and Inflammation in High Cholesterol Diet- Induced Hypercholesterolemia in Rats. Int J Pharma Sci. 2016; 6(3): 1496-1505.
39. Bulur, H., Ozdemirler, G., Oz, B. et al. High cholesterol diet supplemented with sunflower seed oil but not olive oil stimulates lipid peroxidation in plasma, liver, and aorta of rats. J. Nutr. Biochem. 1995; 6: 547–550.
40. Naik, S., Thakare, V. and Patil, S. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. Exp Toxicol Pathol. 2011; 63:419–431.

41. Matusiewicz M., Kosieradzka I., Zuk M. and Szopa J. Effect of Dose and Administration Period of Seed Cake of Genetically Modified and Non-Modified Flax on Selected Antioxidative Activities in Rats. Int. J. Mol. Sci. 2015; 16: 14259-14275. doi:10.3390 /ijms160614259.
42. Jennifer L.A., Susan J.W., Bernhard H.J., Lilian U.T., Jane A. Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. Br. J. Nutr. 2010; 103(7): 929–939.
43. Kawakami, Y., Okumura, H. Y., Kuroki, Y. N., Taketan, Y. and Takeda, E. Flax seed oil intake reduces serum small dense low density lipoprotein concentrations in Japanese men: a randomized double blind, cross-over study. Nutrition Journal. 2015; 14:39.
44. Yassin M.E., Haggag E., Elsanhoty R.M. and Ramadan M.F. Impact of dietary oils and fats on lipid peroxidation in liver and blood of albino rats. Asian Pac. J. Trop Biomed. 2014; 4(1): 52-58.
45. Lillian F. Smith, J. Patterson, L.T. Walker and M. Verghese. Chemopreventive Potential of Sunflower Seeds in a Human Colon Cancer Cell Line. International Journal of Cancer Research. 2016; 12 (1): 40-50.
46. Ramaiyan B., Bettadahalli S. and Talahalli R.R. Dietary omega-3 but not omega-6 fatty acids down-regulate maternal dyslipidemia induced oxidative stress: A three generation study in rats. Biochem Biophys Res Commun. 2016; 2, 477(4): 887-894. doi: 10.1016/j.bbrc.2016.06.153. Epub 2016 Jul 1.
47. Shaban A. and Ravi P Sahu. Pumpkin Seed Oil: An Alternative Medicine. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(2): 223-227.
48. Zeb A. and Ahmad S. Changes in Acylglycerols Composition, Quality Characteristics and In vivo Effects of Dietary Pumpkin Seed Oil upon Thermal Oxidation. Front. Chem. 2017; 5:55. doi: 10.3389/fchem.2017.00055.
49. Reham A. Mohamed, Reham S. Ramadan and Lamiaa A. Ahmed. Effect of Substituting Pumpkin Seed Protein Isolate for Casein on Serum Liver Enzymes, Lipid Profile and Antioxidant Enzymes in $CCl_4$ -intoxicated Rats. Advances in Biological Research. 2009; 3 (1-2): 09-15.
50. Abuelgassim O. Abuelgassim and Showayman I. A. AL-showayman. The effect of pumpkin (cucurbita pepo L) seeds and l-arginine supplementation on serum lipid concentrations in atherogenic rats. Afr J. Tradit Complement Altern Med. 2012; 9(1):131-137.
51. Versari D., Gossl M., Mannheim D., Daghini E., Galili O., Napoli C., Lilach O. Lerman, and Lerman A. Hypertension and Hypercholesterolemia Differentially Affect the Function and Structure of Pig Carotid Artery. Hypertension. 2007; 50:1063-1068.
52. Mokhtar R.A., El-Banna N.H. and Haggag M.H. Protective Effect of Grape Seed Oil on Hypercholesterolemia in Rats. International Journal of Nutrition and Food Sciences. 2016; 5(5): 310-317. doi: 10.11648/j.ijnfs.20160505.11.
53. Goyal A., Sharma V., Upadhyay N. Gill S. and Sihag M. Flax and flaxseed oil: an ancient medicine and modern functional food. J Food Sci Technol. 2014; 51(9):1633–1653. DOI 10.1007/s13197-013-1247-9.

# تعديل ارتفاع كوليسترول الدم الناجم عن الإصابة بالإجهاد التأكسدي من خلال التأثيرات الوظيفية لخليط بعض البذور النباتية في فئران التجارب

**الملخص:** إن بذور الكتان ودوار الشمس واليقطين (القرع) شائعة الاستخدام كبذور صالحة للأكل في تغذية الإنسان نظراً لتأثيرها المعتدل. ارتكزت هذه الدراسة على دراسة خليط من مسحوق بذور الكتان/ دوار الشمس/ اليقطين على الفئران التي تعاني من ارتفاع كوليسترول الدم. تم عمل العديد من التقديرات وهى المأخوذ من الطعام ووزن الجسم المكتسب ومعدل كفاءة الغذاء والوزن النسبي للكبد والقلب ومستويات دهون الدم وإنزيمات الكبد ووظائف الكلى ومؤشرات الإجهاد التأكسدي ومستويات الدهون المتأكسدة ودراسة أنسجة القلب للفئران الطبيعية والمصابة بارتفاع كوليسترول الدم. تم تغذية المجموعة الرئيسية الأولى (6 فئران) على الغذاء الأساسي لمدة 12 أسبوعا. المجموعة الرئيسية الثانية (24فأرا) تغذت على الغذاء مرتفع الكوليسترول لمدة 8 أسابيع، ثم تم تدعيم الغذاء الأساسي بخليط مسحوق البذور بتركيزات 3 و 6 و 9٪ على التوالي لمدة 4 أسابيع متتالية. أوضحت النتائج أن العلاج الغذائي بخليط بذور الكتان/ دوار الشمس/ اليقطين (30 جرام، 60 جرام و 90 جرام) لمدة 4 أسابيع له تأثير تعديل قوي لتحسين التقييم البيولوجي ومستويات تحاليل الدم والتغيرات في أنسجة القلب للفئران المصابة بارتفاع مستوى الكوليسترول. ومن ثم تبين أن خليط هذه البذور لديها القدرة على الحد من مضاعفات أمراض القلب والأوعية الدموية بسبب ارتفاع كوليستيرول الدم.

**الكلمات المفتاحية:** ارتفاع كوليستيرول الدم، مستوى دهون الدم، الانزيمات المضادة للأكسدة، ذكور الفئران البيضاء، دراسة نسيجية للقلب.