Effect of Pomegranate Juice on Lipid Profile and Antioxidant Enzymes in Hypercholesterolemic Rats

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Abstract: Objective: The present study was carried out to investigate the effects of oral administration of Pomegranate juice at three dosage levels (1, 3 and 5 ml/kg b. wt.) to hypercholesterolemic rats for 28 days on body weight gain %, feed efficiency ratio, relative weights of some internal organs, serum levels of total cholesterol (TC), triglycerides (TG), lipoprotein fractions and liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed. Antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx,) were determined in homogenate liver.Histopathological examination of liver and heart were also carried out. **Methods:** Thirty five male Wistar rats were distributed into five equal groups as follows: negative (normal rats), positive (hypercholesterolemic rats) control groups and positive groups orally given Pomegranate juice in doses of 1, 3 and 5 ml/kg b. wt., respectively. **Results:** The results showed that oral administration of Pomegranate juice to hypercholesterolemic rats for 28 days significantly decreased serum levels of TC, TG, low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c) and liver enzymes when compared to the control positive group. Levels of high density lipoprotein cholesterol (HDL-c) and antioxidant enzymes were significantly increased as compared to the control positive group. Histopathological examination of liver and heart of Pomegranate juice-treated groups showed amelioration of histological changes caused by high level of cholesterol in the positive control group. **Conclusion:** Results indicated that Pomegranate juice produces potent antiatherogenic and antioxidant effects in hypercholesterolemic rats. This study recommends that drinking Pomegranate juice may be beneficial for patients who suffer from hypercholesterolemia and/or arteriosclerosis.

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1. Introduction

Hypercholesterolemia has been well known as a proven risk factor for cardiovascular disease (CVD) (**Wang *et al.*, 2011**). The Saudi Arabia Ministry of Health reported that in 2010, CVD are thesecond causes of death with 5,239 deaths (17.3% of the total number of deaths 30,289)(**Health Statistical, 2010**). It is estimated that by theyear 2030 the death rates from CVD will increase and will remain as leading cause of death in the world (**WHO, 2008**). Hypercholesterolemia is generally, associated with an increase in plasma concentrations of low density lipoprotein (LDL-c)(bad cholesterol) and very low density lipoprotein (VLDL-c) and / or a decrease in high density lipoprotein cholesterol (HDL-c) (good cholesterol). Modification of oxidation of LDL-c is thought to play a key role during early atherogenesis i.e. formation of atheroma inside the walls of blood vessels that finally lead to arteriosclerosis (**Kumar *et al.*, 2008**). Because of an increased resistance of LDL-c to oxidation after treatment with various synthetic pharmaceutical drugs (**Breugnot*et al.*, 1992; Pentikainen*et al.*, 1995**), there is a great need to identify natural food products that can offer antioxidant protection against LDL-c oxidation.

Consumption of fresh fruits andvegetables to improve human health has been attributedmainly to their high contents of beneficial phytochemicals andother micronutrients(**Opara and Al-Ani, 2010**). These phytochemicals mainly phenolic compounds (such as flavonoids, phenolic acids, diterpenes, saponins and tannins) have received much attention for their high antioxidative activity by scavenging freeradicals which cause oxidative stress that can lead to cellular damage and manydegenerative disorders (**Lampe, 1999; Boyer and Liu,2004**).

Pomegranate fruit is widely considered as a healthy fruit due to its biological actions, most of these effects were attributed to its high phenolic content (**Lansky and Newman, 2007**). Previous studies on polyphenols of Pomegranate have been shown that these compounds are linked to the prevention of cardiovascular diseases, cancers and neurological damage in humans (**Aviram*et al.*, 2002; Kuskoski*et al.*, 2004; Lansky and Newman, 2007**). Flavonols and anthocyanins showed anti-carcinogenic, antimicrobial (**Opara*et al.*, 2009**), anti-inflammatory and antioxidant activities (**Lansky and Newman, 2007**).

Several studies on Pomegranate extracts and its

active constituents revealed that they have an antioxidant activity by scavenging free radicals, decreasing macrophage oxidative stress and preventing lipid peroxidation in animals as well as increasing plasma antioxidant capacity in elderly humans **(Guo*et al.*, 2008).** Studies in rats and mice confirm the antioxidant property of a Pomegranate by-product extract made from the whole fruit minus, the juice showed a 19% reduction in oxidative stress in mouse peritoneal macrophages, 42% decrease in cellular lipid peroxides content, and 53% increase in reduced glutathione levels **(Rosenblat*et al.*, 2006).**

The present study was designed to investigate the effect of oral administration of Pomegranate juice on hypercholesterolemic rats.

2.Materialand Methods

Material:

Pomegranate:

Ripe Pomegranate fruits used in this study were purchased from a local market, Jeddah, Kingdom of Saudi Arabia. The Pomegranate plant was grown in Taif city, a west province of the Kingdom of Saudi Arabia.

Cholesterol:

Cholesterol was purchased from Sigma-Aldrich Company, St. Louis, Missouri, USA, in the form of white crystalline powder in plastic bottles each contains 100 gram.

Rats:

A total number of thirty five adult male albino rats of Wistar strain of 6-8 weeks old and weighed 150±30 grams were used in this study. The rats were obtained from Faculty of Pharmacy, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Methods:

Preparation of basal diet:

The basal diet was prepared according to **Reeves *et al.* (1993).** It is consisted of 20 % protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch upto100 %.

Induction of Hypercholesterolemia:

Induction of hypercholesterolemia was done by feeding rats on cholesterol containing diet (experimental diet) which was prepared by formulated basal diet with 2% Cholesterol and 0.5% Cholic acid for 4 weeks according to the method described by **Shinnick*et al*. (1990).**

Preparation of Pomegranate Juice:

The fruits of fresh Pomegranate were washedand manually peeled, without separating the seeds. Pomegranate juice was obtained using a commercial blender(Moulinex, France), filtrated with a Buchner funnel **(Faria*et al*., 2007).**

Experimental Designand Grouping of Rats:

The experiment was performed on thirty five male mature Wistar rats. Rats were divided into two main groups; the first main group (7 rats) was fed on the basal diet and kept as a negative control group (C-ve) and received oral gavage of distilled water. The second main group (28 rats) was fed on experimental diet for four weeks to induce hypercholesterolemia. After this period, blood samples were taken for measuring total cholesterol level. Ratswith blood cholesterol level ≥ 5.2 mmol/L were considered to be hypercholesterolmic (**Iqbal*et al*., 2011**). After checking, all rats were distributed into their groups. Group (2) was left as a control positive group hypercholesterolemic rats fed on experimental diet only and received oral gavage of distilled water. and groups (3), (4) and (5) were fed on experimental diet and orally given Pomegranate juice in a dose of 1,3 and 5 ml/kg body weight. Daily feed intake (FI) per group was calculated throughout the experimental period (28 days).The biological values of different diets were assessed by the determination of body weight gain percent (BWG %) which was calculated at the end of the experimental period as well as feed efficiency ratio (FER) was calculated twice a week, according to the method of **Chapman *et al.* (1959).** At the end of the experiment, blood samples were collected to separate the serum. Clear serum samples were carefully separated using Pasteur pipettes, and frozen at - 20° C until biochemical analysis (**Margoni*et al*., 2011**). The liver and heart were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline solution and dried between two filter papers then weighed and they saved for the histopathological examination. Calculation of the relative organs weight was done according to the following equation:

$$\text{Organ relative weight} = \frac{\text{Organ weight}}{\text{Animal final bodyweight}} \times 100.$$

Halves of liver was taken in ice bags and kept frozen till preparation of liver homogenates for estimating lipid peroxidation products and activity of antioxidant enzymes. The other half of liver was preserved in 10% neutral formalin solution till processed for histopathological examination.

Biochemical analyses:

Estimation of Total Polyphenol Concentration in Pomegranate Juice:

Total polyphenol concentrations in Pomegranate juice were determined spectrophotometrically according to the method of **Singleton and Rossi (1965)** and modified by **Narr Ben *et al*.(1996)** for small volumes. equivalents (GAEs).

Serum Analysis:
Serum cholesterol was determined according to the method described by **Allain*et al*. (1974).** Concentrations of serum triglycerides were determined according to the method described by **Trinder (1969).** Serum high density lipoprotein cholesterol was calorimetrically determined according to the method described by **Lopes-Virella*et al*. (1977).** Serum low density lipoproteins cholesterol was calorimetrically determined according to the method described by **Fridewald*et al*.(1972).** Serum very low density lipoproteins cholesterol was calorimetrically determined according to the method described by **Fridewald*et al*. (1972).** Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity were measured according to the method described by **Bergmeyer*et al*. (1978).**

Determination of antioxidant enzyme activity:
The frozen liver samples were thoroughly homogenized on ice with Tri- Hcl buffer solution (PH 7.4) to obtain 10% tissue homogenate. The prepared liver homogenates were used for measurement of activities of antioxidant enzymes such as glutathione peroxidase **Paglia andValentaine (1967),** superoxide dismutase **Kakkar*et al*., (1984)** and catalase **Sinha**, **(1972).**

Histopathological Examination:
Specimens from the halves of liver and heart were taken immediately after weighed the organs of the rats and immersed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, then cleared in xylene, and stained with Hematoxylin and Eosin (H&E) and examined microscopically according to **Bancroft and Gamble (2008).**

Statistical Analysis:
Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 18 (SPSS Inc., Chicago, IL, USA). The obtained data were presented as means ± standard deviation (SD). Statistical analysis of variance between mean values of different groups was performed using one way ANOVA test followed by the least significant difference (LSD) test to determine the variance between all treatments. Differences were considered significant at $P<0.05$.

3.Results and Discussion

Estimation of total polyphenols content of Pomegranate juice:
The total polyphenols content of Pomegranate juice as gallic acid equivalents was 3.9 ± 0.1 mg /ml as recorded in Table 4.1.

Table 4.1. The total polyphenols content of Pomegranate juice as gallic acid equivalents

Beverage	**Gallic acidequivalents GAEs (mg/ml)**
Pomegranate juice	**3.9 ± 0.1**

Mean ± SD of triplicate measurements

Effect of oral administration of Pomegranate juice on initial body weight, final body weight and body weight gain percent (BWG %) in hypercholesterolemicrats:
Oral administration of Pomegranate juice in doses of 1, 3 and 5 ml/kg b. wt., significantly ($P<0.05$) decreased the BWG % when compared to hypercholesterolemic rats(positive control group) as shown in Table 4.2. These findings might be due to decreased appetite (anorexia) of rats and/or reduction of intestinal fat absorption or due to an inhibition of pancreatic lipase activity. It is well known that dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase **(Lei *et al*., 2007).**

Table 4.2 Effect of oral administration of Pomegranate juice on initial body weight, final body weight and body weight gain %(BWG %) in hypercholesterolemicrats

Parameter / Groups	**Initial weight (g)**	**Final weight (g)**	**Body weight gain (%)**
Negative control	**247.54 ± 3.29a**	**280.40 ± 1.87 b**	**13.29 ± 1.15 c**
Positive control	**245.88 ± 3.40 a**	**315.10 ± 4.45 a**	**28.16 ± 1.30 a**
Pomegranate juice 1 ml/kg	**250.08 ± 4.24 a**	**307.02 ± 8.78 a**	**22.76 ± 2.18 b**
Pomegranate juice 3 ml/kg	**248.34 ± 3.89 a**	**290.50 ± 3.28 b**	**17.01 ± 3.05 c**
Pomegranate juice 5 ml/kg	**245.56 ± 6.60 a**	**286.62 ± 3.43 b**	**16.76 ± 1.99 c**

Data are presented as means ± standard deviation, (n = 7 for each group).
Values with different superscripts within each column are significantly different at $P<0.05$.Values with similar or partially similar superscripts are non significant.

Effect of oral administration of Pomegranate juice on feed intake and feed efficiency ratio (FER) in hypercholesterolemicrats:

Feed intake and feed efficiency ratio were significantly ($P< 0.05$) increased in the hypercholesterolemic rats(positive control group), compared to normal rats. These findings were in agreement with those obtained by **Matos *et al.* (2005)**; **Hossin (2009)**; **Otunola*et al*. (2010); Amin *et al.* (2011) and Nwozo*et al.*(2011)** who confirmed our results. The increase in body weight of hypercholesterolemic rats might be due to the increase of feed and caloric intake by rats. Oral administration of Pomegranate juice at three dosage levels 3 and 5 ml/kg b. wt., decreased feed intake and feed efficiency ratio as compared to hypercholesterolemic rats These findings might be due to decreased appetite (anorexia) of rats and/or reduction of intestinal fat absorption or due to an inhibition of pancreatic lipase activity (**Lei *et al.*, 2007**).

Table 4.3 Effect of oral administration of Pomegranate juice on feed intake and feed efficiency ratio (FER) in hypercholesterolemicrats

Parameter / Groups	Mean of daily feed intake (g/d)	Feed efficiency ratio (FER)
Negative control	**26.67 b**	**0.047 ± 0.005d**
Positive control	**27.10 a**	**0.091 ± 0.002 a**
Pomegranate juice 1 ml/kg	**23.10 c**	**0.088 ± 0.009 a, b**
Pomegranate juice 3 ml/kg	**21.50 d**	**0.070± 0.01 c**
Pomegranate juice 5 ml/kg	**20.30 e**	**0.072 ± 0.007 b, c**

Data are presented as means ± standard deviation, (n = 7 for each group).

Values with different superscripts within the column are significantly different at $P< 0.05$. Values with similar or partially similar superscripts are non significant.

Effect of oral administration of Pomegranate juice on liver and heart relative weight to the body weight in hypercholesterolemicrats:

Concerning the relative organs weight to the body weight of rats, the results showed that hypercholesterolemic rats had a significant ($P< 0.05$) increase in the relative weights of liver and heart as compared to normal rats as depicted in Table 4.4. These results might be due to the accumulation of fat in the liver and heart cells leading to an increase in their weight. Our findings were in accordance with those obtained by **Matos *et al.* (2005)** who reported that the increase in liver weight of hypercholesterolemic rats could be a consequence of the higher fat content in liver.

Oral administration of Pomegranate juice in a dose of 3 ml/ kg b. wt., significantly decreased liver and heart weight compared to hypercholesterolemic rats. Our results were in agreement with those of **Chidambara*et al.* (2002)** who reported that Pomegranate peel when given to rats exhibited protective effects on liver and heart weights.

Table 4.4 Effect of oral administration of Pomegranate juice on liver and heart relative weight in hypercholesterolemicrats

Parameter / Groups	Relative liver weight	Relative heart weight
Negative control	**2.75 ± 0.24 b**	**0.28 ± 0.04 b**
Positive control	**3.72 ± 0.58 a**	**0.39 ± 0.05 a**
Pomegranate juice 1 ml/kg	**2.99 ± 0.20 a, b**	**0.35 ± 0.05 a, b**
Pomegranate juice 3 ml/kg	**2.87 ± 0.37 b**	**0.30 ± 0.03 b**
Pomegranate juice 5 ml/kg	**2.97 ± 0.33 a, b**	**0.34 ± 0.04 a, b**

Data are presented as means ± standard deviation, (n = 7 for each group).

Values with different superscripts within the column are significantly different at $P< 0.05$.Values with similar or partially similar superscripts are non significant.

Effect of oral administration of Pomegranate juice on the serum level of total cholesterol (TC) and triglycerides (TG) in hypercholesterolemicrats:

Results of biochemical analyses revealed that hypercholesterolemic rats(positive control group) had a significant ($P<0.05$) increase in total cholesterol (TC) and triglycerides (TG) compared to normal rats as recorded in Table 4.5. The present findings were in the same line as with those reported by **Wang and Chen (2004); Gorinstein*et al.***

(2006); Frantz *et al.*(2012) who demonstrated that lipid metabolism in rats fed high fat - diet (HFD) 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.

Oral administration of Pomegranate juice at the tested doses 1, 3and 5 ml/kg b. wt., significantly (P<0.05) decreased serum TC and TG compared to hypercholesterolemic ratsas shown in Table 4.5. Our results were in accordance with those obtained by **Aviram*et al.* (2000)** who reported that Pomegranate juice consumption by atherosclerotic mice significantly reduced cholesterol accumulation and foam cell formation in heart tissues.Pomegranate juice treatment significantly and substantially inhibited the progression of atherosclerotic lesions by inhibition of atherogenic modifications of LDL-c, including its retention, oxidation, and aggregation.

Effect of oral administration of Pomegranate juice on the serum levels of lipoprotein fractions in hypercholesterolemicrats:

Results in Table 4.6 illustrated the effect of different doses of Pomegranate juice on serum levels of lipoprotein fractions in hypercholesterolemicrats.The obtained data indicated that hypercholesterolemicrats had a significant (P< 0.05) decrease in serum HDL-c of hypercholesterolemic rats while there were significant increases in serum LDL-c and serum VLDL-c when compared with the normal rats. the current results were in agreement with those of **Boden and Pearson (2000)**; **Glass and Witztum (2001)**; **Witztum and Steinberg (2001)** and **Kumar *et al.* (2010)**. The previous authors concluded that oxidation of LDL-c resulted in formation of a wide range of biologically active products, including peroxides and malondialdehyde. Moreover, **Sezer*et al.* (2011)** demonstrated that the oxidative modified lipids and their degradation products are believed to have adverse effects such as pro-inflammatory, immunogenic and cytotoxic activities which contribute to both the initiation and progression of atherosclerotic lesions. Furthermore**, Tebib*et al.* (1994)** found that activity of the lipoprotein lipase enzyme augmented in hypercholesterolemic rats. Lipase transforms VLDL-c into LDL-c that would lead to an increase in serum concentration of LDL-c. However, **Shanmugasundaram*et al.* (1986)** reported that the increment of plasma LDL-c level after HFD 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.

Oral administration of 1, 3 and 5 ml/kg b. wt., of Pomegranate juice to hypercholesterolemic rats significantly (P<0.05) increases serum HDL-c level and decreased serum levels of LDL-c and VLDL-c when compared to hypercholesterolemic rats, as shown in Table 4.6. These results were in the same line with those reported by **Rice-Evans *et al*. (1996); Schwenke and Behr (1998); Gil *et al.* (2000); Aviram*et al*. (2002) and Noda *et al.* (2002)** who concluded that Pomegranate juice is rich in polyphenols and demonstrate high capability in scavenging free radicals and inhibiting LDL-c oxidation *in vitro* and *in vivo*. Phenols and flavonoids are very important plant constituents because of their antioxidant activity **(Annegowda *et al.*, 2010 and Abdel Moneim, 2012)**. The antioxidant activity of phenolic compounds is mainly due to their redox properties which play an important role as free radical scavengers, reducing agents, quenchers of singlet oxygen and complexes of pro-oxidant metals **(Mustafa *et al.*, 2010).**

Table 4.5 Effect of oral administration of Pomegranate juice on the serum levels of total cholesterol (TC) and triglycerides (TG) in hypercholesterolemicrats

Parameter / Groups	TC (mmol/L)	TG (mmol/L)
Negative control	2.04 ± 0.24 c	0.90 ± 0.25 b
Positive control	5.66 ± 0.79 a	1.60 ± 0.19 a
Pomegranate juice 1 ml/kg	3.74 ± 0.62 b	0.96 ± 0.29 b
Pomegranate juice 3 ml/kg	2.32 ± 0.19 c	1.12 ± 0.33 a, b
Pomegranate juice 5 ml/kg	2.50 ± 0.07 c	0.95 ± 0.31 b

Data are presented as means ± standard deviation, (n = 7 for each group).

Values with different superscripts within the column are significantly different at P<0.05.Values with similar or partially similar superscripts are non significant.

Table 4.6 Effect of oral administration of Pomegranate juice on the serum levels of lipoprotein fractions in hypercholesterolemicrats

Parameter / Groups	HDL-c (mmol/L)	LDL-c (mmol/L)	VLDL-c (mmol/L)
Negative control	0.82 ± 0.09 a, b	0.82 ± 0.24 c	0.41± 0.11 b
Positive control	0.64 ± 0.09 c	3.96 ± 0.35 a	0.72 ± 0.19 a
Pomegranate juice 1 ml/kg	0.99 ± 0.23 a, b	2.32 ± 0.68 b	0.43 ± 0.13 b
Pomegranate juice 3 ml/kg	0.96 ± 0.19 a, b	0.96 ± 0.29 c	0.41 ± 0.16 b
Pomegranate juice 5 ml/kg	1.10 ± 0.28 a	0.98 ± 0.24 c	0.43 ± 0.15 b

Data are presented as means ± standard deviation, (n = 7 for each group).
Values with different superscripts within the column are significantly different at $P< 0.05$. Values with similar or partially similar superscripts are non significant.

Effect of oral administration of Pomegranate juice on serum levels of liver function enzymes in hypercholesterolemicrats

It is clear from Table 4.7 that hypercholesterolemic rats(positive control group) had significant ($P<0.05$) increases in serum levels of AST and ALT enzymes of hypercholesterolemic rats whencompared to the normal rats. Our results agreed with those reported by **Lu *et al*. (2007); Prasad (2010) and Saki e*t al*. (2011)** who showed that a high cholesterol diet moderately elevated serum levels of ALT, AST and ALP enzymes in rats. On contrary, **Molgaard*et al*. (1989)** reported that there were no changes in the serum levels of AST and ALT. The discrepancy in the serum levels of these enzymes could be attributed to the levels and duration of hypercholesterolemia **(Lu *et al*., 2007)**.

Oral administration of 1, 3 and 5 ml/kg b. wt., of Pomegranate juice to hypercholesterolemic rats significantly ($P<0.05$) reduced serum AST and ALT compared to the hypercholesterolemic rats as shown in Table 4.7. The present results partially agreed with the results obtained by **Osman *et al*. (2012)** who examined the antioxidant effect of Pomegranate peel and juice on diabetes mellitus induced by alloxan in Female Rats. The results showed that AST and ALT were significantly increased in diabetic group, but after treatment with peel and juice, AST and ALT levels decreased and become near to the control level especially ALT value. This effect is due to antioxidant content of Pomegranate peel and juice.

Kaur*et al*. (2006) reported that Pretreatment with Pomegranate flower extract, at a dose regimen of 50-150 mg / kg b. wt., for a week, have a protective effect against ferric nitrilotriacetate (Fe-NTA)-induced oxidative stress, as well as hepatic injury. The results showed that there was an inhibition in serum of AST and ALT enzymes which may be due to potent antioxidant and hepatoprotective properties of Pomegranate juice.

Table 4.7 Effect of oral administration of Pomegranate juice on serum levels of liver function enzymes in hypercholesterolemicrats

Parameter / Groups	AST (U/L)	ALT (U/L)
Negative control	55.24 ± 2.33c	26.74 ± 0.88d
Positive control	106.38 ± 4.33a	50.00 ± 1.01 a
Pomegranate juice 1 ml/kg	92.72 ± 1.59 b	43.30 ± 0.47 b
Pomegranate juice 3 ml/kg	92.80 ± 1.72b	41.80 ± 0.62 b
Pomegranate juice 5 ml/kg	90.02 ± 0.22b	38.50 ± 1.10 c

Data are presented as means ± standard deviation, (n = 7 for each group).
Values with different superscripts within the column are significantly different at $P< 0.05$. Values with similar or partially similar superscripts are non significant.

Effect of oral administration of Pomegranate juice on liver homogenates levels of catalase (CAT),superoxide dismutase (SOD) and glutathione peroxidase (GPx) in hypercholesterolemicrats:

From data recorded in Table 4.8 it could be noticed that oral administration of Pomegranate juice at doses 3 and 5 ml/kg b. wt., showed significant ($P< 0.05$) increase in CAT,SOD and GPx enzyme levels in liver homogenates compared to hypercholesterolemic rats. The improvement of CAT, SOD and GPx enzyme activities could be possibly explained by antioxidant properties of Pomegranate juice due to presence of bioactive polyphenolic compounds which play a role in scavenging free radicals and also prevent DNA damage **(Fyiad*et al*., 2012)**.

Valadares *et al.* (2010) confirmed the ability of Pomegranate extract to protect DNA and preventing chromosomal damage in mice. In addition, **Kaur*et al.* (2006)** 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.

Table 4.8 Effect of oral administration of Pomegranate juice on liver homogenates levels of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase(SOD) enzymes in hypercholesterolemicrats

Parameter / Groups	CAT (U/mg tissue)	SOD (U/mg tissue)	GPx (U/mg tissue)
Control negative	1.50 ± 0.45 a	1.28 ± 0.21 a	62.90 ± 1.41 a
Control positive	0.97 ± 0.05 b	0.86 ± 0.09 b	48.02 ± 1.19 b
Pomegranate juice 1 ml/kg	1.09 ± 0.11a, b	1.06 ± 0.11 a, b	49.42 ± 0.73 b
Pomegranate juice 3 ml/kg	1.40 ± 0.07 a	1.22 ± 0.05 a	62.50 ± 0.67 a
Pomegranate juice 5 ml/kg	1.45 ± 0.01 a	1.36 ± 0.21 a	62.58 ± 0.97 a

Data are presented as means ± standard deviation, (n = 7 for each group).
Values with different superscripts within the column are significantly different at $P< 0.05$.
Values with similar or partially similar superscripts are non significant.

Histopathological Examination:

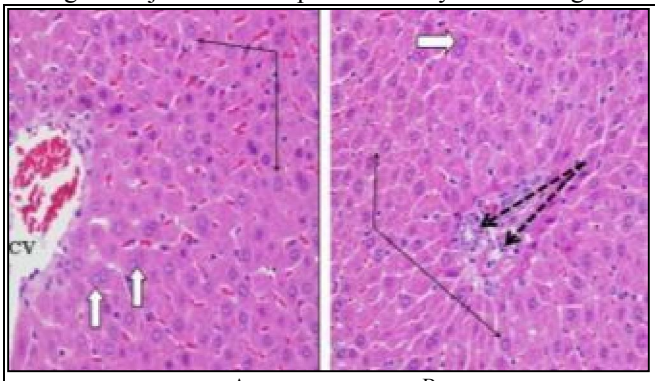
Histological examination of liverof the negative control group demonstrated normal histological pattern where hepatic lobules appeared as hexagonal masses of liver cells (hepatocytes) radiating form a central vein. Blood sinusoids appeared between cords of hepatocytes. The hepatocytes had a hexagonal outline with central rounded nucleus. The cytoplasm showed some vacuoles Figure 1.Compared to negative control Figure 1, examination of liver sections of the positive control (hypercholesterolemic rats) group revealed marked impairment of the normal structural organization of hepatic lobules in many areas and deposition of large lipid droplet in cells. Hepatocytes showed vacuolar degeneration, swollen and vacuolated cells, and some nuclei revealed clear signs of dark small pyknosis as illustrated in Figures 2 and 3.

Examination of liver of hypercholesterolemic rat treated by Pomegranate juice in a dose of 1 ml/kg b. wt., revealed a marked improvement with normal hepatocytes, congested central vein when compared to the positive control group Figure 4. The hypercholesterolemic rat orally given Pomegranate juice in a dose of 3 mg/kg b. wt., showed marked improvement from changes caused by cholesterol except of few residual cells with fine lipid droplets, or scattered dark apoptotic cells and some sections showed bile duct proliferation Figure 5.

Sections from liver of hypercholesterolemic rat after treated by Pomegranate juice in a dose of 5 ml/kg b. wt., showed more improvement in histological structure comparing with section of rats that orally given Pomegranate juice in doses of 1 and 3 ml/kg b. wt., The examination section showed almost normal structure with regular arrangement of hepatocyte cell cords and exhibited a reduction in fat accumulation. The hepatocytes around the central vein (CV) showed rounded nuclei and vesicular indicating active cells. Blood sinusoids between the cells had also normal appearance Figure 6. These histological findings agreed with the study of **Fyiad*et al.* (2012)** who investigated the effect of Pomegranate juice on nucleic acids alterations and oxidative stress in experimentally hepatitis rats. Results of the previous study revealed that pretreatment with Pomegranate juice (20 ml kg-1 b. wt., day-1 for 14 days) effectively hindered the adverse effect of D-Galactosamine / lipopolysaccharide and protected against hepatic damage via suppression of oxidative stress. Histopathological studies of the liver of different groups also supported the protective effects exhibited by Pomegranate juice through restoring the normal hepatic architecture. A significant decrease in the serum level of diagnostic enzyme markers (AST, ALT and ALP) was also detected as compared to the positive control group.

The histological examination of the heart tissue of normal healthy rats showed normal histological architecture manifested by normal cardiac vessels wall thickness, normal size and appearance of cardiac muscles and blood capillaries as illustrated in Figure 7. In rats fed on high - cholesterol diet, the examination of the heart revealed some degenerative changes with inflammatory cell infiltration and marked congestion of blood capillaries as demonstrated in Figure 8. The heart sections of the hypercholesterolemic rat orally given Pomegranate juice in a dose of 1ml/kg b. wt., showed a slight improvement of pathological lesions with presence of thickened walls and cholesterol deposition in cardiac vessels. Degeneratd dark muscles and congested vessels Figure 9. Treatment with Pomegranate juice in a dose of 3 ml/kg b. wt., showed a moderate improvement except cardiac vessels still had focal thickening and some cardiac muscles looked dark Figure 10. Oral administration of Pomegranate juice in a dose of 5 ml/kg b. wt., revealed a marked improvement in histological architecture of the heart tissue except presence of few

apoptotic dark cells in the cardiac muscle as shown in Figure 11. The present results agreed with the study of **Rosenblat and Aviram (2006)** who reported that the antioxidant and free radicals scavenging property of Pomegranate juice seem to protect the myocardium against oxidative damage in heart tissue



A B

Figure 1: (a-b) Section in the liver of a normal rat (negative control) showing liver cells surrounded by central vein (CV) and peripheral portal structures (dotted blackarrows), normal hepatocytes with large central vesicular (blackarrows),some cells are binucleated (white arrows) (H & E x 400).

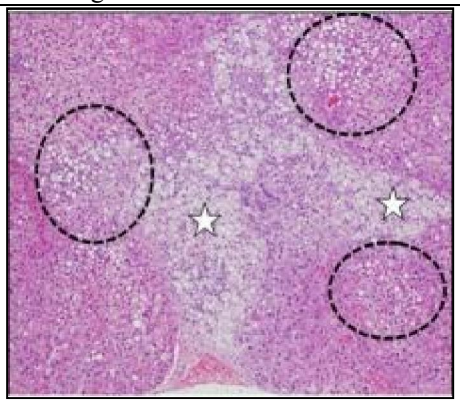
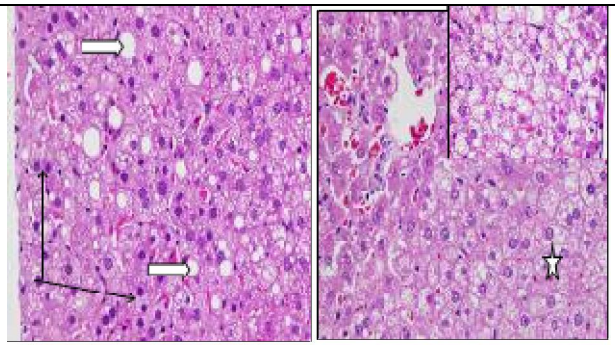


Figure 2: Section in the liver of hypercholesterolemic rat (positive control) showing foci of lipid droplets deposition within hepatocytes (dotted circles) and the neighboring cells showed vacuolar degeneration (stars) (H& E x 100).



A **B**

Figure 3: (a-b) Section in the liver of hypercholesterolemic rat (positive control) showing deposition of large lipid droplet in some cells (white arrows). In other cells marked vacoulations were observed (stars), some cells showed dark small degenerated nuclei (black arrows) (H & E x 400).

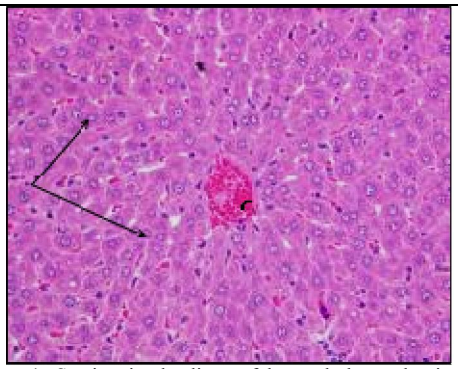
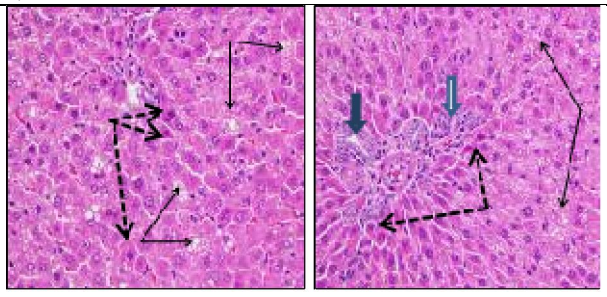


Figure 4: Section in the liver of hypercholesterolemic rats after treatment with 1ml/ kg b. wt., Pomegranate juice showing mild improvement with congested central vein (CV), normal hepatocytes (black arrows) and numerous binucleated cells (white arrows) (H & E x 400).



A **B**

Figure 5: (a-b) Section in the liver of hypercholesterolemic rat after treatment with 3ml/kg b. wt., Pomegranate juice showing moderate improvement from degenerative changes except presence of few residual cells with lipid droplets (thin black arrows) or scattered dark apoptotic cells(dotted arrows) and some showed bile duct proliferation (white arrows) (H& E x 400).

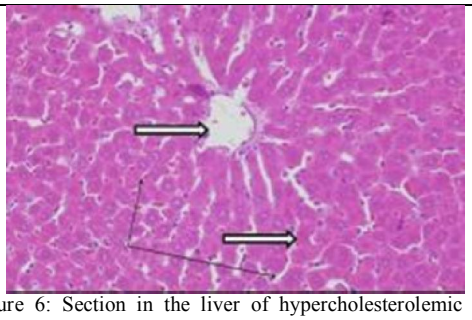
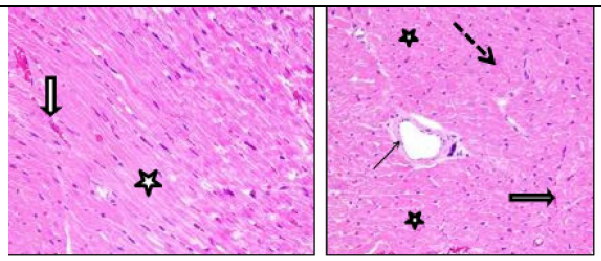


Figure 6: Section in the liver of hypercholesterolemic rat after treatment with 5ml/ kg b. wt., Pomegranate juice showing almost normal structure with regular arrangement of hepatic cell cords (black thin arrows) around the central vein (CV), hepatocytes showed rounded and vesicular nuclei indicating active cells. Hepatic sinusoids between the cells showed normal appearance (white arrows) (H & E x 400)



A B

Figure 7 (a-b) Section of heart of normal rats (negative control) showing cardiac vessels with normal wall thickness (black arrows). Cardiac muscles (stars) with normal size and appearance (no degeneration), Blood capillaries were also normal (white arrows) (H &E x 400).

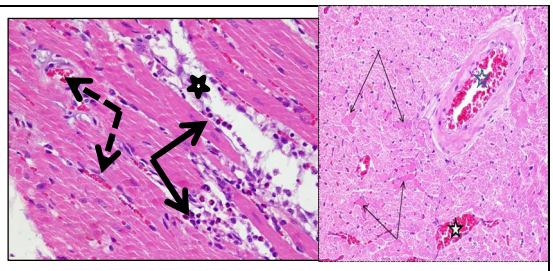
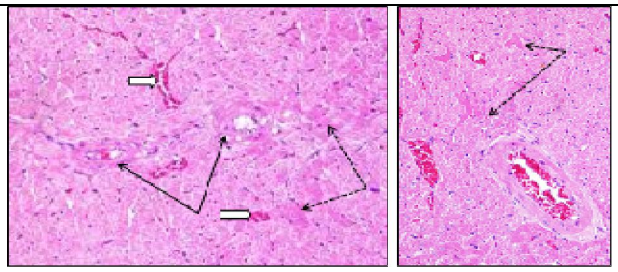


Figure 8 (a-b) Section from rat heart of hypercholesterolemic rat (positive control) showing degenerative changes of some cardiac muscles (stars) with inflammatory cells infiltration (black arrows). Marked congestion of blood capillaries (dotted arrows) was seen (H & E x 400).



A B

Figure 9 (a-b) Section from rat heart of hypercholesterolemic rat after treated with ml/kg b. wt., Pomegranate juice showing a slight improvement except presence of thickened walls and cholesterol deposition in cardiac vessels (black arrows). Degenerated cardiac muscle (dotted arrows) and congested vessels (white arrows) were also seen (H & E x 400).

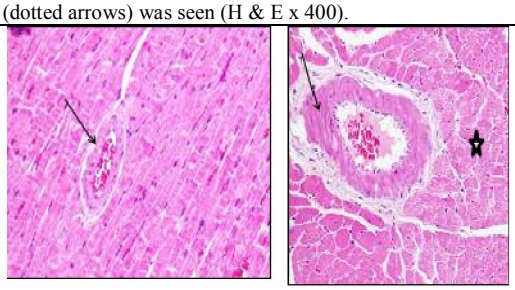


Figure 10 (a-b) Section in cardiac muscles of hypercholesterolemic rat after treatment with 3ml/kg b. wt., Pomegranate juices showing a moderate improvement (white star). Cardiac blood vessels still showed focal thickening (black arrows). Cardiac muscle showed degeneration (dotted arrows) (H&E x 400).

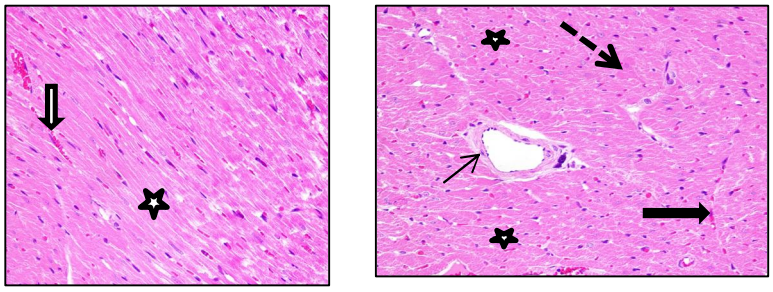


Figure 11 (a-b) Section in cardiac wall of hypercholesterolemic rat after treated with 5ml/kg b. wt., Pomegranate juice showing almost normal appearance of cardiac muscles (stars) except few apoptotic dark cells (dotted arrows). Cardiac blood vessels showed normal thickness (thin black arrow). Blood capillaries were normal and not congested (white arrow) (H & E x400).

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