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EFFECT OF POMEGRANATE (*PUNICA GRANATUM L*) JUICE ON LIPID PROFILE OF HYPERLIPIDEMIC FEMALE MICE

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ABSTRACT

The present study was carried out to investigate the effects of the pomegranate juice (PJ) on lipid profile, lipoproteins and atherogenic index on hyperlipidermic female mice. Twenty four female mice were used, these animals were divided into four groups each group contain six animals as following: group A (control group) was treated with (0.2 mL/animal) of distilled water for 30 days, group B was treated with (0.2 mL/animal) of pomegranate Juice only for 30 days, group C was treated with high cholesterol diet daily for 30 days, group D was treated with high cholesterol diet and (0.2 mL/animal) of pomegranate juice for 30 days. The result indicated that high cholesterol diet caused a significant increase ($P< 0.01$) in the level of the serum total cholesterol (TC), triglyceride(TG), LDL, VLDL and atherogenic index in group C comparison with group A. Also, there was a significant decrease ($P< 0.01$) in HDL in group C comparison with group A. The pomegranate juice caused a significant decrease ($P< 0.01$) in the level of the serum total cholesterol (TC), LDL and atherogenic index in group D comparison with group C. Also, there was a significant increase in triglyceride TG, HDL, VLDL, levels in group D comparison with group C.

KEYWORDS: Pomegranate Juice, Hyperlipidemic, Lipids, Lipoproteins, Mice

INTRODUCTION

Major risk factors for atherosclerosis include high plasma LDL concentrations and LDL modifications such as its retention, oxidation, and aggregation (Williams and Tabas, 1995). Blood platelet activation also contributes to accelerated atherosclerosis (Sinzinger, 1986; Aviram, 1995). Oxidative modification of LDL is thought to play akey role during early atherogenesis. Oxidized LDL is taken up by macrophages at an enhanced rate Via their scavenger receptors (Parthasarathy *et al.*, 1995), leading to the formation of lipid- laden from cells, the hallmark of the early atherosclerosis (Berliner *et al.*, 1995). Cells of the arterial, including endothelial cells, smooth muscle cells, and macrophages, can oxidize LDL in vitro in the presence of catalytic amount of transition metal ions (Aviram *et al*., 1996; Aviram and Fuhrman, 1998). Although increased resistance of LDL to oxidation was observed after treatment with various synthetic pharmaceutical agents (Pentikainen *et al.*, 1995), an effort is being made to identify natural food products that can offer antioxidant protection against LDL oxidation.

Pomegranate fruit (punica granatum L.) cultivated in India, Spain, Iraq and the United states has been rated to contain the highest antioxidant capacity in its juice, when compared to other commonly consumed polyphenol rich beverages (Seeram *et al*., 2008). Edible parts of pomegranate fruit (about 50% of total fruit weight) comprise 80% juice

and 20% seeds. Fresh juice contains 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid, and polyphenolic flavonoids.Pomegranate seeds are arich source of crude fibers, pectin, and sugers. Dried pomegranate seeds contain the steroid estrogen estrone. In Pomegranate Juice (PJ), fructose, and glucose are present in similar quantities, calcium is 50% of its ash content, and the principal amino acids are glutamic and aspartic acids (El-Nemr *et al*., 1990; Cemeroglu, 1992).

The principal antioxidant polyphenols in pomegranate juice include the ellagitannins and anthocyanins (such as cyanidin-3-glucoside, cyanidin-3,5-diglucoside,and dephindin-3-glucoside)(Gil *et al*,2000).Ellagitannins account for 92% of the antioxidant activity of pomegranate juice and are concentrated in the peel, membranes, and piths of the fruit. punicalagins are the major ellagitannins in the whole fruit and can be hydrolyzed to ellagic acid (EA) and other smaller polyphenols in vivo.(Seeram *et al*, 2004).

The present study aimed to examine the effects of pomegranate juice on the plasma lipid profile, including total cholesterol, LDL-Cholesterol, VLDL –Cholesterol, HDL-cholesterol and triglyceride levels of hyperlipidemic female mice.

MATERIALS AND METHODS

Plants Collection

Pomegranates were handpicked and collected from local markets in Al-Nassiriyah city Thi-Qar province, Iraq, then it was washed, chilled to 4°C, and stored in tanks. The seeds of this fruit were then crushed, squeezed, to yield pomegranate Juice (PJ) and kept in dark glass containers for further use.

Method of Food Preparing (High Cholesterol Diet)

50 grams of cholesterol dissolved in 200 grams of olive oil hot in a water bath, and after soluble cholesterol in the oil were added to 1Kg of feed, and then was cut into small pieces fit with the size of the holes in the lid iron to boxes, to facilitate the process taken up by mice (Cook and Thomson, 1950).

Experimental Animals

Twenty four healthy adult female mice aged (8-10) weeks and weighting between (22-30) gm were obtained from Department of Biology, College of Science, Thi-Qar University, Iraq, were used in the present study. The animals were housed under standard laboratory conditions (12h light / 12h dark cycles, 22±2 C°) were fed on standard pellet and tap water ad *libitum.* Experimental animals were divided into four equal groups, each group into consist of (6) mice.

- Group A (control group) was treated orally with (0.2mL/animal) of distilled water, for 30 days.
- Group B was treated orally with (0.2 mL/animal) of Pomegranate juice only, for 30 days.
- Group C was treated orally with high cholesterol diet (prepared according to Cook and Thomson, 1950), for 30 days.
- Group D was treated orally with high cholesterol diet and (0.2 mL/animal) of pomegranate juice, for 30 days.

Blood Samples Collection

After thirty days of treatment, the animals were sacrificed subsequently, the blood samples were collected by cardiac puncture, 1mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA,

Centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20C̊ until the time of essay.

Measurement of Serum Lipid Profile

Serum total cholesterol (TC), Serum triglyceride (TG) and high density lipoprotein (HDL) were analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, Japan using Kits supplied by Biolabo (France). Serum total cholesterol was measured according to (Allan and Dawson, 1979), serum TG was measured according to (Tietz *et al*., 1999), serum HDL was measured according to (Lopes – Virella, 1977) and measurement of LDL and VLDL according to (Fried wald *et al.*, 1972). LDL, VLDL and atherogenic index concentration was measured as follows:

LDL (mg/dL) = total cholesterol – (HDL+VLDL)

VLDL (mg/dL) = TG/5

Atherogenic Index = LDL / HDL

Statistical Analysis:

Statistical analysis was done using the software SPSS version 15, the result were expressed as mean ± standard deviation (means ± SD). One way ANOVA – test was used to compare parameters in different studied groups. P-Values ($P< 0.01$) were considered statistically significant.

RESULTS AND DISCUSSIONS

Effects of Pomegranate Juice on Serum Lipids Levels

Table 1 showed the results of effect of pomegranate juice on lipids levels in hyperlipidemic mice. The results indicated non significant differences ($P< 0.01$) in the total cholesterol (TC) of (B and D) groups compared with the control group (A). While, there was a significant increase ($P< 0.01$) in TC level of group (C) compared with group (A). On the other hand, there was a significant reduced ($P< 0.01$) in TC level of (B and D) groups compared with group (C), figure 1.

Pomegranate Juice is rich in polyphenols and demonstrates high capability in scavenging free radicals and inhibiting low-density lipoprotein oxidation in vitro and in vivo (Gil *et al*, 2000; Aviram *et al*, 2002).Furthermore, Polyphenols of pomegranate may accelerate and promote cholesterol metabolism by reversing cholesterol transport Via HDL (Aviram *et al.*, 2002; Esmaillzadeh *et al.*, 2004). Perhaps the greatest mechanism of PJ (which include ellagic acid that combating cholesterol) is associated with an enzyme known as paraoxonase (PON). Paraoxonase is an HDL-associated enzyme whose activity is related to cholesterol and atherosclerosis, decreased activity of PON is associated with increase cholesterol and increase risk for atherosclerosis, hypocholesterolemic properties of PON may be related to its ability to protect against lipid peroxidation (Aviram *et al.*, 2000; Miguel *et al*., 2004).

In the same table, non significant differences ($P< 0.01$) in (TG) level of group (B) compared with group (A). While, there was a significant increase ($P< 0.01$) in TG level of (C and D) groups compared with group with (A). Also, there was a significant increase ($P< 0.01$) in TG level in group (D) compared with group (C). On the other hand, there was a significant reduced($P< 0.01$) can be observed in TG level of group (B) compared with group (C), figure 2. Polyphenolic compound in pomegranate juice was showed to improve the serum lipid profile this is might be due to it posses a powerful antioxidant activity and it is able to reduce lipid peroxidation. Ellagic acid, tannic acid and other polyphenolic compounds

can remove free radicals and can prevent lipid peroxidation (Devipriya *et al*., 2007).

Table 1: Effect of Pomegranate Juice on Serum Lipids Levels

Animal Groups	Cholesterol (mg/dL)	T.G (mg/dL)
A	120.50 ± 3.27[b]	109.17 ± 3.82[c] . ± 0.09b
B	132.33 ± 7.37[b]	118.83± 3.66[c]
C	267.00 ± 31.78[a]	146.83 ± 5.88[b]
D	135.83 ± 13.93[b]	189.33 ± 26.81[a]

* Each value represents (mean ± S.D), values with non identical superscript (a, b or c... etc.) were considered significantly different (P < 0.01).

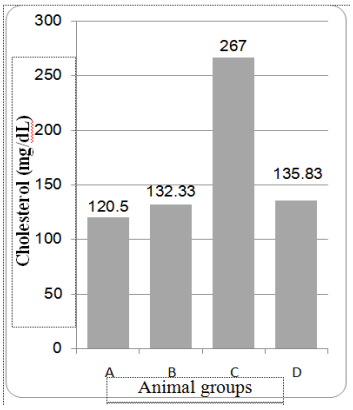

Figure 1: Changes in the Concentration of Serum Cholesterol of Animal Groups

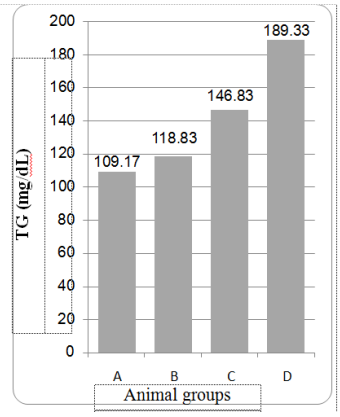

Figure 2: Changes in the Concentration of Serum TG of Animal Groups

Effects of Pomegranate Juice on Lipoproteins and Atherogenic Index Levels

Table 2 explained the effect of Pomegranate Juice on lipoproteins and Atherogenic Index Levels in hyperlipidemic mice. The results showed non significant differences (P< 0.01) of HDL level in (B and D) groups compared with the control group (A). While, the HDL level decreased significantly (P< 0.01) in group (C) compared with group (A). On the other hand, there was a significant increase (P< 0.01) was observed in HDL level of (B and D) groups compared with group (C), figure 3. Various studies have reported that the polyphenols can also increase plasma HDL concentration (Abe et al., 2011). The reason for this rise in HDL may be to the phenolic compounds work to activate Lecithin Acyl Transfrase (LCAT), which works to integrate cholesterol free with high-density lipoproteins (HDL) and thus lead to raise the level of HDL in the blood (Ghule *et al.,* 2006). The phenolic compounds may increase the effectiveness and caused activate lipoprotein lipase enzyme and this enzyme involved in metabolism of protein, high density lipoprotein (Hemla, 1992).

There was non significant difference (P< 0.01) in LDL level of (B and D) groups compared with control group (A). Serum LDL level increased significantly (P< 0.01) in group (C) compared with group (A). Also, there was a

significant reduced (P< 0.01) in LDL level of (B and D) groups compared with group (C), figure 4. Dietary supplementation with polyphenolic antioxidants inhibits low-density lipoprotein oxidation and macrophage foam cell formation and attenuates development of atherosclerosis in animals. Commercial Pomegranate Juice shows potent antioxidant and anti-atherosclerotic properties attributed to its high content of polyphenols (Aviram *et al.*, 2001). Epidemiological studies have demonstrated that the composition of phenol-rich food retards the progression of atheriosclerosis and reduces the incidence of heart diseases by preventing the oxidative stress, that is lipid peroxidation in arterial macrophage and in lipoprotein (Aviram *et al*., 2004).

The results indicated non significant differences (P<0.01) in VLDL level of group (B) compared with group (A). While, there was a significant increase (P<0.01) in VLDL level of (C and D) groups compared with group (A). Also, there was a significant increase (P<0.01) in VLDL level of group (D) compared with group (C). On the other hand, a significant reduce (P<0.01) in VLDL level was observed in group (B) compared with group (C), figure 5. Polyphenolic extract exhibited a significant hypolipidemic effect through the reduction of VLDL levels (Chidambaram, *et al*., 2007). The high level of triglycerides in the blood increases the level of (VLDL) for being contain a large proportion of Triglycerides.

In the same table there was non significant difference (P<0.01) in atherogenic index of groups (B and D) compared with control group (A). While, there was a significant increase (P<0.01) in atherogenic index of group (C) compared with group (A). On the other hand, a significant reduce (P<0.01) in atherogenic index was observed in groups (B and D) compared with group (C), figure 6. The decrease of atherogenic index is due to the low level of HDL with high level of LDL, and this percentage is called risk factor which is important in determining the risk of future patients with heart disease, diabetes and the likelihood of aclot and the proportion of risk increases with the length of the time period of the disease (Stratton *et al*., 2000).

Table 2: Effect of Pomegranate Juice on Serum Lipoproteins Levels

Animal Groups	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic Index
A	80.73 ± 9.68^{a}	17.93 ± 7.85^{b}	21.83 ± 0.76^{c}	0.23 ± 0.11^{b}
B	81.07 ± 2.12^{a}	27.50 ± 6.39^{b}	23.77 ± 0.73^{c}	0.34 ± 0.08^{b}
C	67.70 ± 2.34^{b}	169.93 ± 31.90^{a}	29.37 ± 1.18^{b}	2.51 ± 0.4^{a}
D	78.58 ± 4.79^{a}	19.38 ± 10.34^{b}	37.87 ± 5.36^{a}	0.24 ± 0.12^{b}

* Each value represents (mean SD), values with non identical superscript (a, b or c... etc.) were considered significantly different (P < 0.01).

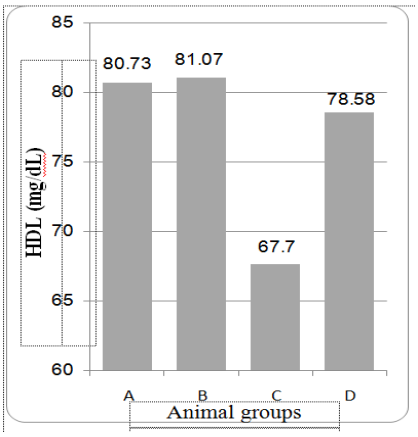

Figure 3: Changes in the Concentration of Serum HDL of Animal Groups

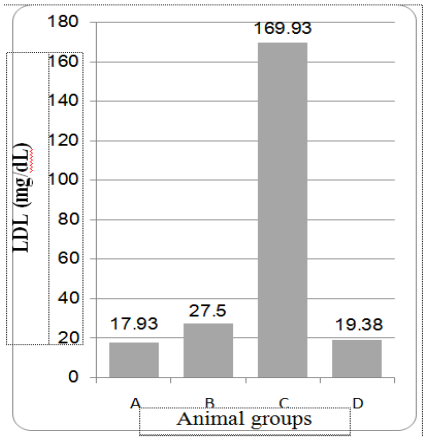

Figure 4: Changes in the Concentration of Serum LDL of Animal Groups

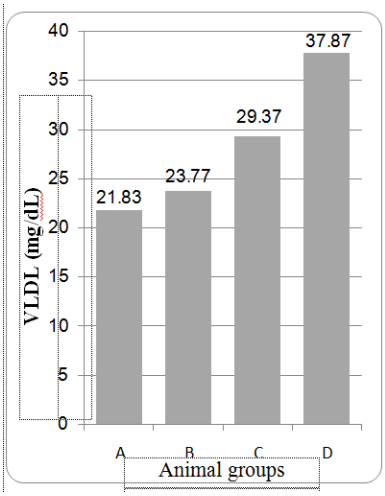

Figure 5: Changes in the Concentration of Serum VLDL of Animal Groups

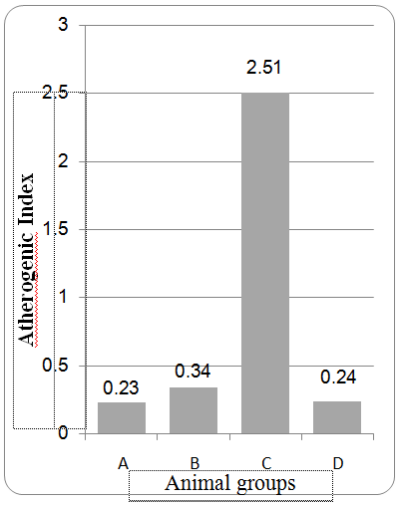

Figure 6: Changes in the Concentration of Serum Atherogenic Index of Animal Groups

CONCLUSIONS

The present study indicated the role of pomegranate (*Punica granatum* L.) juice as hypolipidemic, therefore, it is very important to use of medical plants for treatment of health problems such as hyperlipidemic

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