# EFFECT OF POLYPHENOLIC COMPOUNDS OF POMEGRANATE SEED AND PEEL ON HYPERLIPIDEMIC RATS

Kassem, Siham\*; Zeinab El-Hawary\*; Gamal El-Baroty\*\* and Fawzi El-Shobaki\*

- \* Nutrition Department, National Research Center, Dokki, Cairo, Egypt.
- \*\* Biochemistry Department, Faculty of Agriculture, Cairo University.

#### **ABSTRACT**

The effect of pomegranate seed or peel added as dry matter or extract on lipid parameters and oxidatative state of hypercholesterolemic rats was studied. Hypercholesterolemia was induced in rats by feeding high fat, high cholesterol diet for 10 weeks. Groups of those rats received diet containing either pomegranate dry seed or peel powder. Others received the methanolic extract of either the seeds or peels.

The plasma total cholesterol, HDL-C, LDL-C, VLDL-C, Triglycerides, Lipid peroxides, SOD and catalase activities were followed before and after each dietary regimen.

The results obtained showed a significant increase in each of plasma total cholesterol, LDL, VLDL, Triglyceride lipid peroxides in rats given the HF, HC diet. The plasma HDL, activity of each of SOD and catalase enzymes were decreased. Addition of pomegranate either the seed or the peel in the powder form or as extract corrected that pattern and most of these parameters returned back to near normal level. Analysis of the pomegranate extract by HPLC revealed the presence of compounds including phenolic acids, flavonals, catachin, isoflavones and flavanones.

These findings were interpreted and the conclusion reached is that pomegranate seeds and peels can protect from hypercholesterolemia and in turn atherosclerosis. The methanolic extract contain an appreciable quantities of phenolic compounds that posses antioxidative power. The antioxidative power of the peels is much higher than the seeds since it contains more of these compounds with antioxidant effect.

**Keywords**: Hyperlipidemia; Polyphenols; Pomegranate Seed; Pomegranate Peel

## INTRODUCTION

Atherosclerosis is a disease of global distribution. It has reached alarming epidemic proportion in economically developed countries. In Egypt, although no definite statistical figures for the incidence of the disease are available, yet sporadic studies and observation point to the occurrence of the disease with its consequent complications among a considerable number of the population.

Atherosclerosis is a disease of large and medium sized muscular arteries and the large elastic arteries, such as the aorta and iliac vessels, (Salonen et al., 2000).

Cardiovascular disease is essentially caused by narrowing of arteries "atherosclerosis" which can lead to reduced supply of oxygen to organs such as the heart, skeletal muscle, brain, intestine and kidneys. The presence of cholesterol in the diet is not the sole factor involved in the pathogenesis of the disease. Other nutrients constituting the diet interfere and interact with this dietary cholesterol, thus modifying the metabolic pathway and exert an affect on the manifestation of the disease, (Plotnick et al., 2003).

Antioxidants are defined as any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Epidemiological studies have shown that consumption of fruits and vegetables is negatively associated with morbidity and mortality of cardio and cerebro-vascular disease and certain types of cancers (Johnsen et al., 2003; Rissanen et al., 2003; Temple and Gladwin, 2003). The antioxidant contained in fruits and vegetables, including ascorbic acid, carotenoids, flavonoids, hydrolysable tannins, are supposed to play an important role in prevention of these diseases (Knekt et al., 2002).

Evidence from animal and human experiments also reveals that some natural antioxidants other than ascorbic acid, carotenoids and vitamin E could be absorbed significantly and act as potent antioxidants in vivo. Pomegranate is an important source of active compounds and had been used for Folk medicine for many centuries. The juice has been proved to be effective in prevention of atherosclerosis (Temple and Gladwin 2003). However, few studies were done on the pomegranate seeds or peel. It has been reported that, both the juice and peel are high in antioxidant activity (Aviram et al., 2000, Kaplan et al 2001). Whether the juice, seed or the peel, it is useful to know, the specific compounds in pomegranate that exert the biological action. Flavonoids are believed to be the concerned compounds that exert this antioxidant activity and in turn protect against atherosclerosis.

The present study, aims to analyse the polyphenols content and to investigate the antiatherogenic effect of pomegranate seeds and peels. Also to differentiate between the whole fruit and the methanolic extract.

## MATERIALS AND METHODS

#### Material:

The materials used in the present study are. Pomegranate seed <u>Punica granatum</u>
Pomegranate peel

## Preparation of samples:

The samples were purchased from the local market. Pomegranate was cut and edible parts separated from the peel, each of edible parts "seeds and peel", were dried in an oven till complete dryness, weighted to calculate its water content, then ground to fine powder, preserved in plastic bags, put in a refrigerator till its analysis and using in animal experiments.

Each of the prepared samples were subjected to general analysis for proteins, fats and ash (Table 2).

Table (2): The average composition (g/100 edible portion)

English name	Water content	Ash g%	Fat g %	Protein g%
Pomegranate seed	79	1.97	3.50	2.63
Pomegranate peel	77	3.77	0.80	3.5

## Extraction of polyphenolic compounds:

Each of the dried sample was extracted by methyl alcohol in soxhelet apparatus according to method of Meichael et al., (1992).

The extract was dried in a rotary evaporator, put in brown bottles and preserved in a refrigerator till it was used for formulation of the diet for the animals.

Total polyphenol were determined according to Official method (1990). Total flavonoids were estimated according to the method of Peric et al., (1978).

The individual flavonoids in the extracts, were detrmined by HPLC according to the method of Ben-Hammouda et al., (1995).

Triglycerides were determined according to the method of Fossati and Praancipe, (1982), using kits obtained from Stanhio laboratory. Plasma total cholesterol was determined according to Allain et al., (1974). Plasma HDL-C was estimated as described by (Warnick et al., 1993). Estimation of LDL-C according to Bergmenyer et al., (1985).

## Animal experiments:

#### Diets:

#### Normal control diet:

The control diet is composed of casein (12%) as the source of protein (table 1), salt mixture and vitamins mixture were prepared according to the method of Philip *et al.*, (1993).

#### Animals:

64 Female albino rats (Sprague Daweley) of body weight ranging from 80–100 gm, were obtained from the central animal house of the institute of ophthalmology.

Rats were divided into groups, (each group of 8 rats).

- i. Normal control group was given normal diet (table 1).
- ii. Rats were given high fat diet (HF) with cholesterol (2%) (HC) (table 1).
- iii. Rats given HF, HC diet + pomegranate seed powder 20%.
- iv. Rats given HF, HC diet + extract equivalent to 20% powder.
- v. Rats given HF, HC diet + pomegranate peel powder 20%.
- vi. Rats given HF, HC diet + extract equivalent to 20% powder.

All groups were given high fat diet for a period of four weeks. Heparinized blood samples were taken from each rat from the suborbital vein under slight either anesthesia. Plasma was separated and analyzed for

cholesterol content. A high level of cholesterol was considered as an indication for "hypercholesterolemia".

Rats in each group were put on the designed regimen as shown, and the experiment continued for another 6 weeks. At the end of experiment (ten weeks), the rats were anesthetized with ether. Heparinized blood samples were obtained, plasma was separated, preserved in deep freeze at -20°C till its analysis

Red blood cells (RBCs) were washed three times by saline separated by centrifugation and kept in a deep freeze at -20°C till analysis.

Table (1): composition of control (C)diet and high cholesterol diet (HF)

(Philip et al., (1993):

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In any diamen	С	HF		
ingredients	g/kg			
Casein	120	120		
Sucrose	100	100		
Fat hydrogenated (palm fat)	100	200		
Oil	50	50		
Salt mixture	35	35		
Vitamin mixture	10	10		
L-cystine	1.80	1-8		
Choline chloride	2.50	2-5		
Starch	580.7	480.7		

#### RESULTS AND DISCUSSION:

The major contributors to cholesterol accumulation in arterial cells during development of atherosclerosis include several factors such as the high level of plasma cholesterol (Joan et al., 2005) reduced serum paraoxonase (Li et al., 2006), enhanced macrophage cholesterol estrification rate (Aviram et al., 2000), in addition to the most effective factor which is the increased oxidative stress. This rendered several investigators to look for food rich in antioxidant that can be preventive to atherosclerosis among exposed individuals.

Pomegranate is one of these food items that is believed to possess strong antioxidant property, including it's capability to scavenge or prevent several reactive oxygen species and inhibit lipid peroxidation. (Aviram 2004, Li et al., 2006).

The total polyphenol determined as tannic acid in pomegranate amounted to 509.46 & 645.03 mg/100gm dry weight for both the seeds and peels respectively. Among those polyphenolic compounds, the amount of isoflavonoids determined in both the seeds and peels as catachin amounted to 600 & 1250 mg/100gm dry weight respectively (Table 3). These values are comparable with similar reported values by other authors (Augustin and Gary 2000) and shows that pomegranate contain an appreciable antioxidant power relative to other food items.

Table (3) Total Polyphenois as Tannic acid

English name	Total Polyphenols as Tannic acid			
English hante	Dry weight mg/100g	Fresh weight mg/100g		
Pomegranate seeds	509.46	106.99		
Pomegranate peels	645.03	148.37		

The group of rats fed on the high fat high cholesterol diet, showed a significant increase in the level of plasma total cholesterol, LDL, VLDL, triglycerides and lipid peroxides. The HDL, activity of either SOD or catalase were decreased. A sub group (8 rats) from this HF, HC group was isolated and left on a normal diet for the rest of the experiment. The data obtained for this group showed that the lipid parameters started to change toward normalization, however the values were still very far from those obtained from rats fed on the diet containing pomegranate. This again confirm the role of pomegranate to correct that derangement in either lipid parameters or oxidative stress.

In general, the pomegranate dry matter or the extract, when added to the diet of rats given the high fat, high cholesterol caused a significant drop in the values of lipid parameters which were increased due to the inclusion of the high fat and cholesterol.

An exception to that is the level of plasma HDL which was significantly increased. This effect occurred in both cases, when either the whole dry matter or the extract was added. This proves that the effect exerted on the lipid parameters under such condition is mainly due to the presence of flavonoids, since the extract contain mainly these compounds. With regard to the lowering effect of pomegranate on plasma cholesterol, the effect was more marked in case of the extract either the seeds or peels.

The total flavonoids in peels as determined is about double that in seeds (Table 4), however, the effect was more or less similar. This shows that the effect is limited to certain concentration of flavonoids and excess to that limit seems to be not effective. The lowering effect of phenolic compounds present in pomegranate can be attributed to either their reducing capacity of cholesterol absorption (Kenkt *et al.*, 2002), or due to inhibition of Apo B secretion in hepatocytes. The latter is the precussor of the VLDL-C. It was suggested that polyphenols bind with plasma membrane transport P-glycoprotein, which inhibit cholesterol estrification (Baynes and Thrope, 2000), decreasing the incorporation of cholesterol ester into nasert VLDL.

Table (4) Total Isoflavonoids as Catechin

English name	Total Isoflavonoids as Catechin			
English name	Dry weight mg/100g	Fresh weight mg/100g		
Pomegranate seeds	600	126		
Pomegranate peels	1250	287.5		

The effect of the extract on triglyceride was also more marked than the whole part, similar to the effect on cholesterol. Polyphenol may alter hepatic recreation of triglycerides-rich VLDL. It was reported to cause a reduction of

both plasma Apo B and Apo E concentration. Apo E was found to displace Apo CII from the VLDL particle thereby inhibiting lipoprotein lipase activity and over all lipolysis (Bravo, 1998). It is suggested that the reduction of triglycerides may be partially attributed to decreased Apo E concentration.

In association with the decrease in plasma cholesterol and triglyceride observed due to addition of either the pomegranate dry matter or the extract, a decrease was noticed in lipid peroxides or lipid oxidation products, and a relative increase in the activity of superoxide dismutase and catalase. These changes are indicative to a slower rate of lipid oxidation and show that the (Zern et al., 2005). In conclusion, the fore- mentioned finding showed that, feeding rats with high fat, high cholesterol diet caused a significant increase in lipid parameters except the HDL. The activities of antioxidant enzymes were increased.

Supplementation of the diet with either pomegranate seed or peel caused a marked and observable improvement in lipid parameters and enzyme activities. This effect was more marked in case of supplementation with peel. The same effect was observed when the alcoholic extract of the seed or peel was used. This extract proved to contain appreciable quantities of phenolic compounds which are known to posses antioxidant power.

pomegranate seeds or peels either the dry matter or the extract could exert an antioxidant effect on lipid compartments of these animals. The potent antioxidant activity of pomegranate juice was reported before by Fridovich (1995), Fuhrman et al., (1997) and Kapalan et al. (2001), and may also contribute to the lowering effect of pomegranate seeds or peels, dry matter or extract, on the high plasma cholesterol accumulation, which is indicative to cholesterol accumulation in the retrial cells and development of atherosclerosis (Mohan Kumar et al., 2004). The increased HDL-C is indicative to increased activity of paraoxonase which associates HDL and protects it from oxidation, (Aviram et al., 1998).

Table (5): Lipid parameters in control groups:

Groups		Total cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control I	M	67.63	22.75	30.00	13.35
	± S.E	2.07	1.05	1.77	<u>0.828</u>
	M	401.86	26.04	108.29	36.74
Control II	± S.E	13.99	1.95	13.61	1.56
	Р	0.000	0.101	0.000	0.000
Control III	M	379.00	24.87	103.88	29.84
	± S.E	12 <i>.</i> 49	2.14	12.37	2.66
	P1	0.000	0.000	0.000	0.000
	P2	_0.000	0.000	0.000	0.000

Control I. Given normal diet

Control II. Given HF, HC diet fore 4 weeks.

Control III. Given HF, HC for 4 weeks, then normal diet till 10 weeks.

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Table (6): Triglyceride, Lipid peroxide, SOD and Catalase activities in plasma of those control rats).

Triglyceride Lipid peroxide Catalase SOD U/ml Groups mg/dl mmol/ml U/ml М 1.949 76.13 489.87 9.97 Control I ± S.E 0.113 15.89 0.19 3.96 М 4.94 120.66 1.92 184.00 ± S.E Control II 7.83 0.13 12.87 0.12 Ρ 0.000 0.000 0.000 0.000 М 143.000 2.08 149.25 3.326 ± S.E 13.29 0.122 14.105 0.218 Control III P<sub>1</sub> 0.000 0.000 0.000 0.000 0.000 0.000 0.000 0.000

Table (7): Lipid Parameters in Plasma of Rats Fed on Diet Containing

20% Pomegranate (dry Matter and Extract).					
Groups		Total cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Seed	M±	263.75	59.13	74.00	22.13
Dry	S.E	13.75	4.38	3.61	1.44
matter	P <sub>1</sub>	0.000	0.000	0.000	0.000
	P <sub>2</sub>	0.000	0.000	0.022	0.000
	P <sub>3</sub>	0.000	0.000	0.036	0.023
Seed	М	188.75	45.000	60.000	14.63
Extract	± S.E	7.53	7.82	2.05	0.65
	P <sub>1</sub>	0.000	0.014	0.000	0.247
]	P <sub>2</sub>	0.000	0.001	0.003	0.000
	P <sub>3</sub>	0.000	0.001	0.004	0.000
Peel	М	226.88	47.13	64.88	19.06
Dry	± S.E	14.54	2.16	2.58	1.38
matter	P <sub>1</sub>	0.000	0.000	0.000	0.003
	P <sub>2</sub>	0.000	0.000	0.008	0.008
	P <sub>3</sub>	0.000	0.000	0.000	0.000
Peel	M	204.50	43.38	61.38	16.50
Extract	± S.E	11.30	1.76	3.52	6.8
	P <sub>1</sub>	0.000	0.000	0.000	0.007
	P <sub>2</sub>	0.000	0.000	0.004	0.000
5	P <sub>3</sub>	0.000	0.000	0.000	0.000

P1 = Difference from control I

The antioxidant action of pomegranate is suggested to be the polyphenols reported in the extracted either the seeds or the peels. Antioxidant activities are due to the capacity of these compounds to transfer

P2 = Difference from control II

P3 = Difference from control III

electron ion free radicals, chelae metal catalyst, activate antioxidant enzymes as reported in this study and inhibit oxidases (Spencer et al., 2001).

Analysis of phenolic compounds by HPLC showed the presence of 17 compounds in either the seed or the peel. The concentration in the peel is much more than that in the seed. This goes in agreement with the analysis of total flavonoids as catachin. The compounds present include phenolic acid, flavonals, catachin, isoflavones and flavanones. The most dominant compound is catachin followed by phenol, genestein kaempheral and others. The pattern of analysis seems to be in agreement with that of Li et al., (2006). It is clear that these phenolic compounds detected in the extract of either the seeds or the peel of pomegranate are responsible for the antioxidative effect and cholesterol lowering action. The hypocholesterolemic effect may be either due to retarding effect on cholesterol absorption, or the increase in LDL-receptor mediating cholesterol removal (Estruch et al., 2004). The antioxidant action of most of compounds that were detected in pomegranate seed or peel was reported before by several authors,

Table (8): Triglyceride, Lipid peroxide, SOD, Catalase in Rats Fed on Diet Containing 20% Pomegranate (dry Matter and Extract).

Diet Containing 20% Pomegranate (dry Matter and Extract).						
Groups	!	Triglyceride	Lipid peroxide	SOD	Catalse	
		mg/dl	mmol/ml	U <u>/ml</u>	U/m1	
Seed dry	M	110.75	2.052	208.82	5.79	
matter	± S.E	7.25	0.144	19.96	0.13	
	P <sub>1</sub>	0.000	0.000	0.000	0.000	
· 	P <sub>2</sub>	0.000	0.000	0.000	0.000	
	P <sub>3</sub>	0.023	0.000	0.000	0.000	
Seed	М	71.25	2.394	296.24	6.53	
extract	± S.E	2.80	0.23	10.91	0.27	
	P <sub>1</sub>	0.330	0.000	0.000	0.000	
	P <sub>2</sub>	0.000	0.000	0.000	0.000	
	P <sub>3</sub> `	0.000	0.000	0.000	0.000	
Peel	М	92.25	2.259	318.615	7.152	
Dry matter	± S.E	7.91	0.121	13.71	0.171	
	P <sub>1</sub>	0.091	0.000	0.000	0.000	
	P <sub>2</sub>	0.000	0.000	0.000	0.000	
	P <sub>3</sub>	0.000	0.000	0.000	0.000	
Peel	M	82.50	2.01	200.35	5.883	
extract	± S.E	6.88	0.122	22.42	0.104	
	P <sub>1</sub>	0.435	0.000	0.000	0.000	
	P <sub>2</sub>	0.000	0.000	0.001	0.000	
	P <sub>3</sub>	0.000	0.000	0.052	0.000	

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تأثير مركبات عديد الفينولات الموجودة في الرمان على مستوى الدهون المرتفع في حيوانات التجارب

سُهام قاسم ، زينب الهواري ، جمال الباروطي \* و فوزي الشوبكي \*

قسم التعذية، المركز القومي للبحوث، الدقي، القاهرة، مصر

قسم الكيمياء الحيوية، كلية الزراعة، جامعة القاهرة

تهدف هذه الدراسة إلى معرفة تأثير المركبات عديدة الفينولات الموجودة في الرمان على مدلولات الدهون في الجسم ومدى الإصابة بأمراض تصلب الشرايين. وقد أجريب الدراسة على الفئران البيضاء والتي يتراوح أوزانها ما بين ٨٠ – ١٠٠ جم. وقد تم تقسيم الفئران إلى عدة مجموعات تحتوي مجموعة وضعت لها وجبه قياسية والمجموعات الأخرى وضعت لها وجبه قياسية عالية في محتواها من الدهون مضافا إليها مادة الكولسترول ، وتم دراسة تأثير مستخلصات الرمان من الحبوب والقشر على التغييرات البيوكيميائية واستمرت التغذية لمدة عشرة أسابيع ثم من خلالها متابعة الاستهلاك اليومي من الطعام وأوزان الحيوانات.

يستنتج من الدراسة أنه بالرغم من أن ما تناولته حيوانات التجارب من مادة الكولسترول ارتفعت مدلولات الدهون عندها وبعد المعاملة بمستخلص الرمان سواء الحبوب أو القشر أدى إلى انخفاض مستوى مدلولات الدهون وخاصة القشر "مستخلص القشر" كان له تأثير أعلى من مستخلص الحبوب مما يدل على أن محتوى قشر الرمان من المواد عديدة الفينولات والتي تعتبر مضادات الأكسدة أكثر من الحبوب بالتسالي يمكن الاستفادة من هذه النتائج بوضع أنظمة غذائية لحماية الأفراد المعرضون للإصابة بأمراض تصلب الشرايين أو لمعاونة المصابين بهذا المرض للتخفيف من حدته.