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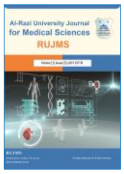

# Antihypercholesterolemia Activities by Functional Effects of Some Mix Plant Seeds in Rats

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## Abstract

***Background****: The moderated effect of flax, sunflower and pumpkin seeds are common used as edible seeds in human nutrition.* ***Aim:*** *To investigate the nutritional effect of flax/sunflower/pumpkin mix seeds on hypercholesterolemia for male albino rats by estimated biological, biochemical and histopathological examinations.* ***Methods****: 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.* ***Results****: 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.* ***Conclusion*****:** *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.*

**Keywords:** Hypercholesterolemia, lipid profile, flax seed, sunflower seed and pumpkin seed.

## 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. 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. 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] . 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[2]. In addition, there is a close correlation between these diseases and lipid abnormalities, especially high level of cholesterol, and blood pressure[3]. 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[4]. 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[5]. 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[6]. 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[7]. 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[8]. 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[9]. 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[10]. 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[11]. 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[12]. Pumpkin seeds are rich natural source of LAn-6, oleic acid, and antioxidant vitamins, such as carotenoids and tocopherols[13].

## Aim of the study

To investigate the nutritional effect of flax/sunflower/pumpkin mix seeds on hypercholesterolemia for male albino rats by estimated biological, biochemical and histopathological examinations.

## Subjects 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)[14] 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 Sana'a city.

- ***Animals and experimental designs***

Male albino rats was weight 140±10 g, aged 8 weeks. Rats were purchased from the laboratory animal house of faculty of science, Sana'a 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.

- ***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[16]. 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[17]. 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[18, 19, 20, 21.] HDL-Cholesterol was measured using a spectrophotometric method [22] and LDL-Cholesterol were calculated by Friedwald formula, VLDL = TG/5, LDL = Total Cholesterol – (VLDL+HDL). Concentration represented in mg/dL[23]. Atherogenic index was calculated by using formula = log (TG/HDL-C)[24]. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were determined according to Reitman and Frankel[25] and alkaline phosphatase enzymes (ALP)[26]. Serum urea was performed measured according to Patton and Crouch[27], serum creatinine was determined according to the method described by Kroll *et al.* [28]. 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.*[29], lipid peroxidation products were assayed by measuring malondialdehyde (MDA) according to Ohkawa *et al.*[30].

- ***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[31].

Data was calculated as mean values with their standard deviation (S.D.) of each group. Values were statistically analyzed according to Armitage and Berry[32] by using (SPSS version 20.0; SPSS, Inc.) one-way analysis of variance (ANOVA), P values >0.05 was 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 feed intake, body weight gain (BWG %) and feed efficiency ratio (FER) 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 < 0.05).***

Results in table 2 recorded relative organs weight % of treated and hypercholesterolemic rats. Heart weight % showed non-significant increases ($p < 0.05$) 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 ($p < 0.05$) 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 <0.05).***

**Biochemical analysis**

***Lipid profile***

Total cholesterol (TC) and triglyceride (TG) serum results data in table 3 illustrated significant increases ($p < 0.05$) 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 ($p < 0.05$).***

Serum LDL-c, VLDL-c and calculated AI data showed significant increases ($p < 0.05$) 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 ($p < 0.05$).***

***Liver enzymes***

Liver enzymes as serum aminotransferases ALT and AST in table 5 illustrated significant increases ($p < 0.05$) 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 ($p < 0.05$).***

***Kidney function***

Table 6 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 ($p < 0.05$) 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 < 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 ($p < 0.05$) 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 ($p<0.05$) 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 <0.05).***

***Heart histopathological examination***

Heart tissues of negative rat group showed the normal cardiac myocytes as found in figure 1. Heart tissues of hypercholesterolemic rat illustrated congestion of myocardial blood vessels as showing in figure 2, intramuscular oedema as showing in figure 3, and intramuscular haemorrhage as showing in figure 4. Rat hearts of mixed flax/sunflower/pumpkin seeds 3% group showed moderate congestion of myocardial blood vessels as illustrated in figure 5. Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group revealed low congestion of myocardial blood vessels in figure 6, and few intramuscular oedema and haemorrhage in figure 7.

Heart tissues of mixed flax/sunflower/pumpkin seeds 12% group revealed to no histopathological changes in Figure 8.

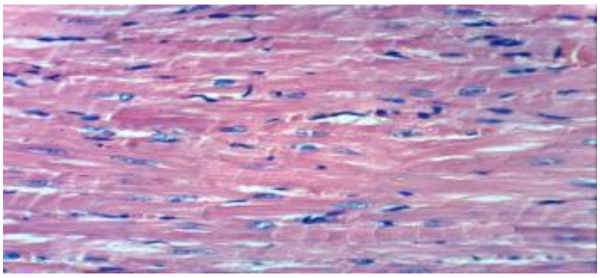

**Figure 1: Cardiac myocytes of negative group.**

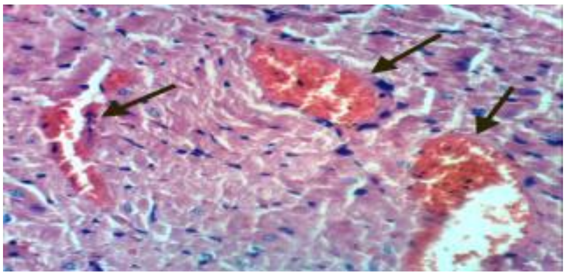

**Figure 2: Heart tissues of hypercholesterolemic group.**

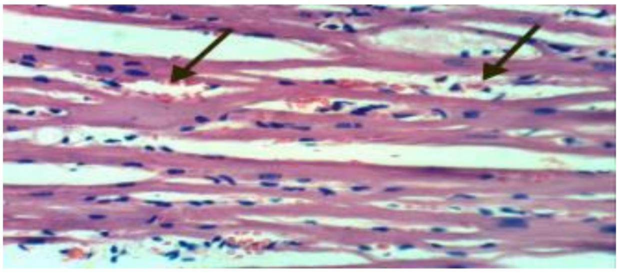

**Figure 3: Heart tissues of hypercholesterolemic group.**

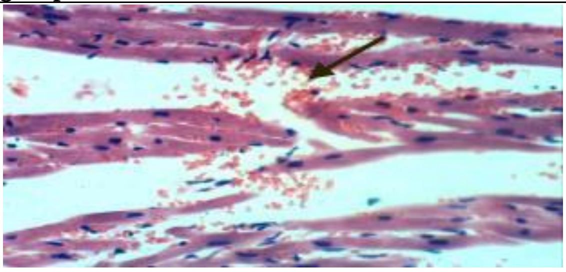

**Figure 4: Heart tissues of hypercholesterolemic group.**

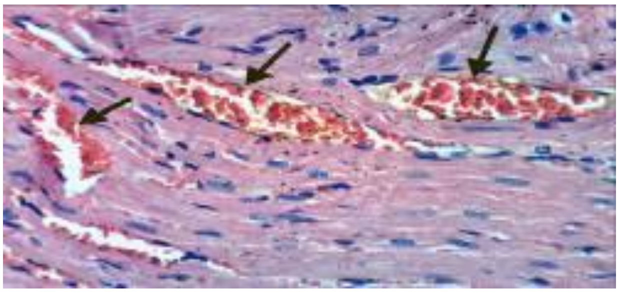

**Figure 5: Heart tissues of mixed flax/sunflower/pumpkin seeds 3% group.**

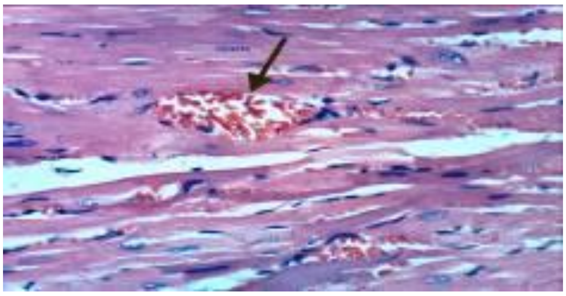

**Figure 6: Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group.**

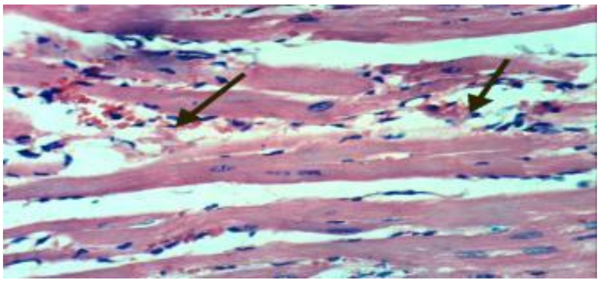

**Figure 7: Heart tissues of mixed flax/sunflower/pumpkin seeds 6% group.**

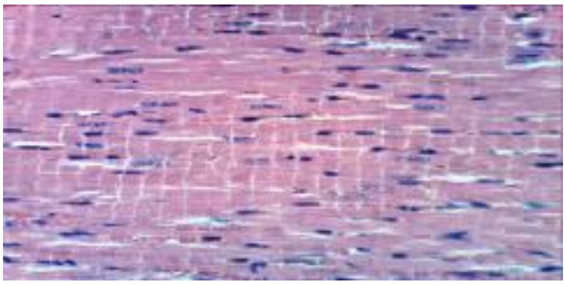

**Figure 8: Heart tissues of mixed flax/sunflower/pumpkin seeds 12% group.**

## Discussion

Hypercholesterolemia is a condition of very high levels of cholesterol in the blood. Recently, hypercholesterolemia has been associated with oxidative stress related to increased lipid peroxidation. High cholesterol levels associated with increased generation of oxidized LDL as a major factor in vascular damage. Hence, 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[33]. Flax, sunflower, and pumpkin seeds have gained interest for their health benefits due to their fatty acid and lignan contents. 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[34]. Feeding animals with pure cholesterol has often been used to elevate serum or tissue cholesterol levels to examine the etiology of hypercholesterolemia-related metabolic disturbances[35]. Antioxidant enzymatic systems include superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase (HO), while enzymatic sources for reactive oxygen and/or nitrogen species (ROS) formation include the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, cyclooxygenases, uncoupled nitric oxide synthase (NOS), and peroxidases [36]. This study agreement with results showed that, 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]. 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[39].

Hypercholesterolemia leads to incremented formation of superoxide and peroxynitrite and thereby results in cardiac dysfunction in hearts of human apoB100 transgenic mice[40]. Flaxseed is emerging as an important functional food ingredient because, its possessive rich contents of αlinolenic acid (ALA, omega-3 fatty acid), lignans, and fiber. Flaxseed oil and flax lignans have many potential health benefits such as reduction of cardiovascular disease, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders. Flax protein helps in the prevention and treatment of heart disease and in supporting the immune system. 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[41]. Flaxseed is also an excellent source of dietary fiber and several micronutrients [42, 43, 44]. Administration of 30% flaxseed cake for 90 days improved total plasma antioxidant status and lowered liver thiobarbituric acid reactive substances[45]. Dietary flaxseed may also offer protection against ischemic heart disease by improving vascular relaxation responses and by inhibiting the incidence of ventricular fibrillation[46]. Supplements of 10 g flax seed oil revealed to significant decreases in serum total cholesterol, LDL-cholesterol, HDL-cholesterol [47]. 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 [48]. Ristic-Medic *et al.*[7] indicated that dietary milled sesame/pumpkin/flax seed mixture added to a habitual diet lowered triglyceride and inflammatory markers C-reactive protein CRP, tumor necrosis factor-alpha TNF-alpha, interleukin-6 IL-6 levels, affect glycemic control and improved fatty acid profile and pruritus symptoms in hemodialysis patients. Pumpkin seeds are rich natural source of linolenic acid LAn-6, oleic acid, and antioxidant vitamins, such as carotenoids and tocopherol[49]. Sunflower seed (*Helianthus annuus* L.) is an oily seed crop that is a good source of protein. High phenolic and flavonoid content revealed to improve cytotoxic and antioxidative potential of sunflower seeds as a chemopreventive agent[50]. 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[51]. Pumpkin seed is a rich natural source of proteins and phytosterols[52]. Pumpkin seed oil has long been considered as an ingredient for its nutritional and medicinal values for the prevention of various diseases. In addition, several studies have suggested the crucial roles and effectiveness of pumpkin seed oil in the treatment of diabetes, anxiety and even cancer. Many reviews provide the chemists, biologists and researchers on the roles of pumpkin seed oil extracts that possess promising biological activities[53]. 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[54].

Pumpkin seed protein isolate could protect the liver cells from $CCl_4$-induced liver damages perhaps, by its antioxidative effect on hepatocytes, hence eliminating the deleterious effects of toxic metabolites from $CCl_4$. Study can recommended that, pumpkin seed protein isolate may be useful for patients suffering from liver diseases due to its hepatoprotective and hypolipidemic activities[55]. Treatment of atherogenic rats with pumpkin seeds significantly decreased serum concentrations of TC and LDL-C. These findings suggest that pumpkin seeds supplementation has a protective effect against atherogenic rats[56]. Versari *et al.*[57] 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[58]. Its well-known proatherogenic effect, hypercholesterolemia may exert direct effects on the myocardium resulting in contractile dysfunction, aggravated ischemia/reperfusion injury, and diminished stress adaptation.

Both preclinical and clinical studies suggested that elevated oxidative and/or nitrative stress plays a key role in cardiac complications induced by hypercholesterolemia. Therefore, modulation of hypercholesterolemia-induced myocardial oxidative/nitrative stress is a practical approach to prevent or treat deleterious cardiac consequences[36].

## Conclusion

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.

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