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Relationships between Pomegranate (Punica granatum) and Paraoxonase Enzyme to Prevent the Development of Atherosclerosis in Male White New Zealand Rabbits

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Abstract: The present study was carried out to investigate the effects of oral administration of Pomegranate Juice (PJ) by which contain of active compounds that stimulate the necessary enzyme and antioxidants production to reduce the effect of added cholesterol to male white New Zealand rabbits diet to initiate and development of atherosclerosis, twenty male white New Zealand rabbits aged 9-11 months, 1500-2000 g had been divided randomly in to four groups (5rabbits/ group), all groups had been given a standard ration with free water, first group had been orally drenched normal saline and considered as a control group, while the second group drenched 6ml Pomegranate juice(PJ) / kg b. wt., third group 0.26 % cholesterol added to rabbit's diet to initiate atherosclerosis, fourth group 0.26 % cholesterol added to rabbit's diet and drenched 6 ml PJ, the treatment for all groups continuous for two months. The results showed positive effects as a significant increase (P≤ 0.0001) in Paraoxonase1 enzyme (PON1), Total antioxidant capacity (TAC), HDL-c, in rabbits serum that drenched 6 ml PJ / kg b.wt. in contrast showed a negative effects as a significant decrease in PON1, TAC, and HDL-c in rabbits that given cholesterol while the results tend in rabbits that given cholesterol and drenched 6ml PJ to be in near with normal concentration in control group, C-reactive protein (CRP), TG, TC, LDL-c and VLDL-c significantly decreased (P≤ 0.0001) in rabbit's serum that drenched 6ml PJ and these parameters significantly increased in rabbit's serum that given cholesterol with diet, while the results tend in rabbit's serum that given cholesterol with diet and drenched 6ml PJ to be in near with normal concentration in control group.

INTRODUCTION

Atherosclerosis, a major degenerative disease of arteries involves a series of inflammatory and oxidative modification within the atrial wall [1], emerging researches shows that obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, aging, diets rich in saturated fats, and reduced activity are the established risk factors for atherosclerosis[2]-[3].

Oxidative stress is a state in which oxidation exceeds the antioxidant systems in the body, it is arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses against them, which intensifies cellular damage. The antioxidant defenses enable the body system to remove ROS, restore the prevailing reducing environment and repair the tissue damage[4]. Oxidative stress plays an important role in the etiology and pathogenesis of many chronic diseases such as atherosclerosis, hypertension diabetes mellitus, and cancers[5]. Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Therefore, it is important to enrich our diet with antioxidants to protect against many chronic diseases. Antioxidants also play an important role in food quality preservation due to their ability to prevent oxidative deterioration of lipids [6].

The Pomegranate tree, which is believed to be Flourished in the Garden of Eden [7], this tree belonging to the Punicaceae family, Pomegranate juice has become increasingly popular because of its important biological actions [8], most of these effects were attributed to its high phenolic content [9], (such as flavonoids, phenolic acids, diterpenes,

saponins and tannins) have received much attention for their high antioxidative activity by scavenging free radicals which cause oxidative stress that can lead to cellular damage and many degenerative disorders[10].Flavonoid significantly reduce oxidative stress by inhibiting formation of LDL lipoproteins and macrophage lipid peroxidation, and in this mechanism atherosclerosis is reduced [11], also PJ decreased LDL susceptibility to aggregation and retention, increased total antioxidant status[12] and increased serum PON1 activity, a HDL associated esterase that can protect against lipid peroxidation by 20%, and suppressed oxidized LDL degradation and cholesterol biosynthesis in macrophage that can lead to reduced cellular cholesterol accumulation and foam cell formation[13]. Paraoxonase-1is a protein of 354 amino acids with a molecular mass of 43 kDa [14]. In serum, it is almost exclusively located on HDL. It is highly conserved in mammals but is absent in fish, birds, and invertebrates, such as arthropods. PON1 has recently emerged as the component of HDL most likely to explain its ability to metabolize lipid peroxides and to protect against their accumulation on LDL. The present review will consider first the antioxidant role of HDL in the context of its other potential antiatherogenic actions and then the evidence that PON1 is indeed responsible for the capacity of HDL to metabolize lipid peroxides before finally discussing the evidence that PON1 is linked with clinically evident atherosclerosis [15].

The Aim of Study

The present study was performed to investigate the effect of oral administration of Pomegranate juice at dosage 6ml / kg body weight (b.w.) on rabbit's serum concentrations of PON1,TAC, CRP, TG, TC, HDL-c, LDL-c and VLDL-c to avoid the initiation and development of experimental atherosclerosis by added cholesterol (0.26%) in rabbit's diet.

MATERIAL AND METHODS

Cholesterol: Cholesterol was purchased from France (BDH) company, in the form of white crystalline powder.

Rabbits: A total number of twenty adult males white New Zealand rabbits aged 9-11 months, and weighed 1500-2000 grams were used in this study. The rabbits were obtained from College of Veterinary Medicine, Mosul University.

Standard ration: The basal diet for rabbits was prepared according to National Research Council [16].

Kits for Biochemical Analysis:

Commercial diagnostic kits for estimating serum PON1 and TAC were obtained from MyBioSource company, U.S.A., and the kit for estimating CRP were obtained from AccuBind company depended on ELISA. TG, TC, HDL-c kits were obtained from refletron company, Germany, while VLDL-c and LDL-c obtained from the mathematical equations :

VLDL-c = TG/ 5

]TG/5 – HDL-c – [TC =LDL-c

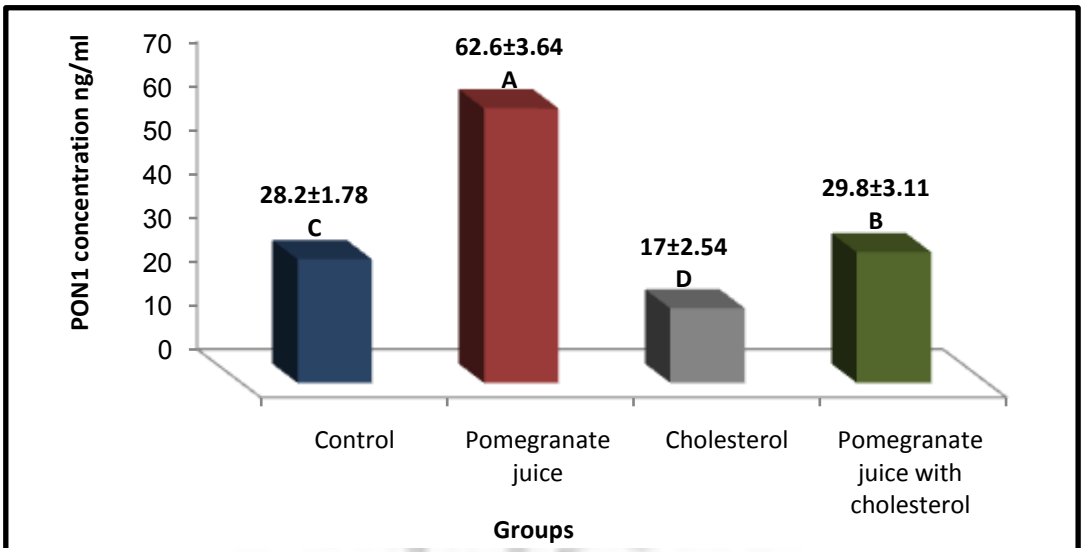
Pomegranate: Ripe Pomegranate fruits used in this study were purchased from a local market, washed and manually peeled without separating the seeds, pomegranate juice was obtained using a commercial blender, filtrated with a Buchner funnel [17]

Statistical analysis

Statistical analysis were done using one way ANOVA analysis of variance using the General Linear Model, SAS software [18] .Duncan, was used to separate the means when significant differences exist. Means and standard deviations were calculated for all parameter. Difference were considered significant at $P \leq 0.0001$.

RESULTS

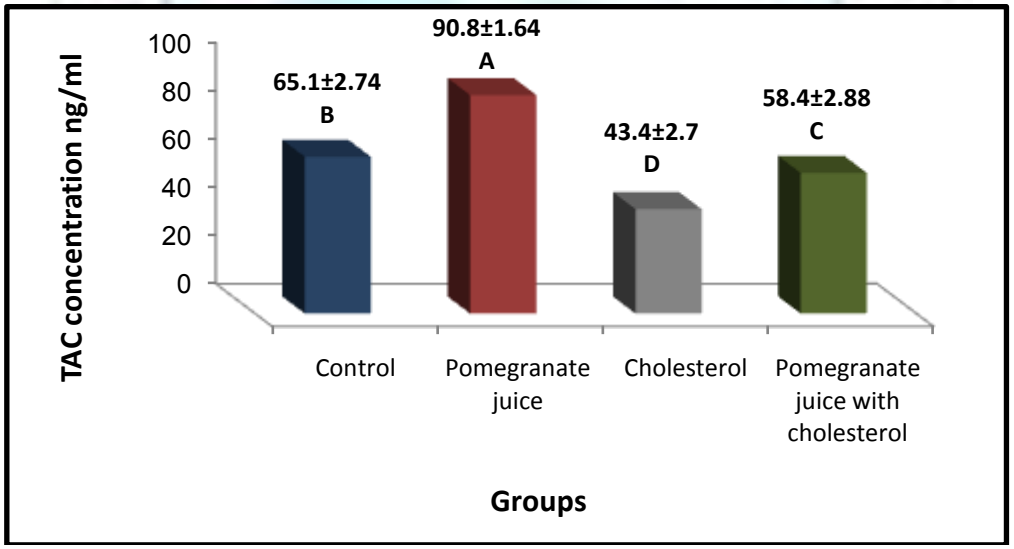
Figure (1) demonstrates a significant increase ($P \leq 0.0001$) in PON1enzyme in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (62.6±3.64) ng/ml, this figure also shows a significant decrease in PON1enzyme in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (28.2±1.78) ng/ml, while the mean of this group was (17±2.54) ng/ml, also this figure explains a significant relative increase in concentration of this enzyme in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (29.8±3.11) ng/ml.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (1): The effect of treatment by PJ and Cholesterol on the concentration of PON1 enzyme

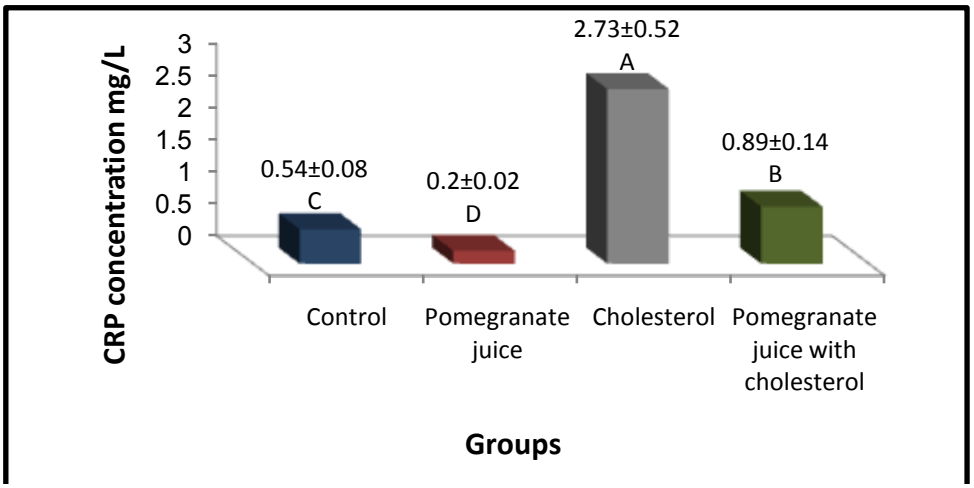
Figure (2) demonstrates a significant increase (P≤ 0.0001) in TAC in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (90.8±1.64) ng/ml, this figure also shows a significant decrease in TAC in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (65.1±2.74) ng/ml, while the mean of this group was (43.4±2.7) ng/ml, also this figure explains a significant relative increase in concentration of this parameter in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (58.4±2.88)ng/ml.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (2): The effect of treatment by PJ and Cholesterol on the concentration of TAC

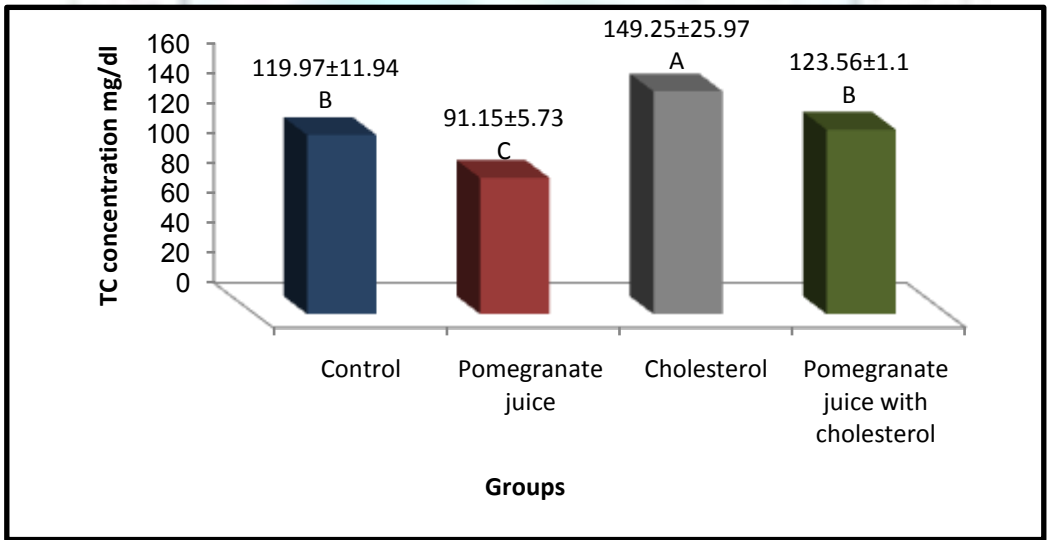
Figure (3) demonstrates a significant decrease (P≤ 0.0001) in CRP in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (0.2± 0.02) mg/L, this figure also shows a significant increase in CRP in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (0.54± 0.08) mg/L, while the mean of this group was (2.73± 0.52) mg/L, also this figure explains a significant relative decrease in concentration of this protein in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (0.89±0.14)mg/L.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (3): The effect of treatment by PJ and Cholesterol on the concentration of CRP

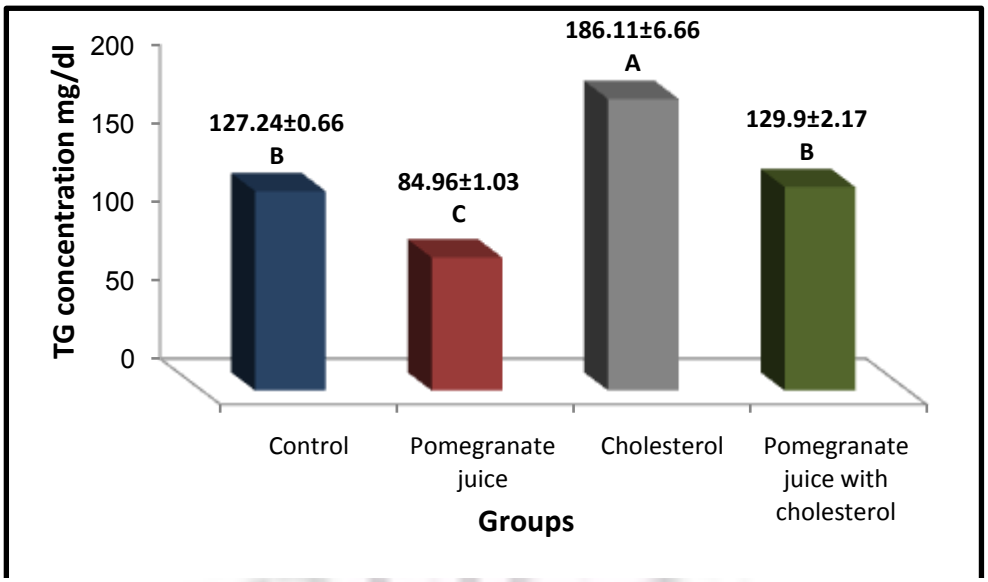
Figure (4) demonstrates a significant increase (P≤ 0.0001) in TC in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (91.15±5.73) mg/dl, this figure also shows a significant decrease in TC in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (119.97±11. 94) mg/dl, while the mean of this group was (149.25±25.97) mg/dl, this figure also explains a decrease not significant in concentration of this parameter in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (123.56±1.1) mg/dl.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (4): The effect of treatment by PJ and Cholesterol on the concentration of TC

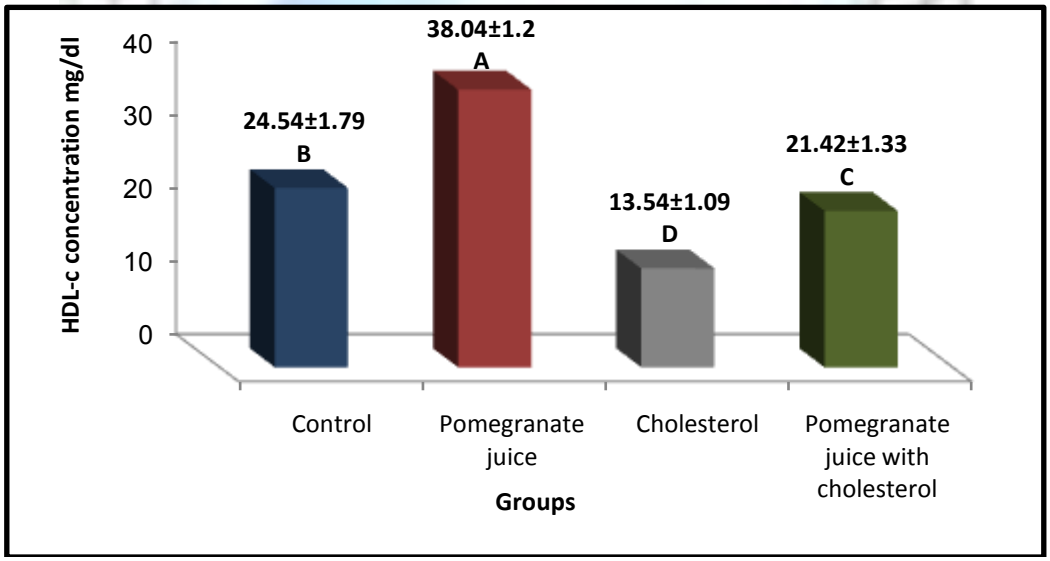
Figure (5) demonstrates a significant decrease (P≤ 0.0001) in TG in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (84.96±1.03) mg/dl, this figure also shows a significant increase in TG in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (127.24±0.66) mg/dl, while the mean of this group was (186.11±6.66) mg/dl, this figure also explains a decrease not significant in concentration of this lipoprotein in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (129.9±2.17) mg/dl.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (5): The effect of treatment by PJ and Cholesterol on the concentration of TG

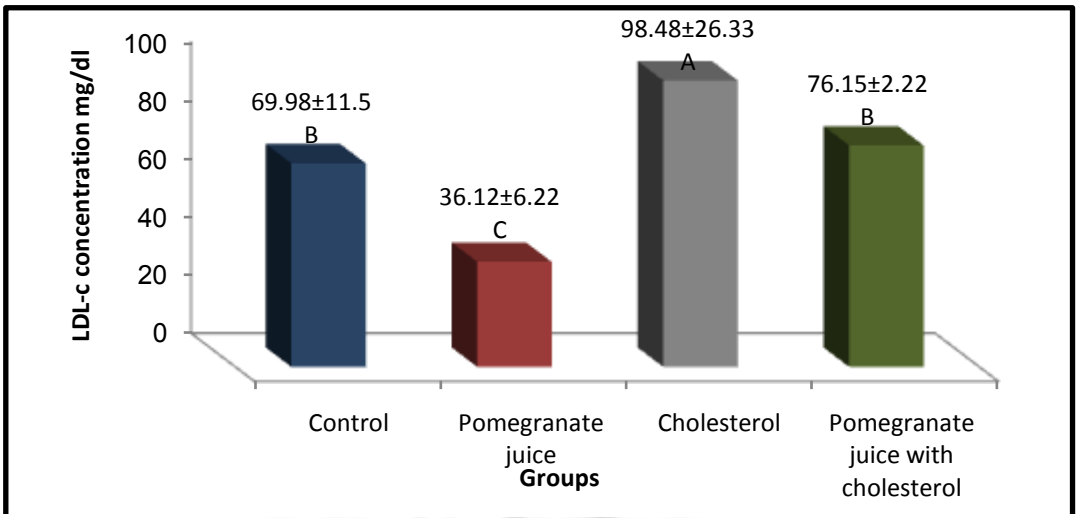
Figure (6) demonstrates a significant increase (P≤ 0.0001) in HDL-c in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (38.04±1.2) mg/dl, this figure also shows a significant decrease in HDL-c in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (24.54±1.79) mg/dl, while the mean of this group was (13.54±1.09) mg/dl, also this figure explains a significant relative increase in concentration of this lipoprotein in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (21.42±1.33) mg/dl.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig.(6): The effect of treatment by PJ and Cholesterol on the concentration of HDL-c

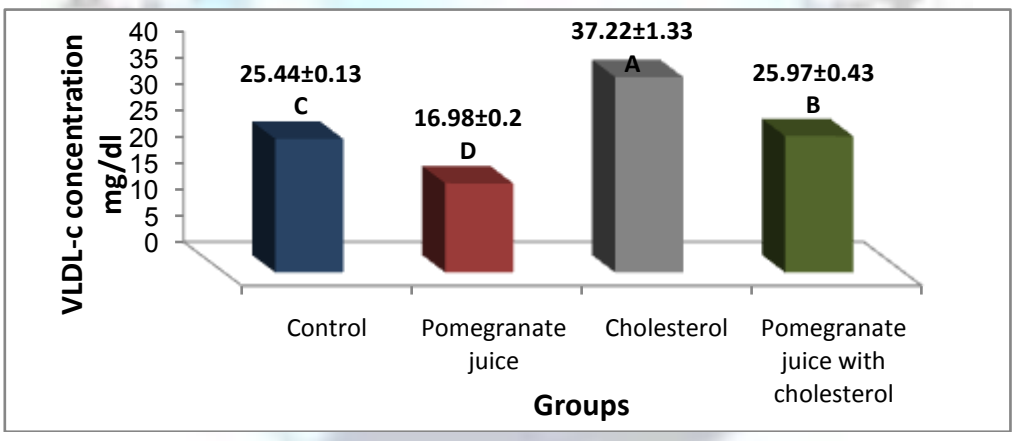
Figure (7) demonstrates a significant decrease (P≤ 0.0001) in LDL-c in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (36.12±6.22) mg/dl, this figure also shows a significant increase in LDL-c in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group (69.98±11.5) mg/dl, while the mean of this group was (98.48±26.33) mg/dl, this figure also explains a decrease not significant in concentration of this lipoprotein in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (76.15±2.22) mg/dl.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (7): The effect of treatment by PJ and Cholesterol on the concentration of LDL-c

Figure (8) demonstrates a significant decrease (P≤ 0.0001) in VLDL-c in a group of rabbits that drenched PJ in dosage 6ml/ kg b.wt. than control group as was the mean of this group (16.98±0.2) mg/dl, this figure also shows a significant increase in VLDL-c in a group of rabbits that given cholesterol with diet by 0.26% compared with control group, as was the mean of control group(25.44±0.13) mg/dl, while the mean of this group was (37.22±1.33) mg/dl, also this figure explains a significant relative decrease in concentration of this lipoprotein in group of rabbits that given cholesterol with diet and drenched PJ to be in near with normal concentrations in control group, the mean of this group was (25.97±0.43) mg/dl.



Means±SD, different letters means there is a significant difference at P≤0.0001

Fig. (8): The effect of treatment by PJ and Cholesterol on the concentration of VLDL-c

Discussion

Most studies considered a major role for anti atherogenic enzyme PON1 in cardiovascular diseases [19] and suggested that this effect is due to the decrease oxidative stress [20], the present study showed the effect of PJ to increase the concentration of PON1 may related to PJ contains polymolecular ellagitannin compounds such as punicalagin which are a potent antioxidant [8]-[21], and PON1 expression and activity can be modulated by dietary polyphenols i.e. LDL receptor deficient mice supplemented with quercitine (a polyphenol contained in PJ) and moderate ethanol inhibited the progression of atherosclerosis by up regulating the hepatic expression with concomitant increased serum PON1 activity [22], also Pomegranate polyphenols seem to have a specific transcriptional role in hepatocyte PON1 expression up regulation[23]. And decreased oxidative stress in serum and macrophage [24], also contributed to PON1 stabilization, increased PON1 association with HDL, and stimulated enzyme catalytic activities, in addition the present study showed a significant decrease in PON1 concentration in rabbit's serum that given cholesterol by diet, Fan and Watanabe, (2003b) [25] showed that rabbits are excellent models for atherosclerosis because they are sensitive to cholesterol diet and rapidly develop atherosclerosis. Pezeshkian et al. (2011) [26], showed that cholesterol rich diet decreased serum PON1 concentration which in turn led to a reduction in formation and progression of atheroma, and the level of PON1

was significant reduced in sixtieth day as expected atherogenic diet as an accelerating factor of progression of atheroma lead to this condition in 80% rabbits.

Several enzymes such as (super oxide dismutase SOD, Catalase CTA, Glutathione peroxidase GPx), vitamins such as(vitamin E, and C), and ß carotene comprise the complex antioxidants system within the blood, these substances work in concert to protect the body from the harmful effect of ROS [27]. Because of the difficulty in measuring each component of the antioxidants system separately, methods have been developed to assess the total antioxidant capacity of the serum [28].

The antioxidant activity of Pomegranate components has been the subject of many studies [29], most conducted in vitro and in vivo. All these activities may be related to the diverse phenolic compounds present in Pomegranate, including punicalagin isomers, and anthocyanins (delphinidin, cyaniding, pelargonidin 3- glucosides, and 3, 5-diglucosides). These compounds are known for their properties to scavenging free radicals, decreasing macrophage oxidative stress and preventing lipid peroxidation in animals as well as increasing plasma antioxidant capacity in elderly humans [30].

Aviram et al. (2004) [12] showed that serum total antioxidant status (TAS) was increased by 130% after one year of PJ consumption also Guo et al. (2008) [30], found that consumption of 250 mL Pomegranate pulp juice daily for four weeks by healthy elderly subjects resulted in increased plasma antioxidant capacity, while subjects consuming apple juice experienced no significant increase. Flavonoids also make a great contribution to the antioxidant activity of Pomegranate due to their effect on free radicals elimination [31]. The principal antioxidant polyphenols in PJ include the ellagitannins and anthocyanins which have been shown to be the antioxidant responsible for the free radicals scavenging ability of PJ [8]. Chidambara et al. (2002) [32] concluded that Pomegranate extract has also been shown to protect the antioxidant enzymes CAT, GPx and SODfrom the effects of toxic chemicals. Turk et al. (2008) [33], reported that there was a significant decrease in malondialdehyde (MDA) level and marked increases in reduced glutathione (GSH), GPx and CAT activities, and vitamin C level were observed in rats treated with different doses of PJ. The improvement of CAT, SOD and GPx enzyme activities could be possibly explained by antioxidant properties of PJ due to presence of bioactive polyphenolic compounds which play a role in scavenging free radicals and also prevent DNA damage [34]. Valadares et al. (2010) [35] confirmed the ability of Pomegranate extract to protect DNA and preventing chromosomal damage in mice. In addition, Kaur et al. (2006) [36] demonstrated that Pomegranate extract afforded up to 60 % protection against hepatic lipid peroxidation due to maintenance of the GSH and serum levels and activities of CAT, GPx and glutathione reductase (GR) enzymes.

CRP, a marker of inflammation, has been recognized as an indicator of atherosclerotic and cardiovascular risk [37], synthesized by the liver, mostly under the regulation of the pro-inflammatory cytokines such as IL-6, IL-1, and TNF-α [38]. The present study showed a significant decrease in serum CRP concentration, (Esmaillzadeh et al.,2006) [39] showed that a higher consumption of fruits and vegetables is associated with lower CRP. Melo et al. (2014) [40] and De Clerq et al.(2012) [41], demonstrated that α-linolic acid one of the pomegranate's fatty acid, it is reduces CVD risk and CRP a strong associations of athero-thrombotic disease [42], also there is a genetic basis for different CRP responses to diet because recent studies showed that CRP gene polymorphism influences CRP level [43]. Other studies reported that changes in CRP were inversely correlated with changes in HDL-c [44], and positively correlated with serum TG [45].

The present study showed positive relationship between PJ and serum HDL-c concentration and negative relationship between PJ and TG, TC, LDL-c and VLDL-c, this results may be related to PJ content of polyphenol, ingestion of polyphenol decrease the level of total cholesterol, LDL-Cholesterol, apolipoprotein B, or an increase of the level of high-density lipoprotein (HDL)-cholesterol and apolipoprotein A-I [46], also as mentioned Ohtsuki and Kondo (2003) [47] glycosides have the ability to decreased TC, TG, LDL-c, VLDL-c and atherogenic index in addition to its active role in reduced the vascular diameter and media -intimal cross- sectional area of the aorta. Pomegranate is an important source of phenols and flavonoids such as anthocyanins, hydrolysable tannins punicalagin and punicalin [48], ellagic and gallic acids [9], Pomegranate also contains vitamin C [33],that have capability in scavenging free radicals and inhibiting LDL-c oxidation in vitro and in vivo [49]. Aviram et al. (2000) [11] reported that PJ consumption by atherosclerotic mice significantly reduced cholesterol accumulation and foam cell formation in heart tissues, also PJ treatment significantly and substantially inhibited the progression of atherosclerotic lesions by inhibition of atherogenic modifications of LDL-c, including its retention, oxidation, and aggregation. In addition Rosenblat and Aviram (2006) [50] demonstrated that Pomegranate juice can inhibit LDL-c oxidation in 3 ways:

1. Pomegranate juice polyphenols inhibit copper ion-induced LDL-c oxidation, and thus reduce the oxidized LDL (ox-LDL) content.
2. Pomegranate juice polyphenols also increase the activity of serum HDL-c associated (PON1).
3. (PON1) can in turn hydrolyze lipid peroxides in ox-LDL and convert them to a less atherogenic LDL-c thus causing further reduction in ox-LDL content.

Results of the present study revealed that feeding of rabbits on cholesterol diet resulted in significant increases in serum levels of TC, TG, LDL-c and VLDL-c accompanied with a significant decrease in HDL-c level as compared to the control group, Moghadasiam (2000) [51], demonstrated that added cholesterol to diet increase the enzymes activities

which have an important role in lipoproteins synthesis such as Cholesterol acyl COA Carboxylase and reduced the activity of Cholesterol Acyl transferase and Lecithin and inhibiting the activity of lipoprotein lipase and liver lipase activity that led to increased synthesis of VLDL-c and decreased the concentration of HDL-c, Frantz et al.(2012) [52], who demonstrated that lipid metabolism in rats fed fat diet presented disorders and levels of serum TC and TG increased significantly, compared with the negative control group**.** These results could be explained on the basis that feeding of rats on atherogenic diet leads to increase in cholesterol absorption and hence serum cholesterol increment. Shanmugasundaram et al. (1986) [53] reported that the increment of plasma LDL-c level after cholesterol consumption could be explained via involvement of two enzymes namely cholesterol ester hydrolase (CEH) and cholesterol ester synthetase (CES). These enzymes balance the cholesterol levels in the blood. Hence, it is logical to assume that the elevation in plasma cholesterol is mediated through increased cholesterol turnover and influenced by the relative balance between CEH and CES activity, with increased estrifying activity (when CEH: CES is lowered) cholesterol will be predominantly in its ester form (as in LDL-c) and can lead to the development and progression of atherosclerosis.

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