Hypolipidemic, Antioxidant and Renal Protective Effect of Seeds Mixture Rich in Omega-3 and Omega-6 Fatty acids in rats

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Abstract: Aim of the work: Assessing the hypolipidemic, antioxidant and renal protective activities of seeds mixture rich in omega-3 and omega-6 fatty acids in rats. **Material and Methods:** 64 male albino rats were divided into 8 groups: control group, hypercholesterolemic rats, fed the balanced diet supplemented with cholesterol at a dose level of 2 g/100 g diet; the other 6 groups of animals fed the same previous hypercholesterolemic diet supplemented with either mixture of Flax / pumpkin (F/P), Flax/Sesame (F/S), Flax/Peanut (F/A), purslane / pumpkin (P/P), purslane / Sesame (P/S) and purslane /Peanut (P/A) to ascertain the claim of its utilization against diseases. The seeds mixture rich in unsaturated fatty acids were prepared at ratio of (5/1) (ω-6 and ω-3) and were orally administered to rats diet for 30 days. **Results**: High cholesterol fed diet rats (2%) showed a significant increase in total cholesterol, total lipids, and triacylglycerol in both serum and liver. Serum phospholipids, LDL-C, MDA and atherogenic index also significantly increased compared to (BD) group. On the other hand, High cholesterol (HCD) fed diet rats showed a significant decrease in serum high-density lipoproteins (HDL), Superoxide dismutase (SOD) and liver glutathione peroxidase (GPX). Cholesterol-enriched diet also significantly increased serum urea, creatinine, sodium and potassium levels compared to healthy control. Consumption of seeds mixture rich in omega-3 and omega-6 fatty acids by hypercholesterolemic rats resulted in a significantly decrement in lipid parameters and improvement in antioxidant status and renal function as compared with hypercholesterolemic rats. **Conclusion:** The results suggest that seeds mixtures had Hypolipidemic, Antioxidant and renal protective effect, which were probably mediated by unsaturated fatty acids present in seed mixture.
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1 – Introduction

Hyperlipidemia is the current medical as well social problem, leading to increasing morbidity and mortality. The major risk factors of hyperlipidemia are associated with atherosclerosis, which predisposes ischemic heart disease and cerebrovascular disease (Brown and Goldstein, 1990). Most patients who present with hyperlipidemia have a polygenic predisposition to raised blood lipids aggravated by dietary or lifestyle indiscretion.

It is well known that lifestyle and diet play a role in the development of kidney disease. Several studies indicated that abnormalities in lipid metabolism can often accompany and exacerbate renal disease (Vazquez-Perez *et al.,* 2001) Hypercholesterolemia is well-known to be an independent risk factor for renal injury (Oda *et al.,* 1999) and to aggravate the pathogenesis of a variety of clinical and experimental renal diseases (Kivipelto *et al.,* 2001). High cholesterol diet (HCD) was found to increase blood pressure and to induce renal injury (Zou *et al.,* 2003). Moreover, many accumulating evidences support the idea that HCD exacerbates kidney damage in animal models of kidney disease (Mori *et al.,* 2012). Previous data showed that even a short exposure to HCD supplementation is associated with an increase in oxidative stress and renal inflammation (Wilson *et al.,* 2003). Indeed, HCD supplementation to animals was reported to significantly increase kidney oxidative stress parameter and to significantly reduce kidney antioxidant parameters (Vijayakumar *et al.,* 2004). Therefore, the inhibition of oxidative stress under hypercholesterolemic conditions is considered to be an important therapeutic approach for kidney related diseases.

Omega-3 and ω-6 fatty acids are eicosanoid precursors that regulate immune and inflammatory functions. Some essential fatty acid (EFA) derivatives, such as dihomo-gamma- linolenic and arachidonic acid, both from the ω-6 series, and EPA, from the ω-3 series, are especially important because they are lipid mediators involved in many physiological functions (Krummel, 2007).

Dietary intakes of ω -3 and ω -6 fatty acids are critical determinates of the proportions of bioactive 20- and 22-carbon n26 and n23 highly unsaturated fatty acids (HUFAs) in tissue phospholipids (Lands *et al.,* 1992)). Tissue HUFAs, in turn, have been shown to affect multiple disease states (Leaf, 2007 and Rao *et al.,* 2008) ranging from psychiatric (Hibbeln., 2009) and cardiovascular disease (Harris., 2008) to neurodevelopmental deficits (Hibbeln and Davis., 2009). The omega-3 index, which is a direct measure of erythrocyte EPA + DHA as a percentage of total fatty acids, has been proposed as a risk biomarker for cardiovascular disease (Harris., 2008).

Omega-3 PUFAs are primarily found in fish, especially in twait shad, salmon, tuna, and anchovies (Whelan and Rust, 2006). Another important source of PUFA is flaxseed.

An important source of PUFA is flaxseed obtained from *Linum usitatissimum* plants (Linaceae family), cropped mainly in Argentina, Brazil, Canada, China, India and Turkey (Sammour, 1999). Flaxseed is a flat, oval-shaped seed (Marques *et al.,* 2011) whose oil contains 53% alpha-linolenic acid (ALA), an essential ω-3 fatty acid. Flaxseed is also a good source of dietary fiber (20-25%) (Vijaimohan *et al.,* 2006) and lignans (>500 μg/g), which are plant steroids analogous to mammalian estrogen (Stodolnik *et al.,* 2005).

Purslane (*Portulaca oleracea*) is a nutritious vegetable used for human consumption, and it was mentioned in Egyptian texts from the time of the Pharaohs (Mohamed and Hussein, 1994). Purslane is eaten raw as a salad and also is eaten cooked as a sauce in soups or as greens. Purslane provides a rich plant source of nutritional benefits (Sudhakar *et al.,* 2010). It is one of the richest green plant sources of omega-3 fatty acids and a- linolenic acid (Simopoulos and Salem, 1986).

Portulaca oleracea L. (Portulacaceae) is an edible plant and has been used as a folk medicine in many countries, acting as a diuretic, febrifuge, antiseptic, antispasmodic, and vermifuge (Mohanapriya *et al.,* 2006). It has been shown to play pharmacological roles, including antibacterial (Zhang *et al.,* 2002), analgesic (Chan *et al.,* 2000), skeletal muscle-relaxant (Parry *et al.,* 1993), and wound-healing (Rashed *et al.,* 2003) activities. Many studies have also shown that the major bioactive components of *Portulaca oleracea* are flavonoids, coumarins, monoterpene glycoside, and alkaloids (Sakai *et al.,* 1996) Some research results indicated that *Portulaca oleracea* could also be used to reduce the incidence of cardiovascular diseases (Liu *et al.,* 2000)

Polyunsaturated fatty acids from the n-6 (ω-6) family, found in nuts, seeds, and vegetable oils such as corn and soybean oils (Institute..., 2005), are also important. While ω-3 PUFAs are precursors of 3-series prostanoids and 5-series leukotrienes (associated to anti-inflammatory and antithrombotic properties), ω-6 PUFAs are precursors of 2-series prostanoids and 4-series leukotrienes (associated to pro-inflammatory and prothrombotic activity) (McKenney and Sica, 2007).

Pumpkins (Cucurbit sp.) belonging to the Cucurbitaceae family are grown widely around the world as a vegetable. The phytochemical composition renders the seeds valuable for nutritional purposes. Stevenson *et al.,* 2007 studied several pumpkin cultivars (Cucurbita maxima D.), for their seed oil content, fatty acid composition and tocopherol content. The oil content ranged from 11 to 31%. Total unsaturated fatty acid content ranged from 73 to 81%. The predominance of linoleic, oleic, palmitic and stearic acids was observed. The a-tocopherol content of the oils ranged from 27 to 75 mg/g, while c-tocopherol ranged from 75 to 493 mg/g.

Sesame seed (*Sesamum indicum* L.), another widely consumed seed, is a good ω-6 source. This Pedaliaceae is cropped in both tropical and subtropical countries. India and China are the major producers accounting for 70% of world production. In Brazil, 13,000 tons of sesame seeds are produced over nearly 20,000 ha, yielding approximately 650 kg/ha (Arriel *et al.,* 2005). Sesame oil has advantages over other vegetable oils owing to its high nutritional and therapeutic value. Sesame seeds, which are used in traditional Indian and Chinese medicine, contain 57% highly stable oil (Reshma *et al.,* 2010). Due to its high oxidative stability, sesame oil is added to margarines, salads, and frying oils (Yen and Lay, 1990). Saturated fatty acid (SFA) content in sesame oil is nearly 14%, comparable to soy and corn oil. Oleic and linoleic (LA) acid levels are approximately 45%, which is close to that found in corn, soy, and cottonseed oil (Embrapa, 2001).

Peanuts are legumes and grow underground. They are similar to tree nuts in form and fat content. Approximately 60% of the energy in nuts and peanuts is derived from fat, and greater than 75% of this fat is unsaturated(Kris-Etherton *et al.,* 1999). Much of the health benefit attributed to nuts stems from the lipid-lowering effects Mukudden-Petersen *et al.,* 2005) of their high unsaturated fatty acid profile as well as actions of other constituents like fiber, vitamin E, and phytochemicals Maguire *et al.,* 2004).

Peanuts are rich source of Mg, folate, fibre, a-tocopherol, Cu, arginine and resveratrol. All of these compounds have been shown to reduce CHD risk in various ways, and this suggests that peanut consumption might benefit those at risk for CHD. However, most studies to date have been performed in either healthy or hypercholesterolaemic subjects in combination with low-fat diet. (O'Byrne *et al.,* 1997).

This study aimed to assess the *In vivo* Hypolipidemic, antioxidant and renal protective activities of seeds mixture rich in omega-3 and omega-6 fatty acids in rats.

2- Material and Methods

Materials:

Chemicals:

All chemicals including cholesterol and Kits were fine grade chemicals purchased from local distributer (Sigma chemical) Cairo, Egypt.

Preparation of seeds mixture and diets

Flax (*L. usitatissimum* L.), pumpkin **(*Cucurbita pepo*)**, Sesame (*S. indicum*), Peanut (*Arachis hypogaea*) and purslane (*Portulaca oleracea*) seeds were purchased from local market, Cairo – Egypt, crushed at ambient temperature and stored at 4 °C prior to use.

Seed mixture of Flax / pumpkin (F/P), Flax/Sesame (F/S), Flax/Peanut (F/A), purslane / pumpkin (P/P), purslane / Sesame (P/S) and purslane /Peanut (P/A) rich in omega-3 and omega-6 were prepared. The ratio of omega-6/omega-3 fatty acids was 5/1 as recommended by the WHO and according to Grigg (2004), Blandeau and Schneider (2006).

Flax seeds and purslane seeds were used as ω-3 fatty acids rich sources, while pumpkin, Sesame (S. indicum) and Peanut seeds used as ω-6 fatty acids rich sources.

Animals:

Sixty-four male albino rats (Sprague Dawley strain) of body weight ranging from (160 **± 7 g)** were obtained from the Institute of Ophthalmology (Cairo, Egypt). Animals were housed individually in stainless-steel cages fitted with a wire mesh bottoms and fronts. They maintained in an environmentally controlled animal house temperature (24±3^{o}C) and relative humidity (50 ± 10) on a daily photoperiods of light/dark. Animals were acclimatized for ten days prior to experiment.

Experimental design:

The rats were randomly enrolled into eight experimental groups with eight rats in each and were treated as following:

Group 1: (control): Rats were received standard basal diet according to AIN-93 formulation (Reeves *et al.,* 1993). (BD).

Group 2: Rats were received standard basal diet +2% cholesterol (HCD).

Group 3: Rats were received hypercholesterolemic diet supplemented with Flax / pumpkin seed mixture (F/P).

Group 4: Rats were received hypercholesterolemic diet supplemented with Flax/Sesame seed mixture (F/S).

Group 5: Rats were received hypercholesterolemic diet supplemented with Flax/Peanut seed mixture (F/A).

Group 6: Rats were received hypercholesterolemic diet supplemented with purslane / pumpkin seed mixture (P /P).

Group 7: Rats were received hypercholesterolemic diet supplemented with purslane / Sesame seed mixture (P/S).

Group 8: Rats were received hypercholesterolemic diet supplemented with purslane /Peanut seed mixture (P/A).

After 30 days, the rats were sacrificed after overnight fasting under diethyl ether anesthesia. Liver was removed immediately, washed with ice- cold saline solution, dried between filter paper, weighed and stored at -20^{O}C for biochemical analysis,

Biochemical Analysis:

Serum total cholesterol was assayed by the method of Richmond, (1973), serum triacylglycerol according to Fossati and Prencipe, (1982), serum HDL by the method of Steele *et al.*(1976) while serum low-density lipoprotein-cholesterol (LDL-C) fraction and atherogenic index (AI) were determined according to the Friedewald equations (Friedewald *et al.,* 1972):

LDL-C = TC–(triacylglycerol/5+HDL-C).

AI = (TC – HDL-C) / HDL-C.

Serum very low-density lipoprptein cholesterol (VLDL-C) concentration was calculated according to Friedewald *et al*., (1972) by the following equation:

Serum VLDL-C (mg/dl) = Triacylglycerols/5.

For liver lipid analysis, total hepatic lipids were extracted with a mixture of chloroform: methanol (2:1) and measured according to Folch *et al.* (1957). Liver TAG and TC were measured enzymatically as described above. Serum phospholipids was assayed according to (Connerty *et al.,* 1961).

MDA was measured as an indication of lipid peroxidation using the colorimetric method described by Draper and Hadly, (1990). Serum catalase was assayed according to Vanizor *et al.* (2003). Liver glutathione peroxidase (GPX) was determined according to Tapple (1978).

Blood urea nitrogen (BUN) was analyzed using kits from Bioanalytics Company following the method described by Tabacco *et al*. (1979). Serum creatinine concentration was analyzed using kits from Bioanalytics Company following the method described by Fabing and Ertingshausen (1971).

Serum sodium, potassium, were estimated by the colorimetric method of Berry *et al.* (1988), Sunderman and Sunderman (1958), respectively.

Statistical analysis:

Statistical analyses were performed by using the SPSS software (version 16; SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviation (SD). Differences between treatment groups were analyzed by one-way analysis of variance (ANOVA) with post hoc analysis using

Bonferroni multiple test. Differences were considered significant when $P < 0.05$.

3- Results:

High cholesterol fed diet rats (2%) showed a significant increase in total cholesterol, total lipids, and triacylglycerol in both serum and liver. Serum phospholipids, LDL-C, and atherogenic index, also significantly increased compared to (BD) group. On the other hand, High cholesterol fed diet rats showed a significant decrease in high-density lipoproteins (HDL). Cholesterol-enriched diet also significantly increased serum urea, creatinine, sodium and potassium levels compared to healthy control (BD). Consumption of seeds mixture rich in omega-3 and omega-6 fatty acids by hypercholesterolemic rats resulted in a significantly decrement in lipid parameters and improvement in renal function as compared with hypercholesterolemic rats.

Table (1): Effect of seeds mixture on serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol VLDL-C and high density lipoprotein cholesterol (HDL-C), in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	TL (mg/dl)	TC (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)
Group 1 Normal control(BD)	298.13±7.51	98.94±1.47	144.13±3.04	25.74±1.24	44.38±2.13[a]	28.83±0.61
Group 2 High cholesterol diet(HCD)	470.18 ± 5.42	180.04±2.09	209.50±3.55	106.89±1.13	31.25±2.19	41.90±0.71
Group 3 (F/P)	321.14±5.54	128.68±1.79[a]	162.00±3.25[a]	54.53±1.90	41.75±2.12[ab]	32.40±0.65a
Group 4 (F/S)	370.13±4.39	140.13±1.88[b]	173.13±5.25[b]	67.50±1.68[a]	38.00±2.14[bc]	34.63±1.05[b]
Group 5 (F/A)	403.63±4.41[a]	146.00±1.31[c]	179.38±3.38[c]	73.38±2.90[b]	36.75±2.49[c]	35.88±0.68[c]
Group 6 (P/P)	333.63±5.53	131.13±2.75[a]	163.50±3.34[a]	58.55±4.45	39.88±4.19[bc]	32.70±0.67[a]
Group 7 (S/P)	384.13±7.21	139.13±2.17[b]	176.38±3.70b[c]	63.73±1.39[a]	40.13±2.36[abc]	35.28±0.74[bc]
8group (A/P)	403.13±9.69[a]	147.00±1.69[c]	180.14±4.98[c]	71.48±3.36[b]	39.38±3.58[bc]	36.15±0.98[c]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Table (2): Effect of seeds mixture on liver total lipid, total cholesterol (TC) triacylglycerol (TAG), serum Phospholipid and atherogenic index in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Liver TL (g/mg)	Liver TC (mg/g)	Liver TAG (mg/g)	Serum Phospholipids (mg/dl)	AI
Group 1 Normal control(BD)	0.466±0.031	43.02±2.59	48.83±2.80	141.44±2.53	54.56±1.09
Group 2 High cholesterol diet(HCD)	0.99±0.089	108.17±5.79	116.68±3.39	195.25±3.73	148.78±0.72
Group 3 (F/P)	0.68±0.019[a]	66.88±4.36[a]	92.92±2.75[ab]	152.50±4.44[ab]	86.93±2.03
Group 4 (F/S)	0.74±0.019[b]	74.38±2.67[b]	96.36±2.88[ac]	157.75±3.41[a]	102.13±0.99[a]
Group 5 (F/A)	0.83±0.017[c]	75.25±6.14[b]	99.02±4.75[cd]	158.50±4.21[a]	109.25±2.31[b]
Group 6 (P/P)	0.67±0.033[a]	66.88±3.09[a]	92.63±3.42[b]	150.75±2.25[b]	91.25±4.62
Group 7 (S/P)	0.77±0.033[b]	76.00±3.85[b]	98.88±3.64[cd]	156.50±3.66[ab]	99.00±1.06 [a]
Group8 (A/P)	0.85±0.035[c]	78.63±4.63[b]	101.00±4.89[d]	156.75±5.97[ab]	107.63±2.50[b]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Effect of seeds mixture on serum lipids as well as hepatic lipids:

Rats fed HCD diet exhibited several metabolic abnormalities as shown in Table 1 and Table 2. The rats developed hypolipidemia, indicated by a significant increase in total cholesterol, total lipids, and triacylglycerol in both serum and liver. Serum phospholipids, LDL-C, and atherogenic index, also significantly increased compared to control group. On the other hand, High cholesterol fed diet rats showed a significant decrease in high-density lipoproteins (HDL). In rats receiving HCD plus seed mixture, serum lipids including serum total cholesterol, total lipids, and triacylglycerol, LDL - cholesterol, liver lipids including total lipid, cholesterol, triacylglycerol and Serum phospholipids, were markedly (*P*< 0.05) decreased in comparison with rats receiving HCD diet only. On the other hand, seed mixture resulted in an increase of serum HDL-cholesterol concentration as compared to HCD fed rats.

Effect of seed mixture on renal function:

Serum blood urea nitrogen (BUN), Creatinine, sodium and Potassium were determined to assess the kidney function. As shown in table 3, HCD diet-fed rats had elevated serum blood urea nitrogen (BUN), Creatinine, sodium and Potassium compared to the control rats (BD) (P<0.05). However, treatment with seed mixture markedly reduced these levels compared to HCD diet-fed rats, implying that seed mixture had executed a protective effect against the HCD diet-induced kidney injuries.

Table (3): Effect of seeds mixture on serum blood urea nitrogen (BUN), serum Creatinine, serum sodium and Potassium, in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)
Group 1 Normal control(BD)	39.16±1.72	0.68±0.014	137.28 ± 4.3^{a}	4.35± 0.639
Group 2 High cholesterol diet (HCD)	60.03±2.26	1.87±0.099	150.03 ± 3.68^{c}	7.48±0.327
Group 3 (F/P)	43.77±2.07	0.77±0.038	140.29 ± 4.05^{ab}	5.36 ± 0.243^{a}
Group 4 (F/S)	47.91 ± 2.97^{ab}	0.91 ± 0.028^{a}	142.47 ± 4.48^{bd}	6.31 ± 0.186^{cd}
Group 5 (F/A)	51.72 ± 3.02^{cd}	0.95 ± 0.030^{a}	143.86 ± 4.21^{bd}	6.62 ± 0.159^{c}
Group 6 (P/P)	46.55 ± 2.28^{a}	0.88 ± 0.094^{a}	142.24 ± 6.09^{bd}	5.69 ± 0.269^{ab}
Group 7 (S/P)	49.44 ± 2.44^{bc}	0.94 ± 0.019^{a}	143.53 ± 4.56^{bd}	6.13 ± 0.151^{bd}
Group (A/P) 8	53.55 ± 2.37^{d}	1.12±0.065	146.22 ± 4.89^{cd}	6.57 ± 0.139^{cd}

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Effect of seed mixture on oxidative status:

In order to explore the effect of seed mixture on oxidative stress status, serum MDA, serum SOD and liver GPX contents were assessed. HCD diet feeding resulted in a significant increase in serum MDA level, on the other hand serum SOD and liver GPX were greatly decreased compared to control group (BD). Lipid peroxidation was efficiently counteracted by the treatment with seed mixture as compared with the untreated HCD diet-fed rats. However, as shown in table 4, administration of seed mixture significantly increased both serum SOD and liver GPX compared to untreated HCD diet-fed rats.

Table (4): Effect of seeds mixture on serum MDA, SOD, and liver GPX in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	**Serum MDA (nmol\l)**	**Serum SOD** (mmol/l)	**Liver GPX** (µ /mg)
Group 1 Normal control(BD)	1.85±0.071	338.87±3.48	0.98±0.089
Group 2 High cholesterol diet(HCD)	3.67±0.063	227.87±4.26	0.33±0.031
Group 3 (F/P)	2.30 ± 0.068^{a}	324±4.21	0.69±0.028
Group 4 (F/S)	2.39 ± 0.031^{b}	309.88 ± 3.68^{a}	0.58±0.047
Group 5 (F/A)	2.45 ± 0.024^{cd}	293.25 ± 3.06^{b}	0.49 ± 0.032^{a}
Group 6 (P/P)	2.28 ± 0.041^{a}	311.25 ± 3.49^{a}	0.64±0.033
Group 7 (S/P)	2.41±0.024bc	305.25±2.49	0.53 ± 0.027^{a}
Group 8 (A/P)	2.46±0.028d	291.88 ± 2.85^{b}	0.45±0.026

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

4- Discussion:

Consuming diets enriched with animal fat increases the risk of cardiovascular diseases causing hyperlipidemia and Arteriosclerotic vascular disease (ASVD) in addition to augmenting LDL-cholesterol levels over time (Onody *et al.,* 2003).

Determination of the ω6:ω3 ratio is important for human heath since excessive consumption of ω6, accompanied by decreased ingestion of ω3, is a risk factor for cardiovascular disorders. These fatty acids compete for enzymes involved in desaturation reactions and chain elongation. Although these enzymes have greater affinity for fatty acids from the ω-3 series, the conversion of linolenic acid into long-chain PUFA is strongly affected by dietary linolenic acid levels (Ramadan *et al.,* 2009).

As shown in Table 1 and in Table 2 High cholesterol fed diet rats (2%) showed a significant increase in total cholesterol, total lipids, and triacylglycerol in serum and liver. Serum phospholipids, LDL-C, and atherogenic index also significantly increased compared to control group (BD). On the other hand, High cholesterol fed diet rats showed a significant decrease in high-density lipoproteins (HDL)

Our results are in agreement with Akpolat *et al,*. 2011 who reported that, the levels of total cholesterol and LDL were increased in the serum of HCD-fed rats, and these results were consistent with earlier findings (Joles *et al*., 2000; Sudhahar *et al*., 2008).

Animal fat consumption stimulates phospholipid biosynthesis, possibly because it decreases phospholipase activity or increases phospholipid volume triggering an inflammatory process (Basbag *et al.,* 2009).

Fat is an important constituent of diet, which regulates plasma and hepatic lipid levels. So amount and type of dietary fat influence lipid parameters. In fact, according to Khosla and Sundram (1996), diets rich in saturated fatty acids (SFA) raise LDL-C compared to those observed in poly-unsaturated fatty acids (PUFA)-rich diets. Other studies (Hegsted *et al.,* 1993) showed that SFA rises but PUFA lowers TC and LDL-C. Cardiovascular diseases may be caused by an inadequate and imbalanced intake of ω - 6 and ω - 3 EFAs, coupled with inadequate rates of D6-desaturation of both linoleic (18:2n -6) and a-linolenic (18:3n - 3) acids Khosla and Sundram (1996).

Consumption of seeds mixture rich in omega-3 and omega-6 fatty acids by hypercholesterolemic rats resulted in a significantly decrement ($p<0.05$) in lipid parameters

Our results are in agreement with those of Makni *et al.,* 2010 who investigated improvement in lipid metabolism and reduction of the risk of free radical damage in the hypercholesterolemic rats with consumption of seeds mixtures of Flax/Sesame and Flax/Peanut.

The results of the present study are also in agreement with those of Makni *et al.,* 2008 who illustrated that seed's oil rich in PUFAs and antioxidative compound could prevent and act in a lipid-lowering diet.

The normalizing effect of flaxseed on the elevated serum concentrations of total cholesterol and LDL and depressed HDL levels was previously reported (Ratnayake *et al*., 1992; Prasad, 1997), and may be due to their effect of decreasing the cholesterol absorption.

Makni *et al.,* 2008 evaluated the effect of flax and pumpkin seed mixture intake in rats fed with a 1 % cholesterol diet. In the seed-fed group, significant increase in poly- and monounsaturated fatty acids was observed. Plummeted malondialdehyde level and bolstered antioxidant defence system indicated the anti-atherogenic potential of the seed mixture. Gossell-Williams *et al.,* 2008 examined the effect of pumpkin seed oil supplementation on the total cholesterol, and low-density and high-density lipoprotein cholesterol, and systolic and diastolic blood pressure in rats. Both non-ovariectomized and ovariectomized rats were supplemented with corn oil or pumpkin seed oil for 5-days/ week for 12 weeks (40 mg/kg given orally). Blood analysis showed healthy lipid level in the pumpkin seed oil-supplemented group.

Flax, Sesame and Peanut seeds have long been used extensively as a traditional food in the orient for their various purposes. Flaxseeds (*Linum usitatissimum* L. member of Linaceae family) contain 32–45% of their mass as oil, where 51–55% are alpha-linolenic acid (ALA; 18:3n -3, **ω** -3 fatty acid), a precursor of EPA and DHA.

Both flaxseed and sesame seed are nutritional supplements, representing an excellent source of PUFA that can promote cardio protective effects if consumed daily (Chung et al., 2005).

It has been reported that many health benefits are associated with consumption of peanuts including weight gain control (Alper and Mattes, 2002), prevention against cardiovascular diseases (Feldman, 1999), protection against Alzheimer disease (Peanut-Institute, 2002), and cancer inhibition (Awad *et al.,* 2000). Benefits are mainly attributed to the fact that peanuts do not contain trans-fatty acids (Sanders, 2001), but they are rich in mono- and poly-unsaturated fatty acids (Kris-Etherton *et al.,* 1999), micronutrients such as vitamin E, folate, minerals (potassium, magnesium, and zinc), fibers and health promoting phytochemicals, particularly resveratrol (Sanders *et al.,* 2000) and other phenolic compounds.

Flax/Sesame and Flax/Peanut seeds mixture are known for their preferable organoleptic properties. It is clear also that fibers present in seeds play major roles in intestinal transit of animal models and humans. Seeds mixture contained also significant amounts of important minerals.

The antioxidant activity of seed mixture is related to contents of bioactive molecules such as total phenols (Litridou *et al.,* 1997), which make these mixture seeds a preferable supplement to hypercholesterolemic prevention diets. Results of the present study supports the suggestion of others who showed that seed' rich in PUFAs and antioxidative compound could prevent and act in a lipid-lowering diet (Makni *et al.,* 2008).

Purslane is best used for human consumption as a green vegetable rich in minerals and Omega-3 fatty acids (Mohamed and Hussein, 1994). Omega-3 fatty acid is a precursor of a specific group of hormones (prostaglandins) and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life.

Although humans and other animals can synthesize SFA and MUFA, they lack the enzyme that inserts *cis*-double bounds in position 3 and 6 of fatty acids chain to synthesize ALA and LA, respectively. Both acids are part of the same metabolic pathway, competing for Δ^6-desaturase, but they display different mechanisms of action. ALA exerts a major effect on the modulation of lipoproteins, whereas EPA and DHA decrease the synthesis of triglycerides and adiposity (Poudyal *et al.,* 2011). Moreover, as an essential fatty acid, ALA can be converted into EPA and DHA, and LA is a direct precursor of pro-inflammatory arachidonic acid (AA).

A reduction in the dietary ω-6: ω-3 ratio can decrease the risk factors for developing metabolic syndrome. The effect of ω-6 fatty acids on cardiovascular disease is still controversial. Some studies attribute the cardio protective properties of ω-6 fatty acids to their ability to decrease LDL-c levels, while others argue that the pro-inflammatory action of specific eicosanoids derived from AA is harmful. Irrespective of the amount of dietary ω-6 fatty acid, there is growing acceptance that the inclusion of high levels of metabolically more active ALA and EPA, in addition to DHA, is important to reduce the risks of cardiovascular diseases (Broughton *et al.,* 2010).

The results of table 3 in the present study illustrated that,Cholesterol-enriched diet significantly (p<0.05) increased serum urea, creatinine, sodium and potassium levels compared to healthy control (BD). These results are in accordance with those of Coritsidis *et al*., 1991 who demonstrated that Hyperlipidaemia might mediate renal injury by directly acting on the resident cells of the kidneys. The glomerulus and the renal tubulointerstitium may be preferred locations for lipid deposition and interaction with resident cells because of the lack of a basement membrane separating the mesangium and the capillary stream, and the presence of fenestrated epithelium lining the glomerular and peritubular capillaries. Thus, lipids can easily access these areas and influence local metabolism.

Abnormalities in lipid metabolism appear to play a pathogenic role in progressive renal diseases (Vazquez- Perez *et al*., 2001) and hypercholesterolaemia is considered an independent risk factor for renal injuries (Oda and Keane, 1999). Some studies based on animal models have provided evidence that there is a pathogenetic relationship between the elevated plasma lipid levels and renal injuries (Diamond, 1991; Schlondorff, 1993)

In the kidney, abnormal lipid metabolism can modify and accelerate glomerular and vascular damage, and the loss of the glomerular filtration barrier function is manifested by a clinical sign, proteinuria. Recent studies have shown that the slit diaphragms between interconnecting foot processes of glomerular epithelial cells (podocytes) are directly involved in the maintenance of an effective filtration barrier (Kasiske, 1987).

HCD is well known to cause nephrotoxicity and renal injury in different animal models. In the present study, signs of increased renal oxidative damage induced by HCD supplementation for four weeks were studied. Serum creatinine and urea levels were significantly increased (p<0.05) in HCD group as compared to control animals (BD).

On contrast to our results Kasiske *et al.* (1990) reported that, HCD supplementation to rats did not change the creatinine levels although kidney injury was reported (Kasiske *et al.,* 1990) Moreover, HCD feeding for eight weeks could not significantly altered the plasma creatinine levels in rats (Gamal El-din *et al.,* 2011).

The HCD induced-nephrotoxicity reported in the present study may be due to increased rate of oxidative stress and lipid peroxidation in the kidneys, which are kwon to potentiate generation of reactive oxygen species (ROS) and renal injury.

Consumption of seeds mixture rich in omega-3 and omega-6 fatty acids by hypercholesterolemic rats resulted in a significant improvement in renal function. These results are in agreement with a recent study which indicated that flaxseed oil treatment markedly reduced the degeneration in the renal tissue of hypercholesterolaemic rats (Kpolat *et al.,* 2011).

Flaxseed has demonstrated useful anti-inflammatory and antioxidative properties in a number of animal models and human diseases. Flaxseed may also inhibit sclerosis and formation of scar tissue. Flax lignans were highly protective "in a dose-dependent fashion, by a significant delay in the onset of proteinuria with preservation in glomerular filtration rate and renal size." The study suggested that flax lignans "may have a therapeutic role in lupus nephritis."((Velasquez *et al.,* 2001).

Also it was reported that, the administration of Flax and Pumpkin seeds mixture through the diet of diabetic rats improved the renal histological alterations induced by alloxan, which could be attributed to its antiradical/antioxidant activities (Makni *et al.,* 2010).

Decreased levels of urea and creatinine in the purslane treated animals may be due to its antioxidant potential (Shirwaikar *et al.,* 2003).

In this study, sesame seed supplement might attenuates oxidative-stress-associated renal injury by reducing oxygen free radicals and lipid peroxidation.

Sesame oil has been reported to inhibits oxidative stress and shorten the recovery period and allow the regeneration of renal tubules after the onset of gentamicin-induced renal injury in rats (Periasamy et al., 2010).

In this study the peanut added to seed mixture lower, serum urea and creatinine levels; this may be explained by the ability of some antioxidant in peanut to scavenge free radicals generated by HCD, which would otherwise cause kidney damage.

Previous Studies suggested that there might be an association between polyunsaturated fatty acids (PUFA) and the development of chronic kidney disease. PUFA supplementation has been shown to reduce renal inflammation and fibrosis in animal models. Both, omega-3 and omega-6 fatty acids increase levels of prostacyclin PGI3 and PGI2, respectively, which are active and potent vasodilators. Omega-3 polyunsaturated fatty acids are generally considered more beneficial than omega-6 fatty acids. However, data showed that both omega-6 and omega-3 fatty acids have anti-inflammatory properties, that a diet rich in PUFA may be protective against the decline in renal function (LauretanIi et al., 2009)

The results of the present study illustrated a significant increase in serum MDA and significant decrease in serum SOD and liver GPX on hypercholesterolemic rats. In accordance with our results kidney level of MDA, a specific lipid peroxidation marker was elevated while GSH level was decreased after four weeks of HCD diet administration to rats. Previous data showed HCD administration to cause hyperlipidemia and to be associated with oxidative stress and nitric oxide inactivation by ROS, which diminishes nitric oxide (NO) bioavailability leading to renal dysfunction (Amin *et al.,* 2011) Furthermore, HCD elevated brain, kidney and erythrocytes levels of lipid peroxidation products while decreased GSH content (Montilla *et al.,* 2006). It was reported that HCD induces modification in lipid composition of cell membranes and the extracellular matrix to be more prone to free radical generation (Gwinner *et al.,* 2000).

Oxidative injury, due to free radicals, is associated with several diseases including diabetes, cardiovascular diseases and hypertension (Russo *et al.,* 1998; Vendemiale *et al.,* 1999). The administration of antioxidants resulted in improved status in both patients and animal model (Galley *et al.,* 1997).

The protective role of glutathione, as an antioxidant and detoxifying agent, has been demonstrated in various clinical studies (Simopoulos, 2004). It is a ubiquitous compound that is synthesized rapidly in the liver, kidney and other tissues, including the gastrointestinal tract. In animal cells, glutathione acts as a substrate for glutathione peroxidase, which reduces lipid peroxides that are formed from polyunsaturated fatty acids (PUFA) in the diet and as a substrate for glutathione-S- transferase, which conjugates electrophilic compounds. Many evidences showed that glutathione obtained from the diet is directly absorbed by the gastrointestinal tract and thus dietary glutathione can readily increase the antioxidant status in humans (Jones *et al.,* 1989).

The antioxidant enzymes such as GPx, GR, SOD and GST, take part in maintaining GSH homeostasis in tissues (Abdel-Moneim *et al.,* 2010).

Makni *et al.* (2010) investigated the hypoglycaemic and antioxidant effects of flax and pumpkin seed mixture on the kidney of alloxan-induced diabetic rats. The characteristic histopathological changes were less pronounced as the supplement ameliorated the antioxidant enzymes CAT, SOD and GSH and decreased MDA levels. The increases in glucose, total lipid, total cholesterol and triglycerides in plasma were significantly subdued. Further, Makni *et al.,* 2011 observed that a pumpkin seed oil diet attenuated the increased levels of the plasma enzymes aspartate aminotransferase and alanine aminotransferase that pose a risk of diabetes. Its use in regular food may be effective in the prevention of diabetes and its complications.

It have been described that plasma and liver antioxidant enzyme activities may be modulated by consumption of seeds mixture of Flax and Pumpkin in hypercholesterolemic rats (Makni *et al.,* 2008). The antioxidant defence system in plasma and liver including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) enzymes may be modulated by nutritional factors (Huang *et al.,* 1994).

F/S and F/A diets have exerted marked antioxidant effect as compared to hypercholesterolemic rats. These data corroborated with other findings and support suggestions that seeds with high antioxidant capacity are biologically more active than other seeds with low antioxidant capacity, (Ruiz-Gutierrez *et al.,* 1999).

In agreement with previous reports (Visavadiya and Narasimhacharya, 2007), this study revealed a decrease in the activities of the antioxidant enzymes serum SOD, and liver GPx in plasma and liver of hypercholesterolemic rats, as compared to those of controls. Such decreases may be associated with the production of a-, b-unsaturated aldehydes during lipid peroxidation. These compounds have the ability to increase oxidative stress by promoting the cellular consumption of glutathione and by inactivating selenium-dependent glutathione peroxidase (Kinter and Roberts, 1996).

In our study, increased levels of serum SOD and liver GPX were found to correlate depressed MDA in rats, showing the antioxidant activity of seeds mixture.

Purslane is also reported as an excellent source of the antioxidant vitamins α-tocopherol, ascorbic acid and β- carotene, as well as glutathione. Purslane is considered as a rich source of many amino acids like isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine and valine. Purslane has been described as a "power food of the future" because of its high nutritive and antioxidant properties (Dkhil1 *et al.,* 2011)

purslane is a plant with good nutritional and medicinal potential and it is used for its beneficial effects. Hao *et al.* (2009) reported that purslane can be used as a medicinal plant where it is used for anti-aging, thereby increasing the level of SOD and decreasing the level of MDA in the brains of mice treated with D-galactosamine.

Purslane is a potent antioxidant and is reported to contain omega-3 fatty acids (Mohamed and Hussein, 1994). The increase in antioxidant enzyme activities in serum and liver in the current study were possibly due to the antioxidants present in purslane which act against oxidative stress.

The ratio of omega-6 to omega-3 EFA is an important determinant of health, because both omega-6 and omega-3 fatty acids influence gene expression. The balance of omega-6 and omega-3 fatty acids is very important for homeostasis and disease prevention.

5- Conclusion:

In summary, it appears that consumption of diets rich in **ω**-3 and **ω**-6 PUFAs (seeds mixtures of F/P, F/S, F/A, P/P, P/S or P/A), increase the activity of some antioxidant enzymes, improve lipid metabolism, improve renal function and reduce the risk of free radical damage in the hypercholesterolemic rats.

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