



### RESEARCH ARTICLE

#### EFFECT OF POMEGRANATE JUICE AND PEEL ON ANTIOXIDANT ENZYMES AND LIPID PROFILE IN CARBON TETRACHLORIDE-INDUCED HYPERLIPIDEMIC RATS.

Doaa M. Genena<sup>1</sup> and Neveen F. Agamy<sup>2</sup>.

1. Fellow of Nutrition, Medical Research Institute - University of Alexandria
2. Assistant Professor of food analysis, High Institute of Public Health- University of Alexandria

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#### Abstract

Lipid peroxidation is a crucial step in the pathogenesis of free radical-related disease states, including glomerulonephritis, rheumatoid arthritis, gastrointestinal and cardiovascular diseases. Antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation. Pomegranate is a good source of polyphenols and other antioxidants. The purpose of the present study is to evaluate the effect of pomegranates juice and peel administration on antioxidant enzymes activities and lipid profile in carbon tetrachloride-induced hyperlipidemic rats. Hyperlipidemic rats were treated with pomegranates juice and peel for 8 weeks. Results showed that all hyperlipidemic rats administrated juice and peel had significant decrease in TBARS concentration and increase in GSH, GPx, GST, CAT and SOD in plasma compared to CCl<sub>4</sub> group. Lipid profile parameters (except HDL-C) decreased significantly with juice and peel administration compared to CCl<sub>4</sub> group. In conclusion, consumption of pomegranate juice and peel showed significant improvement of lipid parameters and antioxidant enzyme activities in hyperlipidemic rates suggesting therapeutic potential of using pomegranate as a medicinal plant for managing hyperlipidemia and modifying the risk of CVD.

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#### Introduction: -

Human are continuously exposed to different kindsof chemicals such as food additives, industrialchemicals, pesticides and other undesirablecontaminants in the air, food and soil(Stavric, 1994).These substancesmostly induce a freeradical-mediated lipid peroxidation leading todisruption of biomembranes and cellular dysfunction. Lipid peroxidation is thought to play an important role in the pathogenesis of free radical-related disease states, including glomerulonephritis, rheumatoid arthritis, gastrointestinal and cardiovascular diseases(Cho et al., 2003). Oxidative stress has been linked to an altered lipid profile and endothelial damage which is a crucial event in the most common pathological processes underlying cardiovascular diseases (CVD)(Halperin et al., 2006). Additionally, the well-known side effects of the current synthetic lipid-lowering drugs have increased the need to move toward alternative treatment for the prevention of hyperlipidemia including medicinal plants and natural antioxidants(Pahan, 2006).

Antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation(Sheweita et al., 2001). Studies have demonstrated that there are approximately 5000 known plant phenolics and many of them have antioxidant activity(Pace et al., 2006). During last years, the

interest in the antioxidant properties of the phenolic compounds in vegetables and fruits among agricultural and food scientists, nutritionists, food industry professionals and consumers had increased, derived from their strong activity and low toxicity compared with those of synthetic phenolic antioxidants, such as butylated hydroxytoluene (Dolatabadi et al., 2010).

*Punica granatum* L., commonly called pomegranate has an ancient history; sculptured representations of the fruit are found on the ancient monuments of Egypt and the Assyrian ruins. The tree is native to the region of Persia and the Himalayan ranges of India and has been cultivated in Iran, Afghanistan, Pakistan, North India, Armenia, Azerbaijan, Georgia, and the Mediterranean region for several millennia (Jurenka et al., 2008). Pomegranate has been used in various regions and folk or traditional medicine because of its enormous compounds with lots of activities and without toxicity (Lansky et al., 2007). Pomegranate juice is an affluent source of polyphenols with high antioxidative potential. Moreover, its antihypertensive, and anti-inflammatory effects have been shown in limited studies in human and murine models. Pomegranate juice showed an antioxidant activity three times higher than red wine and a green tea infusion (Gil et al., 2000). Pomegranate juice also displayed potent antiatherogenic action in atherosclerotic mice and humans (Aviram et al., 2000).

Carbon tetrachloride ( $\text{CCl}_4$ ), a well-known model agent for producing chemical hepatic injury, requires biotransformation by hepatic microsomal cytochrome P450 to produce toxic metabolites, namely trichloromethyl free radicals ( $\text{CCl}_3\cdot$ ) and subsequent derivative ( $\text{Cl}_3\text{COO}\cdot$ ) (Brautbar et al., 2002). These radicals may interact with membrane lipids leading to peroxidation (Aamir et al., 2011).  $\text{CCl}_4$  causes an imbalance between the synthesis and degradation of lipids (Boll et al., 2001). In view of this, this study was carried out to evaluate the effect of pomegranate juice and peel administration on antioxidant enzymes activities and lipid profile in carbon tetrachloride-induced hyperlipidemic rats.

### Materials and Methods:-

The component used in the preparation of products and standard diet were purchased from local markets in Alexandria, Egypt.  $\text{CCL}_4$  was purchased from Merck; Darmstadt. The dose of  $\text{CCL}_4$  used was 1 ml/kg BW twice a week (Zuinen et al., 2007). Commercial kits were obtained from Biosystems S.A. (Spain), Diamond (Germany) and Randox (United Kingdom). Methanol, Folin-Ciocalteu (Prolabo) reagent, Sodium Carbonate, Gallic Acid, Rutin,  $\text{AlCl}_3$ , DPPH reagent were obtained from Central laboratory unit for advanced environmental and biological analyses, High institute of public health, Alexandria University. The study was approved by the ethical committee of the High Institute of Public Health, Alexandria University.

### Preparation of Pomegranate juice:-

Pomegranate was purchased from local markets in Alexandria, Egypt. The fresh pomegranate fruits, free of blemishes or obvious defects were washed and cut into pieces, the rind was removed, and seeds were separated. Juice was obtained using a commercial blender (Braun blender, Germany), filtrated with a Buchner funnel and immediately diluted with distal water to volume of 1:3 and stored at  $20^\circ\text{C}$ . The chosen dose of Pomegranate juice for the experiment was 5 ml/Kg BW (Osman et al., 2011).

### Preparation of pomegranate peel powder:-

The pomegranate peel was collected, washed, cut into small pieces and dried in air then in an oven at  $50^\circ\text{C}$  till complete dryness. The dried peel was grounded to fine powder in a mechanical blender and stored at  $3 - 4^\circ\text{C}$  until used. For the experimental protocol, 5% of pomegranate peel powder were added to the standard diet (Abdou et al., 2012).

### Experimental animals and protocol:-

Fifty male rats about 130-150 gm were obtained from the Medical Research Institute, Alexandria University, Alexandria, Egypt. The animals were housed in cages under standard condition of illumination with a 12-h light-dark cycle at  $25 \pm 1^\circ\text{C}$  (Childs et al., 2002). Animals were housed 5 per cage and fed on standard rodent pellet diet (Gold Mohar, Lipton - India, Ltd), and tap water ad libitum for two weeks before the beginning of the experiment for rehabilitation. Diets were presented to rats in special non-scattering feeding cups to avoid loss of food and contamination. Every day the animals were observed for the external appearance, shape, color, distribution of hair and physical activity. The animals were then divided randomly into the following five groups:

**Group (1):** Ten rats served as control group given the standard diet for 8 weeks.

**Group (2):** Ten rats were given the standard diet and treated with CCL<sub>4</sub> alone (1 ml/Kg BW) twice a week for 8 weeks.

**Group(3):** Ten rats were given the standard diet and treated with combination of CCL<sub>4</sub>(1ml/kg BW) and pomegranate juice(5 ml/Kg BW) for 8 weeks.

**Group (4):** Ten rats were treated with combination of CCL<sub>4</sub>(1 ml/Kg BW) and pomegranate peel alone added to standard diet by 5% for 8 weeks.

**Group (5):** Ten rats were treated with combination of CCL<sub>4</sub> (1 ml/KgBW), pomegranate juice (5 ml/Kg BW) and pomegranate peel by 5% for 8 weeks.

#### Measured parameters and techniques:

At the end of the experiment, rats were fasted overnight and anesthetized with chloroform. The blood samples were collected in tubes with heparin (anti-coagulant). The heparinated blood samples were placed immediately in ice. Plasma samples were obtained by centrifugation at 860xg for 20min, and were stored at -20°C until analyses. Liver was immediately removed at the end of the experiment; weighed and washed using chilled saline solution. Tissues were minced and homogenized (10% w/v) in ice-cold sodium potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 ×g for 20 min at 4°C. The resultant supernatant of the organs was used for the measurement of different enzyme activities.

#### Estimation of antioxidant enzymes in plasma and liver:-

The activity of Glutathione S-transferase (GST; EC 2.5.1.18), Glutathione reductase (GSH) Glutathione peroxidase (GPx; EC. 1.1.1.9) was determined by the methods of **Habig et al.,(1974)**, **Beutler et al., (1963)**, and **Chiu et al., (1976)** respectively. Catalase (CAT; EC 1.11.1.6) activity was assayed using the Luck method involving the decomposition of hydrogen peroxide (**Luck, 1971**). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to **Misra and Fridovich (1972)**. Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method of **Tappel and Zalkin (1959)**.

#### Lipid profile:-

Plasma concentrations of total lipids, total cholesterol (TC) and triglycerides (TG) were determined per the methods of **Frings et al., (1972)**, **Watson (1960)**, and **Fossati et al., (1982)**, respectively. High-density lipoprotein (HDL-C) was measured according to the methods of **Warnicket al., (1983)**. Low-density lipoprotein (LDL-C) was determined by the calculation (cholesterol-(TG/5+HDL-C). Very low-density lipoprotein (VLDL-C) was calculated by dividing the values of TG by factor of 5.

#### Statistical analysis:-

Data were analyzed using SPSS software package version 20 (SPSS, Chicago, IL, USA). P value (< 0.05) was considered significant. The data were assessed for normality using Kolmogorov-Smirnov test. The data were normally distributed and presented as mean ± standard deviation. One way ANOVA test was used to compare the mean for different measures among the different experimental groups, followed by post-hoc pairwise comparison with adjustment of P value using Bonferroni correction.

#### Results and Discussion:-

Data in Table 1 shows the effects of pomegranate juice and peel on antioxidant enzymes activities in CCl<sub>4</sub>-induced hyperlipidemic rats. CCl<sub>4</sub> significantly (p<0.05) increased TBARS and decreased GSH levels as well as GPx, GST, CAT and SOD activities in plasma of the rat group treated only with CCl<sub>4</sub> as compared to control group. In other words, CCl<sub>4</sub> induced oxidative stress in plasma as manifested by the alterations observed in antioxidant defense systems both enzymatic and non-enzymatic. Lipid peroxidation is one of the principal causes of CCl<sub>4</sub> toxic effect and is mediated by the free radicals' derivatives of CCl<sub>4</sub>. Our results are in consistence with the results of **Shih et al. (2005)** who confirmed that the hepatic activity of superoxide dismutase was markedly decreased by CCl<sub>4</sub> treatment. Also, **Comporti (1985)** indicated a substantial increase in ROS, MDA levels and depletion of GSH levels in CCl<sub>4</sub> intoxicated rats.

On the other hand, our study demonstrated that treatment of hyperlipidemic rats with combination of CCL<sub>4</sub>, pomegranate juice and peel caused significant (p<0.05) decrease in TBARS concentration and increase in GSH, GPx, GST, CAT and SOD activities. In other words, pomegranate juice and peel reduced the toxic effects of CCl<sub>4</sub> (Table1). The significant elevation in GSH, GST, SOD, GPx and catalase of plasma indicates the protection offered

by juice and peel against CCl<sub>4</sub> induced toxicity. It is known that some flavonoids are able to reduce xenobiotic - induced toxicity in animals and counteract the damaging effects of oxidative stress, cooperating with natural systems like glutathione and other endogenous protective enzymes (Kadarian et al., 2002). These results are in agreement with Mohieldin et al. (2011) who revealed that treating rats with carbon tetrachloride and pomegranate peel ethanolic extract had significantly decrease the GST, catalase and SOD activity, and significantly increase the GST, catalase and SOD activity when compared with CCl<sub>4</sub>-treated group during the hepatoprotective and curative periods. Our findings are also confirmed by the findings of Aviram et al., (2004) who reported that total antioxidant status in serum of patients with carotid artery stenosis was substantially increased, by 130% after 12 months of juice consumption. The effect of juice may be related to its potent tannins and anthocyanins which scavenge wide spectrum of free radicals. (Gil et al., 2000), (Aviram et al., 2000).

Pomegranate peel administration showed insignificant higher levels of plasma GSH, GPx, GST, CAT, and SOD when compared to juice, which is may be due to the slightly higher content of polyphenols in peel than in juice. Aviram et al., (2000) found that the inner and outer peels contain 20–30 fold more polyphenols than do the aqueous fractions of the seeds and Juice (566 and 739 µmol polyphenols/mg compared with 22 and 25 µmol polyphenols/mg, respectively).

The decrease of TBARS in plasma of the hyperlipidemic rat groups treated with juice and peel (Table 1) agrees with the results of Naveena et al., (2008) who reported that the free radical scavenging activity of pomegranate peel phenolics involves electron donation to free radicals that converts them to relatively more stable compounds. Several studies have confirmed the antioxidant and free radical scavenging activity of phenolic compounds derived from pomegranates, the antioxidant activity of plants extracts depends on the concentration of phenolic compounds, and the antioxidant power of pomegranate peel extract has been found to linearly increase with the concentration of peel phenolics up to the level of 400 µg/g (Sönmez et al., 2005), (Rosenblat et al., 2006).

Data listed in Table 2 shows the effect of pomegranate juice and peel on lipid profile of CCl<sub>4</sub>-induced hyperlipidemic rats. Treatment of rats with CCl<sub>4</sub> caused a significant increase ( $p < 0.05$ ) in plasma TG, TC, LDL-C and VLDL-C, while HDL-C was significantly decreased ( $p < 0.05$ ) compared to control group. These findings are in agreement with Uličná et al. (2003) who reported that the long-term intraperitoneally administration of CCl<sub>4</sub> to experimental animals induced, as expected, pathological changes in the liver. CCl<sub>4</sub> treatment of rats also caused a severe increase of hepatic triacylglycerol, total cholesterol and Malondialdehyde. The highly reactive free radical (trichloromethyl, CCl<sub>3</sub><sup>•</sup>) in the presence of oxygen leads to auto-oxidation of fatty acids and causes functional and morphological changes in the cell membrane. Scavenging of free radicals is one of the major anti-oxidation mechanisms to inhibit the chain reaction of lipid peroxidation. ROS are highly reactive and can react with many intracellular molecules, mainly unsaturated fatty acids (phospholipids, glycolipids, glycerides and sterols) and transmembrane proteins with oxidizable amino acids. The oxidation of these molecules causes the increase in the cellular membrane permeability. ROS can attack the unsaturated bonds of the membrane lipids in an autocatalytic process, with the genesis of peroxides, alcohol and lipidic aldehydes as by-products of the reaction. Thus, the increase of free radicals in cells can induce the lipid peroxidation by oxidative breakdown of polyunsaturated fatty acids in membranes of cells (Henke et al., 2005).

Meanwhile, our study reported that treatment of hyperlipidemic rats with pomegranate juice caused a significant decrease ( $p < 0.05$ ) in plasma lipids except HDL-C, which was significantly increased ( $p < 0.05$ ) compared to control group. These results are in agreement with Esmailzadeh et al., (2006) who found that pomegranate juice significantly reduced TC, LDL-C, the ratio of LDL-C/HDL-C, and the ratio of TC to HDL-C. Another study on mice fed pomegranate juice, plasma lipid peroxidation was markedly lower, and this effect was dependent on concentration (Aviram et al., 2000). Furthermore, Aviram et al., (2004) showed that pomegranate juice consumption for 3 years on patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL-C oxidation. Khoo et al., (1990) reported that LDL-C aggregation was inhibited in vitro by pomegranate juice; this inhibition may be related to hydrophobic interactions between constituents of pomegranate juice and the lipoprotein. Pomegranate juice was indeed shown to possess impressive antioxidative properties due to its polyphenolics, tannins and anthocyanins (Gil et al., 2000). Additionally, phenolic compounds derived from pomegranate, vitamins C and E, melatonin and lycopene have been used as antioxidant agents to prevent various lipid-peroxidation-induced damages in different organs (Sönmez et al., 2005), (Rosenblat et al., 2006), (Sönmez et al., 2007), (Türk et al., 2007). Polyphenolic flavonoids not only affect cellular oxygenases but also cause

conformational changes in plasma membrane constituents, such as cellular receptors for lipoproteins(Aviram et al., 2000).

The decrease in plasma lipid profile of hyperlipidemic rats treated with peel which is showed in the present study is in agreement with Hossin(2009)who evaluated the pomegranate peel powder as a dietary fiber source for the treatment of hypercholesterolemia and atherosclerosis and found that dietary supplementation with peel powder at a concentration of 5, 10 and 15 g/100 g for a period of four weeks significantly reduced serum TC, TG, LDL-C, VLDL-C and lipid peroxidation levels in Hypercholesterolemic rats. Results of the present study confirm these findings(Table2).He also reported that all Hypercholesterolemic groups administered with different level of pomegranate peel extract (1, 2 and 3%) had significant decrease in serum TC, LDL-C-, VLDL-C, lipid peroxidation and atherogenic index and triglycerides comparing with control positive. Additionally, Ibrahim (2010)noticed that feeding the rats on standard diet containing 400 or 800 mg/Kg BW pomegranate peel extract for 4 weeks obviously decreased ( $P<0.05$ ) levels of TC, TG and TL.Pomegranate peel polyphenolic extract is effective in lowering serum and hepatic lipids(Cheng et al., 2005).

In the current study pomegranate peel was shown to be more influential in lowering level of lipid profile than juice, which is consistent with the findings of Aviram et al., (2000) who compared between the juice and the peel in terms of total polyphenol content. The researchers analyzed the antioxidant properties of pomegranate constituents other than the juice; they prepared aqueous solutions of the inner and outer peels and the crushed seeds and found that the aqueous extracts of the inner and outer peels were more powerful antioxidants than the juice, suggesting that the inner and outer peels may contain more potent antioxidant polyphenols. The aqueous extract obtained from the crushed seeds was found to be a weak antioxidant against LDL-Coxidation.

Based on the previous results, the authors concluded that the consumption of pomegranate juice and peel caused significant improvement of lipid parameters and antioxidant enzyme activities in hyperlipidemic rats suggesting therapeutic potential of using pomegranate as a medicinal plant for managing hyperlipidemia and modifying the risk of CVD.

**Table 1:-** Effect of pomegranate juice and peel on antioxidant enzymes activities in CCl<sub>4</sub>-induced hyperlipidemic rats.

Parameter	Experimental groups				
	Control	CCl <sub>4</sub>	CCl <sub>4</sub> +J*	CCl <sub>4</sub> +P*	CCl <sub>4</sub> +J*+P*
TBARS (nmol/ml)	0.54±0.01 <sup>d</sup>	0.97±0.03 <sup>a</sup>	0.71±0.01 <sup>b</sup>	0.67±0.02 <sup>b</sup>	0.63±0.01 <sup>c</sup>
GSH (U/ml)	0.549±0.05 <sup>b</sup>	0.250±0.02 <sup>e</sup>	0.406±0.03 <sup>d</sup>	0.457±0.03 <sup>cd</sup>	0.498±0.03 <sup>cb</sup>
GPx (U/ml)	16.63±1.09 <sup>b</sup>	7.39±0.60 <sup>d</sup>	12.16±0.65 <sup>c</sup>	13.92±1.17 <sup>bc</sup>	14.98±1.18 <sup>bc</sup>
GST (μmol /hr./ml)	1.03±0.02 <sup>b</sup>	0.49±0.03 <sup>e</sup>	0.82±0.02 <sup>d</sup>	0.86±0.02 <sup>cd</sup>	0.92±0.02 <sup>c</sup>
CAT (u/min/ml)	51.83±3.90 <sup>b</sup>	22.71±0.99 <sup>d</sup>	40.58±3.63 <sup>c</sup>	43.75±4.02 <sup>bc</sup>	45.63±3.66 <sup>bc</sup>
SOD (U/ml)	2.73±0.02 <sup>b</sup>	1.40±0.10 <sup>d</sup>	2.08±0.03 <sup>c</sup>	2.39±0.03 <sup>bc</sup>	2.55±0.10 <sup>b</sup>

\*J: Pomegranate juice, \*P: Pomegranate peel.

Values are expressed as means ± SE; n = 10 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c, d, e, f) were significantly different,  $p<0.05$ .

**Table 2:-**Effect of pomegranate juice and peel on lipid profile in CCl<sub>4</sub>-induced hyperlipidemic rats.

Parameter	Experimental groups				
	Control	CCl <sub>4</sub>	CCl <sub>4</sub> +J*	CCl <sub>4</sub> +P*	CCl <sub>4</sub> +J+P
TL(mg/dl)	508.75±11.31 <sup>c</sup>	677.88±10.00 <sup>a</sup>	574.98±11.91 <sup>b</sup>	557.94 ±6.32 <sup>b</sup>	555.13±24.43 <sup>b</sup>
TC(mg/dl)	147.72±5.45 <sup>d</sup>	198.76±4.40 <sup>a</sup>	181.93±7.86 <sup>ab</sup>	168.67±6.47 <sup>bc</sup>	160.35±6.31 <sup>cd</sup>
TG(mg/dl)	123.00±5.43 <sup>b</sup>	159.30±4.24 <sup>a</sup>	149.13±10.79 <sup>a</sup>	146.15±12.27 <sup>a</sup>	138.87±8.11 <sup>ab</sup>

<b>HDL-C(mg/dl)</b>	54.48±2.63 <sup>b</sup>	36.21±2.87 <sup>e</sup>	40.31±1.62 <sup>ed</sup>	45.87±2.57 <sup>cd</sup>	48.06±2.98 <sup>bc</sup>
<b>LDL-C(mg/dl)</b>	68.64±1.73 <sup>c</sup>	130.69±0.68 <sup>a</sup>	111.79±4.08 <sup>b</sup>	93.57±1.45 <sup>b</sup>	84.42±1.71 <sup>b</sup>
<b>VLDL-C(mg/dl)</b>	24.6±1.09 <sup>d</sup>	31.86±0.85 <sup>a</sup>	29.83±2.16 <sup>ab</sup>	29.23±2.45 <sup>ab</sup>	27.87±1.62 <sup>cd</sup>

\*J: Pomegranate juice, \*P: Pomegranate peel.

Values are expressed as means ± SE; n = 10 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c, d, e, f) were significantly different, p<0.05.

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