Comparison the Anti-atherogenic and hypolipidaemic effect of Cod liver oil, pomegranate seed oil and Rosuvastatin in hyperecholes-trolemic rats

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Abstract

Rosuvastatin known is the more statin moderating the lipid profile by reduction the low density-lipoprotein than the other statin such as atorvastatin, simvastatin and par-vostatin . The present study was designed to investigate the anti-atherogenic and the hypolipidemic effect of cod liver oil, pomegranate seed oil and rosuvastatin in hyper-cholesterolemic rats. Thirty five albino wister rats were divided randomly into five equal groups, the first group served as negative control, all rats were administrated normal saline. The second group as positive control were rats administrated high cho-lesterol diet for six weeks. The animals in the third, fourth and fifth groups were ad-ministrated for 6 weeks as fallow: cod liver oil (0.5 mg/kg rat per day), pomegranate seed oil (200 mg/kg/day) and rosuvastatin (0.5 mg/kg/day, orally), respectively; In addition high cholesterol diet. The result revealed the high cholesterol diet in the pos-itive control group caused a significant ($p \leq 0.05$) alteration in the lipid profile by in-creasing the serum total cholesterol (TC), Low density lipoprotein(LDL-c), very low density lipoprotein (VLDL-c) and triglyceride (TGs) as well as significant ($p \leq 0.05$) decrease the High-density lipoprotein. Also, the result showed a significant ($p \leq 0.05$) increase atherogenic indices by increase the coronary risk index (TC/HDL-C), ather-ogenic Coefficient (TC-HDL-C/HDL-C) and Atherogenic index of serum Log(TG/HDL-C) compare with negative control group. Indicated results significant ($p < 0.05$) decrease in the level of serum glutathione (GSH) and significant ($p \leq 0.05$) increase in the level of malondialdehyde (MDA). While the animals in the group 3,4 and 5 were administrated CLO, PSO and statin the result clarified a significant ($p \leq 0.05$) improvement in studied parameters , through decrease the TC, LDL-C, VLDL-C, TG, MDA and atherogenic indices , and increase the HDL-C and GSH compared with negative and positive control groups.

Key words : Cod liver oil, Pomegranate seed oil, Lipid profile, Rosuvastatin, Rats

مقارنة التأثير المضاد لتصلب الشرايين والخافض للدهون بين زيت كبد سمك القد، زيت بذور الرمان و الروزوفاستاتين في الفئران عالية الكولسترول

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المستخلص

يعرف عقار ال Rosuvastatin بأنه من اكثر انواع العقاقير التي تعمل على تحسين الصورة الدهنية عن طريق انخفاض البروتين الدهني واطئ الكثافة LDL–C مقارنة مع بقية العقاقير المستخدمة لهذا الغرض مثل atorvastatin ، Simvastatin و Parvostatin . صممت الدراسة الحالية لمقارنة التأثير المضاد للتصلب العصيدي والمقلل للدهون بين كلا من زيت سمك القد CLO ، زيت بذور الرمان PSO و ال Rosuvastatin في الجرذان المختبرية المعاملة بوجبة عالية الكولسترول . تم استخدام 35 من الجرذان البيض المختبرية وقسمت عشوائيا الى خمسة مجاميع متساوية . المجموعة الاولى عدت مجموعة سيطرة سالبة حيث تم اعطاء الحيوانات المحلول الفسيولوجي فقط . المجموعة الثانية عدت مجموعة سيطرة موجبة وعوملت الحيوانات بوجبة عالية الكولسترول ولمدة 6 اسابيع . اما المجاميع 3 ، 4 ، وال 5 جرعت ب زيت سمك القد (0,5 ملغم /كغم) ، زيت بذور الرمان (200 ملغم /كغم) وال Rosuvastatin (0,5 ملغم/ كغم) على التوالي بالاضافة الى معاملتها بوجبة عالية الكولسترول ولمدة 6 اسابيع . اضهرت النتائج بأن الغذاء العالي الكولسترول في مجموعة السيطرة الموجبة عمل على تغيير معنوي ($P\leq0.05$) في الصورة الدهنية عن طريق زيادة مستوى الكولسترول الكلي TC ، الكلسريدات الثلاثية TG ، االبروتين الدهني واطئ الكثافة LDL–C والبروتين الدهني الواطئ الكثافة جدا VLDL–C بالاضافة الى انخفاض معنوي في مستوى البروتين الدهني عالي الكثافة HDL–C . كذلك اضهرت النتائج زيادة معنوية ($P\leq0.05$) في مؤشرات التصلب العصيدي عن طريق ارتفاع مؤشر خطورة الشرايين التاجية ، معامل التعصد و مؤشر التصلب العصيدي في المصل مقارنة مع مجموعة السيطرة السالبة . بالاضافة الى ذلك اشارت النتائج الى انخفاض معنوي ($P\leq0.05$) في مستوى الكلوتاثايون المختزل GSH وزيادة معنوية ($P\leq0.05$) في مستوى المالندايلديهايد MDA . في حين جاءت النتائج حيوانات المجموعة 3، 4 و 5 والتي جرعت يوميا CLO , PSO و Rosuvastatin بتحسين العايير التي تم دراستها اعلاه من خلال الانخفاض المعنوي ($P\leq0.05$) في TG, TC، LDL–C ، VLDL–C ، MDA وال دلائل التصلب العصيدي ، وزيادة معنوية ($P\leq0.05$) في مستوى GSH وال HDL–C بالمقارنة مع مجموعة السيطرة الموجبة والتي غذيت على وجبة عنية بالكولسترول .

الكلمات المفتاحية : زيت كبد سمك القد ، زيت بذور الرمان ، فحص الدهون ، روزوفاستاتين، الجرذان .

Introduction

Cardiovascular disease (CVD) is the most common health problem worldwide. This disease often manifested as coronary heart disease (CHD). Atherosclerosis is the main cause of CHD, atherosclerosis and cardiovascular disease associated with hyperlipidemia and disturbance of lipoprotein and plasma lipids (7) . Dyslipidemia as a risk factor of cardiovascular disease, is manifested by elevation of plasma lipoprotein concentration. Thus, several studies revealed that hypercholesterolemia and possible coronary atherosclerosis are suggested as the sole risk factor of ischemic heart disease (21) . These facts proven by Musunuru (32) who shown that many individual with normal LDL-c level nevertheless develop cardiovascular disease . Statin are usually the drug of choice as they are easly to take and have lesser interaction with other drugs. Statin are HMG-Coa reductase inhibitor . HMG-CoA reductase is an enzyme located in the liver tissue and produce mevelonate which is a small molecule used in the synthesis of cholesterol . In spite of its benefits, it has many side effect such as stomach upset, joint pain and liver damage. Statin lower cholesterol level in people at risk of coronary vascular disease because of hypercholesterolemia . As atherosclerosis is considered to be inflammatory disease (4) . Rosuvastatin is a widely used HMG-CoA reductase inhibitor (statin). This drug were reported to reduces LDL-C concentration and help patients to achieve the LDL-C target in the japan atherosclerosis society, it is interesting to note that the decrease of LDL-c and increase of HDL-C achieved by rosuvastatin seen to be greater than those achieved with other statin (29, 55).

The medicinal plants widely used against several disease, because of their popularity, low adverse effect make the medicinal plant alternative sources of medicinal compound . Pomegranate as a medicinal and nutritional fruit that has many chemical compound include vitamins, poly saccharides, minerals, poly phenols and carbohydrate (2,19). Pomegranate seed oil is important source of poly unsaturated fatty acid (PUFA) including linolenic acid such as punicic acid which is contained about 72% in pomegranate seed oil (51) , which has anti atherogenic effects by decreasing Triglyceride and TAG/HDL-c after 4 week of treatment with 400 mg of pomegranate seed oil (26) , as well as, PSO contain a low saturated fatty acid which is important for therapeutic uses in human health (12). The presence of different substances with various chemical structures reveals multiple therapeutic effect of pomegranate. "Pomegranate seed oil have chemo-preventive activity against many types of cancer such as prostate, breast and colon cancer (6, 37) . pomegranate seed oil has antioxidant and anti-inflammatory activity , due to present many poly phenolic compound such as Ellagic acid and Gallic acid (23). Many references revealed that PSO consist of about 80% conjugated fatty including punicic acid that have hypolipidemic role (54, 56). The disturbance of lipoproteins particularly Low density-lipoprotein (LDL-C) the main cause in the development of atherosclerosis" (20). Tahmasbi et al (52)"showed that the PSO could suppress the ovarian deficiency induced serum lipid and antioxidant stress and improve the antioxidant status to the overiectomized rats".

Cod liver oil a rich source of omega -3 poly unsaturated fatty acid (PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) they "are play important and vital role in the normal function of many tissue cells such as heart , blood vessels , eyes, and nerve and it has lowering lipid effect by increase HDL-c and decrease LDL-c in plasma and particularly have anti-thrombotic, anti-inflammatory effect and providing anti atherosclerosis properties (8, 29, 35).several studies clarified that Polyunsaturated fatty acids (PUFAs) play an important role in treatment and prevention of certain disease such as coronary heart disease, hypertension, and renal disease and have anti arrhythmic,hypolipidemic and vasodilator properities (8, 35, 49).The aim of the present study to compare which of the above medical substances has a potent beneficial effects as hypolipidemic and ati-atherogenic effect in hypercholesterollemic rats.

❖ Materials and methods

• Experimental design and blood collection

Thirty five albino wister rats were used , 2.5-3 months age, were obtained from national center for drug control and research . the rats were haused in a plastic cages 7 rats/cage under constant environmental condition (22-25 c) , 12 h/ dark/light cycle and free access to drink water *ad libitum*.Animal were fed on cotrol diet for 6 weeks as follow : Group1 (negative control) administrated normal saline and were fed on normal control diet.Group2 (positive control)(atherogenic control) animals were fed with high cholesterol diet for 6 weeks , animals in group 3,4 and 5 administrated, in addition to the cholesterol-rich diet, were administrated for 6 weeks as fallow: cod liver oil (0.5 mg/kg rat per day)(44) pomegranate seed oil (200 mg/kg/ day)(52) , and rosuvastatin (0.5 mg/kg/day, orally)(15) , respectively. At the end of the experiment, the animals were anesthetized by chloroform and blood was drawn via cardiac puncture technique using disposable medical syringe (5 ml.) blood samples were kept with sterilized tube without anticoagulant, and left for 15-30 mints at room temperature 25C to allow to blood coagulation, and serum was collected by centrifugation the samples at (3000 rpm) for 15 minutes and frozen at -20 C for biochemical testes

Biochemical analysis

Measurement of total cholesterol"(TC), triglyceride (TG), High density-lipoprotein (HDL-C) by using commercial kits [Biomerieux, france and Randox, UK] , low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) was calculated by Friedwald equation (13) as follow : LDL-C=TC — HDL-C — VLDL, were VLDL = TG/5 . Serum reduced Glutathione"(GSH) and serum malondialdehyde (MDA) were measured by method of Roberts and Francetic (42)

Atherogenic indices calculated as follow :

- Coronary Risk Index = TC/HDL-C (22).
- Atherogenic Coefficient TC – HDL-C/HDL-C (5).
- Atherogenic Index of plasma = Log (TG/HDL-C) (9) .

Statistical analysis

Statistical analysis was performed by using SPSS with P value < 0.05 as statistically significance, one way anova was used to compare between the means of different groups. The data was expressed as mean ± slander error (48) .

❖ Results and Discussion

Lipid profile

Table and figure (1) illustrated the mean values of lipid profile of negative control and other treated groups. The positive control (atherogenic control) treated with high lipid content diet show a significant ($p < 0.05$) increased in the mean values of serum total TC, TG, LDL-C and VLDL-C and decrease HDL-C compare to the mean value of negative control rats treated with normal diet . on other hand , treatment of rats in the groups 3,4 and 5 with CLO, PSO and Rosuvaststin ,respectively shown improvement of studying parameters and there were no significant ($p > 0.05$) differences compared with negative control, particularly rats treated with PSO and rosuvastatin in which they revealed a significant ($p < 0.05$) decrease in TC, and LDL compare with other treated groups.

Table (1):Effect of CLO, PSO and Rosuvastatin in lipid profile of hyperecholestrolemic rats .

Test / Group	Total Cholesterol mg/dl	Triglyceride mg/dl	LDL-C	VLDL-C	HDL-C
–Ve Control	75.83±1.47 A	57.76±2.53 A	24.41±1.69 A	11.55±0.50 A	39.86±2.59 A
+Ve Control	103.6±3.15 B	105.66±3.40 B	62.83±4.02 B	21.13±0.68 B	19.48±0.92 B
CLO Group3	71.65±1.93 AC	59.03±2.85 A	22.06±1.90 AC	11.80±0.57 A	37.7±1.98 A
PSO Group4	69.55±1.54 C	58.01±2.30 A	18.94±1.30 AC	11.12±0.42 A	39.0±1.66 A
Rosuva. Group5	68.56±1.53 C	53.70±1.40 A	16.06±0.97 C	10.74±0.39 A	42.15±1.43 A

Values are expressed as mean ± SE , n= 7 each group; -Ve Control : Animals received normal saline .+ Ve Control: Rats administrated high cholesterol diet .;Group3(CLO): animals administrated high cholesterol diet and Cod liver oil (0.5 mg/kg rat per day) ; Group 4(PSO): animals administrated high cholesterol diet and Pomegranate seed oil (200 mg/kg/day) Group5(Rosuv.): animals administrated high cholesterol diet and rosuvastatin (0.5 mg/kg/day)Capital letters denote differences between groups, P<0.05.

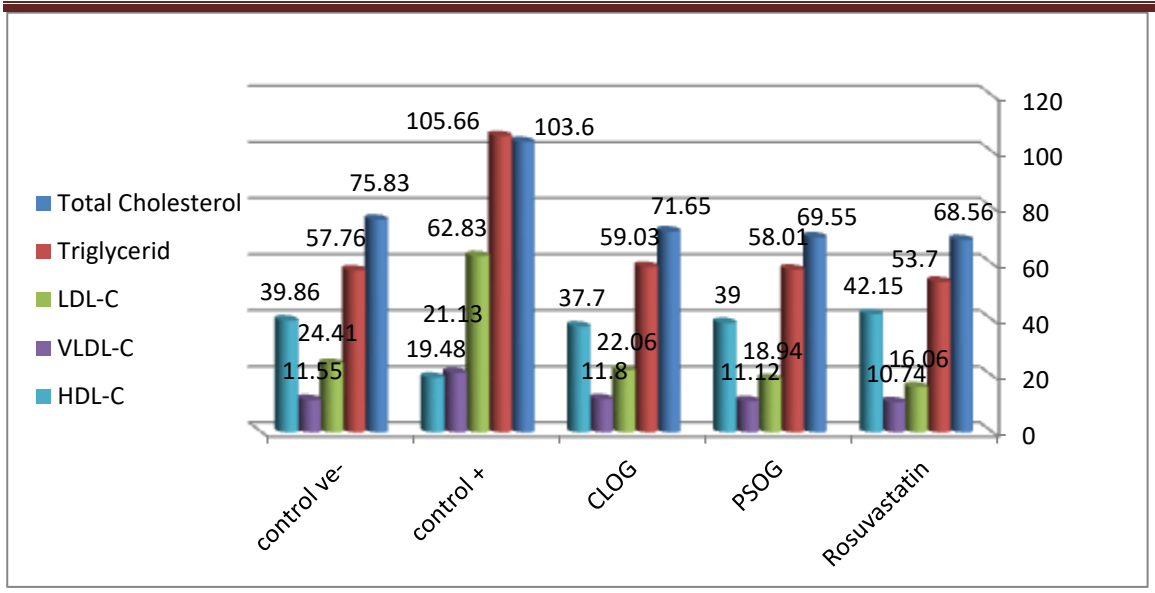

Figure (1): the effect of dyslipidemia, CLO, PSO and Rosuvastatin on lipid profile.

Atherogenic Indices

Statistical analysis of the results of atherogenic indices clarified in the table and figure (2). Treatment of rats in a positive control group (atherogenic control) with high cholesterol diet lead to dyslipidemia of animals which represented by significant ($P< 0.05$) increase in the all atherogenic indices parameters which include Coronary risk index (TC /HDL-C), Atherogenic Coefficient (TC-HDL-C/HDL-C) and Atherogenic index of plasma , Log (TG /HDL-C) as comparing with negative control . The data pointed to the ameliorating and the absence of statically differences ($P>0.05$) between group3 , group 4 and group5 after treated the rats with CLO, PSO and rosuvaststin , respectively comparing with negative control group, especially, the pomegranate seed oil and rosuvastatin treated animals as mean values compared with control negative group .

Table (2) : Effect of CLO, PSO, and Rosuvastatin on atherogenic indices of hyperecholestrolemic rats.

Test / Group	Coronary risk index	Atherogenic Coefficient	Atherogenic index of plasma
–Ve Control	1.93±0.10 A	0.935±0.10 A	0.163±0.04 AC
+Ve Control	5.41±0.42 B	4.410±0.42 B	0.735±0.02 B
CLO Group	1.92±0.09 A	0.920±0.09 A	0.195±0.03 A
PSO Group	1.79±0.04 A	0.792±0.05 A	0.173±0.02 AC
Rosuva. Group	1.64±0.04 A	0.646±0.04 A	0.105±0.02 C

Values are expressed as mean ± SE , n= 7 each group; -Ve Control : Animals received normal saline .+ Ve Control: Rats administrated high cholesterol diet .;Group3(CLO): animals administrated high cholesterol diet and Cod liver oil (0.5 mg/kg rat per day) ; Group 4(PSO): animals administrated high cholesterol diet and Pomegranate seed oil (200 mg/kg/day) Group5(Rosuv.): animals administrated high cholesterol diet and rosuvastatin (0.5 mg/kg/day)Capital letters denote differences between groups, P<0.05.

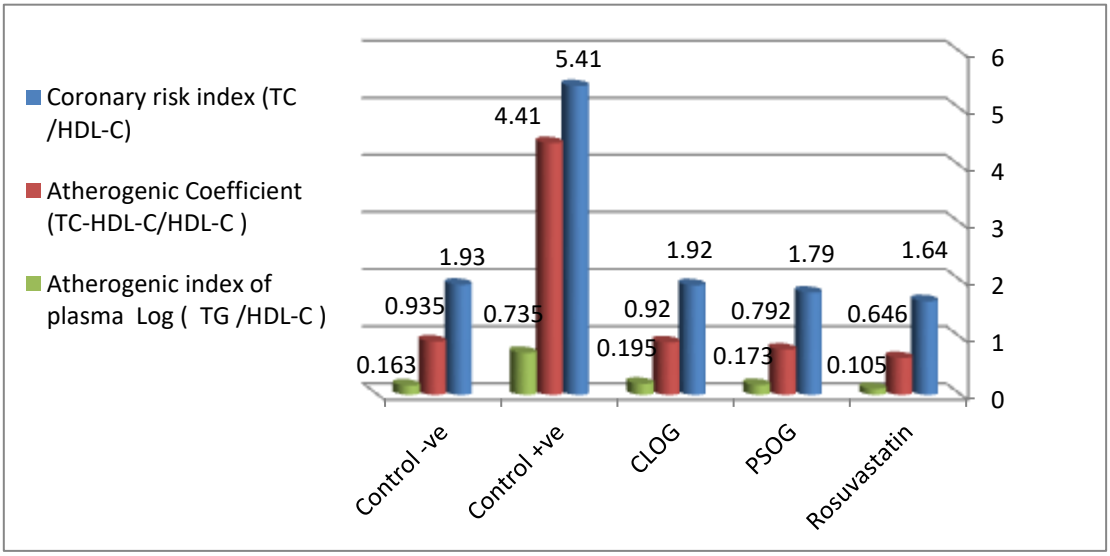

Figure (2): the effect of dyslipidemia , CLO, PSO and Rosuvastatin on Atherogenic indices

Oxidative stress parameters

The data pertaining to the mean values of serum GSH concentration and MDA concentration in the treated groups along the experimental period were depicted in the table and figure (3).The results revealed that there were a significant (P<0.05) differences in the mean values of serum GSH concentration and MDA concentration in the positive control group via decrease of serum GSH and Increase MDA concentration Compare to the negative control, while the results has been shown improvement of Serum oxidative status in the animals of others treated groups that administrated with Cod liver oil (group3) , pomegranate seed oil (group 4) and rosuvastatin (group 5) via

significant (P<0.05) decrease in MDA and increase of GSH as comparing with hypercholesterolemic rats in group 2.

Table (3) : Effect of CLO, PSO, and Rosuvastatin on reduced glutathione (GSH) and malondialdehyde (MDA) of hyperecholestrolemic rats.

Test \ Group	–Ve Control	+Ve Control	CLO Group	PSO Group	Rosuva. Group
MDA (mol/L)	0.34±0.02 A	1.10±0.05 B	0.44±0.03 C	0.38±0.02 AC	0.30±0.02 A
GSH (µmol/L)	20.75±1.22 A	14.80±0.82 B	18.38±1.08 AC	16.37±0.87 BC	19.15±0.67 AC

Values are expressed as mean ± SE , n= 7 each group; -Ve Control : Animals received normal saline .+ Ve Control: Rats administrated high cholesterol diet .;Group3(CLO): animals administrated high cholesterol diet and Cod liver oil (0.5 mg/kg rat per day) ; Group 4(PSO): animals administrated high cholesterol diet and Pomegranate seed oil (200 mg/kg/day) Group5(Rosuv.): animals administrated high cholesterol diet and rosuvastatin (0.5 mg/kg/day)Capital letters denote differences between groups, P<0.05.

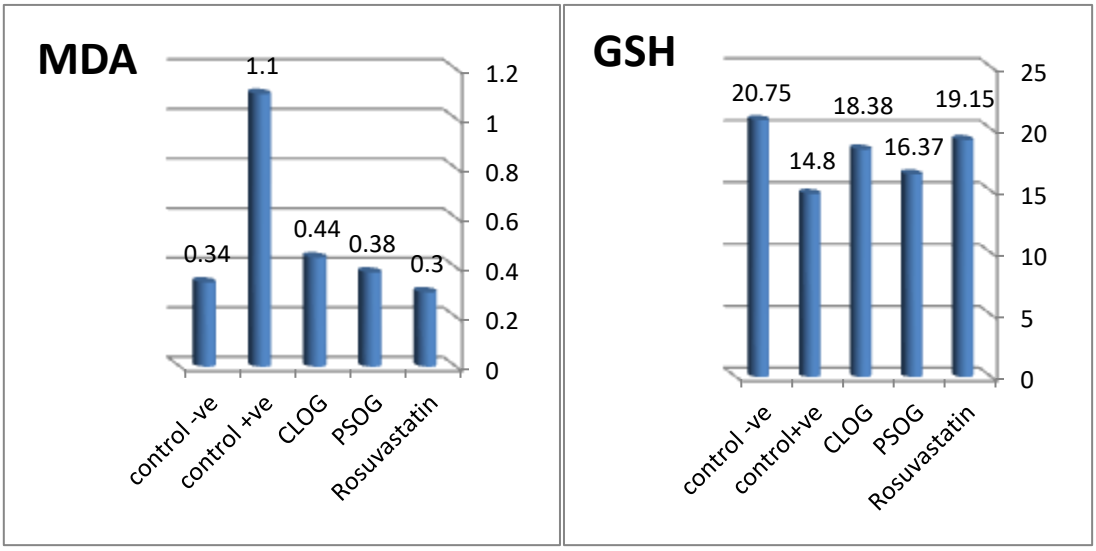

Figure (3) : the effect of dyslipidemia , CLO, PSO and Rosuvastatin on MDA and GSH

Discussion

Treatment of animals with high cholesterol diet led to increase of serum lipid profile and atherogenic indices, lipid profile often used in predicting diagnosis lipid relating disorder such as atherosclerosis. There is extensive evidence that relation between hypercholesterolemia and oxidative stress . The oxidative modification of lipoprotein particularly LDL-C has emerged as a fundamental process in the development of atherosclerosis (20) . Several studies have shown that blood level of LDL-C significantly predict incident atherosclerotic cardiovascular disease (CVD) , and LDL-C lowering therapy has been repeatedly demonstrated in many populations to

reduce CVD risk (16, 32) . An increase level of TG is both independent and synergistic risk factors for CVD (24). Many individual with normal LDL-C level nevertheless develop CVD (32). Many studies showed that atherogenic index of plasma (AIP) has been proposed as indicator of plasma atherogenicity, because inversely correlated with LDL-C and increased in people with cardiovascular disease (25) and this test easily available to measure response of treatment (14). "Recent study slowed the direct relation between high cholesterol / HDL-C (coronary risk index) with coronary artery disease (33). Treatment of animals with cod liver oil in group 3 led to improvement of lipid profile through decrease TC, TG, LDL,VLDL and increase HDL-C . Cod liver oil , a natural compound, is a particularly rich sources of omega-3-fatty acid include Ecosapentaenoic acid (EPA) and doxosahexaenoic acid (DHA) , there for the results of current study agreement with many authors who shown that cod liver oil improvement lipid profile in diabetic rats"(53). Cod liver oil has been antidyslipidemic and hypocholesterolemic activities through elevated HDL-C/LDL-C ratio which associated with low risk of disease events (18).EPA and DHA play essential role in the lower lipid effect and decrease TG and increase HDL-C in plasma and have anti-thrombotic and anti-inflammatory, protection against atherosclerosis (8, 35). The "hypocholesterolemic effect of CLO appear to be due mainly to a reduction in LDL-C concentration resulting from an increase in hepatic receptor dependent LDL-uptake"(25) , other possible contributor include reduced liver LDL secretion and increased liver receptor-mediated VLDL clearance (47) , as well as an increased cholesterol excretion via bile (46) . Dietary fish oils rich in omega-3 fatty acids have been proved to be effective in the lowering of the plasma triacylglycerol and lipoprotein (specially VLDL) levels in experimental animals, thereby being attributed a role in the prevention of cardiovascular disease (34)". On other hand PSO have lipid lowering effect on rats of group4. The beneficial effect of PSO may be related to the presence of a variety of biologically active compounds, particularly polyphenols which have been studied for their antioxidant effects "(27). The present results agreement with another study clarified that administration of diabetic rats for 8 weeks with pomegranate juice improve lipid profile and significant decrease in the total cholesterol and LDL-C and improve the TC/HDL-C , LDL-C /HDL-C ratio and increase the HDL-C ,these beneficial effects attributed to decreased absorption and increase fecal excretion of cholesterol as well as possible affects on HMG-CoA reductase and sterol acyltransferase, two enzymes key to cholesterol metabolism (11) .

The results of current study showed a significant increase ($p \leq 0.05$) in the serum concentration of Malonialdehyde (MDA) and sharp decline in the concentration of serum reduced glutathione (GSH) in rats treated with high cholesterol diet, these results may be due to stimulation of enzyme fatty acyle CoA oxidase and increase oxidation of fatty acids lead to increase reactive oxygen species (ROS) production involving H_2O_2 which in turn lead to increase lipid peroxidation that caused disturbance of cell membrane homeostasis and damage of cell, so MDA will be increased (3). A study was carried out by Murry etal., (31) shown that lipid peroxidation happen due to increase production of free radical , which lead to destruction of polyun-

saturated fatty acids of cell membrane, thus increase concentration of MDA. GSH is a water-soluble tripeptide; it is an important antioxidant and plays a major role in the detoxification of endogenous metabolic products, including lipid peroxides, and xenobiotic compounds including heavy metals, pollutants, and drugs. Intracellular glutathione exists in both the oxidized disulfide form (GSSG) or in reduced (GSH) state (1). GSH, by the catalytic action of glutathione peroxide, detoxifies peroxides and hydroxyl radicals into nontoxic forms. As a result, it converts into oxidized GSSG, and then by the action of glutathione reductase it recycles from GSSG to GSH (38). on other hand, Dyslipidemia lead to disturbance of antioxidant status causing oxidative stress, thus decrease of serum GSH may be due to participation of GSH in preventing of oxidation in oxidative stress status either by direct scavenger of free radicals or due to formation glutathione peroxidase (40, 50). hypercholesterolemia could increase the release of platelet activating factor (PAF), which in turn could increase the synthesis and release of IL-1 and TNF. PAF, IL-1, and TNF are known to stimulate polymorph nuclear leukocytes to produce free radicals, which would increase the lipid peroxidation products (36) . Treatment of rats in the group 3,4 and 5 with CLO, PSO and Rosuvaststin caused significant improvement of antioxidant parameters by decrease of MDA and increase GSH , the result of this study agreement with the results of Rivellese etal .(41) were they slowed that fish oil improvement the lipid profile and antioxidant enzymes in hypercholesterolemic patients . The antioxidant activity of pomegranate seed oil is contributed to tochopherol and poly phenolic compound contents (10) . Oxidative stress reducing agents of PSO have metallic chelating potential singlet oxygen quencher and hydrogen donors (45) . PSO contains ellagic acid, an antioxidant compound that removes peroxy radicals and prevents lipid peroxidation induced by Cu2+" (39) . Pomegranate has been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation which lead to decrease MDA . "The antioxidant proprieties of pomegranate confirmed by studies in rats and mice showing that increase the plasma antioxidant capacity and increased reduced glutathione GSH" (17, 43).

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