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Protective Effects of Pomegranate Juice, Peel and Their Molasses on Aging

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Abstract

Purpose: Pomegranate fruit could be considered a functional food because it has valuable phytochemicals that display medicinal effects. These can act as antioxidant and anti-hepatotoxic. The purpose of this study was to investigate evaluate total phenolic contents, antioxidant capacity in Pomegranate juice, pomegranate peel and their molasses and study the effect of these products on the lipids profile, lipid peroxidation and liver functions of aging rats.

Methods: Thirty aged male albino rates, Sprague Dawley Strain, weighting (315 + 10 g) were divided to five groups; the first group (control) was fed with the basal diet. The second and the third groups were fed with pomegranate juice (2 and 4 ml /day), The fourth and fifth groups were fed with pomegranate molasses (0.5ml/day) and Pomegranate peel (10% /day) , respectively. After four weeks blood samples were taken for analysis.

Results: pomegranate peel flowed by pomegranate molasses contained the highest polyphenols and flavonoids contents. pomegranate juice (2 and 4 ml), pomegranate molasses(0.5ml) and pomegranate peel (10%) showed higher GSH-px activates than aged rats control.The findings showed a significant reduction in Glutathione peroxidase (GPx) and Superoxide dismutase (SOD), and an enhancement in malondialdehyde (MDA) values after treatment ($p < 0.05$). The supplemented by pomegranate juice (2 and 4 ml), pomegranate molasses(0.5ml) and pomegranate peel (10%) induced significant decrease in serum total cholesterol and triglyceride, the

higher reduction percentage belonged to the aged rats group supplemented with PP (10%). The highest reduction of LDL-C and VLDL-C were achieved by using Pomegranate peel (10%).

Conclusion: The results of this study showed the protective effects of pomegranate juice, pomegranate molasses and pomegranate peel on Symptoms of aging-induced serum oxidative stress (SOD and GPx) and lipid profile (TC, HDL, and LDL) changes in aging rats. The findings of this study also showed considerable antioxidant activity, total phenolic, and total flavonoid contents. However, further studies are needed to investigate the mechanisms of oxidative stress induction and protection, some un-expected results.

Key words: Pomegranate Juice; Molasses; Antioxidant activity; lipids profile, lipid peroxidation.

INTRODUCTION

Aging is described as the changes that, occur in living organisms with the passage of time that lead to functional impairment and ultimately to death. A general decline in various biochemical and physiologic functions is noted in most organs during agine, resulting in increased susceptibility to age-associated diseases **(Srinivasan, 1999)**. In its original form, the free radical theory of aging proposed that aging is due to the accumulation of unrepaired damage from free radical attack on cellular components. Modern thinking interprets the free radical hypothesis in terms of oxidative stress, and the reformulated theory proposes that aging is caused by a shift in the balance between the pro-oxidative and anti-oxidative processes in the direction of the pro-oxidative state. It is postulated that aging results from an increase in oxidative damage to lipids, proteins, or DNA or, alternatively, from the effect of the oxidative stress on the regulation of genes that govern developmental processes, including differentiation and aging. **(Harman, 1992; Beckman and Ames 1998 and Cadenas and Davies 2000).** Considerable importance is given to functional foods, which apart from their basic nutritional functions, provide physiological benefits and play an important role

in disease prevention or slow the progress of chronic diseases. There has been much more interest in the pomegranate as a medicinal and nutritional product because of its multi functionality and its great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction (**Viuda-Martos *et al.* 2010 and Jaiswal *et al.* 2010**).

Pomegranate could be considered a functional food it has valuable phytochemicals that display medicinal effects. These can act as anti-diabetic, antimicrobial, antioxidant, anti-hepatotoxic and anti-inflammatory and improve cardiovascular health (**Celik *et al*. 2009 and Lee *et al*. 2010**). Polyphenols as dietary antioxidants may affect various aspects of both innate and adaptive wings of the immune system by shifting pro-oxidant/antioxidant balance. Complement system, for instance, has been shown to be inhibited by polyphenols and this complement inhibitory effect may have some role in anti-inflammatory properties of polyphenols. Pomegranate rich concentration of diverse, free-radical scavenging bioflavonoids, made it recommended in the treatment of acquired immune deficiency syndrome (**Moneim, 2011**).

Pomegranate juice is an important source of phenolic compounds: the soluble polyphenols content varies from 0.2 to 1.0g/100g, being anthocyanins one of the most important. Together with lignans, gallagyl-type tannins, ellagic acid derivatives and other hydrolysable tannins which contribute to the antioxidant activity of the juice (**Heber, 2008**).

Pomegranate molasses is a thick syrup made from cooked-down pomegranate juice, which is a slightly astringent, sweet–sour condiment that is deep and dark (and slightly ruby) in color (**Kaya and Sözer, 2005**). Pomegranate syrup had various applications as a flavoring agent, a salad dressing or soft drink ingredient. Pomegranate molasses have 2 to 3 folds high content of mineral and antioxidant than pomegranate juice (**Yilmaz *et al*. 2007**).

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Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids and tannins. These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit (**Zahin et al., 2010**). Pomegranate peel has been known for many years for its health benefit, including antibacterial activity. More recently, research indicated that pomegranate peel extracts also inhibit tyrosinase activity an enzyme that induces the production of melanin which leads to hyperpigmentation of the skin.

The present study was undertaken to investigate evaluate total phenolic contents, antioxidant capacity in Pomegranate juice, pomegranate peel and their molasses and study the effect of these products on the lipids profile, lipid peroxidation and liver functions of aging rats.

MATERIALS AND METHODS

Materials:

Pomegranate fruits were purchased from local market of Giza governorate, Egypt. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and common commercial suppliers. Total cholesterol, HDL-cholesterol, LDL-cholesterol, total lipids, alkaline phosphatase (AP), aspartate amino transferase (AST), alanine amino transferase (ALT), glutathione peroxidase (GSH) and malonaldehyde (MDA) kits were obtained from Randox Laboratories Ltd, England.

Methods:

Pomegranate juice: Pomegranate fruits were washed, drained and manually cut-up and the outer leathery skin, which encloses hundreds of fleshy arils, was removed. The juice that is localized in the arils was manually pressed and extracted and stored at freezer for further analysis.

Pomegranate molasses: The pomegranate juice was concentrated by using an electromagnetic heater. A 500 ml of the juice sample was put in a beaker and replaced

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on the heater open to atmosphere. The sample was continuosly heated and stirred during this process. Samples were taken for measurement of °Brix and replaced again after used.

Total phenolic: con tents of pomegranate juice and molasses were determined according to **Singelton et al. (1999)** using Folin–Ciocalteu reagent and gallic acid as standard and the resu lts expressed as mg gallic acid equivalent/100 ml. Free-radical scavenging acti vity of the tested samples against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed according to the method described by **Karioti et al. (2004)**. Total flavonoids were measured spectrophotometrically at 510 nm and expressed as mg gallic acid equivalents/100 ml according to **Yang et al. (2009)**.

Experimental animal:

Thirty aged male albino rates, Sprague Dawley Strain, weighting (315 + 10 g) as well as (5) adult male weighting (young rats) (125 + 5 g) were used. All rats were fed standard diet for four consecutive days. Aged rats were divided into six groups, 5 rats each with similar total body weight and were housed individually in the wire cage. All groups of rats were fed the experimental diet four weeks according to following groups; the first group (young control) was fed with the basal diet. The second group (aged rats) were divided to five groups; the first group (control) was fed with the basal diet. The second and the third groups were fed with pomegranate juice (2 and 4 ml /day), The fourth and fifth groups were fed with pomegranate molasses **(** 0.5ml /day) and pomegranate peel(10%) respectively. The blood samples were obtained from orbital plexus venus by means of fine capillary glass tubes according to the method described by **Schermer (1967)**. The blood samples were placed in dray and clean centrifuge tubes and allowed to clot for 1 - 2 h at room temperature. Serum was removed using a Pasteur pipette and centrifuged for 20 min at 1100 x g. The clean supernatant serum was kept frozen until analysis.

Biochemical analysis:

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Total lipids, triglycerides, total cholesterol, low density lipoprotein (LDL) and high-density lipoprotein (HDL) were determined according to the methods described by **Frings and Dunn (1979); Richmound (1973); Fossati and Prencipe (1982); Friedwald et al. (1972) and Demacker et al. (1980),** respectively. The alkaline phosphatase (AP); Alanine amino transferase (ALT) and aspartate amino transferase (AST) enzymes were measured according to the methods described by **Varley et al. (1980) Bergmeyer and Harder (1986); Kachmar and Moss (1976),** respectively**.** The superoxide dismutase (SOD) activity was measured as the degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH by the method of **Marklund and Marklund (1974).** Glutathione peroxidase enzyme (GSH) and malonaldehyde (MDA) were determined according to the methods described by **Hu (1994); Jentzsch et al. (1996).**

Statistical analysis:

The results were recorded as the mean ± SD of the three replicates. The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system. Duncan's multiple range tests were used to determine the differences among means at the level of 5%.

RESULTS AND DISCUSSION

Polyphenols, total flavonoids and antioxidant activity:

As shown in **Table 1**, the polyphenol content of pomegranate juice (6.97) were significantly ($P \leq 0.05$) lower than that of pomegranate molasses and peel (12.75 and 16.86 mg/100g), respectively. Also, the content of flavonoids of pomegranate juice were significantly ($P \leq 0.05$) lower than that of pomegranate molasses and peel. The increase in total phenolic and total flavonoids content of pomegranate molasses is mostly due to the evaporation of water during processing. Pomegranate fruit is a rich source of polyphenols which ranged from 290-450 mg/100 ml juice and the total flavonoids contents were significantly different between pomegranate cultivars with approximately 1.6-folds difference between the highest and the lowest contents (**Fawole *et al*. 2011**).

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Antioxidant activity of pomegranate products measured by DPPH method revealed that, the antioxidant activity of pomegranate peel (86.45%) was considerably higher than that of pomegranate juice (76.83%), but no significant difference was detected between the pomegranate molasses (83.71%). **Orak (2008)** determined the antioxidant activity of pomegranate juice and molasses as 79.06% and 85.91%, respectively, which were in accordance with the present results. It is stated that the higher antioxidant activity of pomegranate molasses is due to the phenolic content in spite of anthocyanin being destroyed by the effect of heating.

Table (1): Polyphenols, total flavonoids and antioxidant activity of pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP).

Groups	Polyphenols (mg/100g)	Total flavonoids	DPPH (%)
Pomegranate juice (PJ)	6.97^{c}	19.74^{c}	76.83^{b}
Pomegranate molasses (PM)	12.75^{b}	26.57^{b}	83.71^{ab}
Pomegranate peel (PP)	16.86^{a}	37.94^{a}	86.45^{a}
LSD	1.54	1.83	2.84

Food intake, body weight and FER

Table (2) shows the effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on food intake and body weight in aged rat, also the effects on body weight gain was studied. The experiment aged rats supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) showed lower than food intake and the mean value were 23.8, 23.4, 22.9 and 21.5 gm/day, respectively. Data in Tables (2) indicated that, the mean values of initial body weight of aged group rats after adoption feeding on basal diet, were nearly the same and ranged between 271.38 to 275.24 gm. At the end of experiment (4 weeks) the final body weight of aged rats control was 276.46 gm. The

aged rat supplemented on PJ, PM and PP high body weight than the negative control rats.

The obtained results that the feed efficiency ratio (FER) at the end of period for the aged control rat was (0.063) while the groups supplemented by (2 and 4ml) PJ were increased the (FER). Also feed on 0.5 ml PM and 10% PP were increased the FER at 0.252 and 0.550 respectively.

Table (2): Effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on food intake body weight and FER of aging rats.

Groups	Initial-B/W (gm)	Final-B/W (gm)	Body weight gain (g)	Food intake (g/day)	FER
Aged rats (control)	274.86a	276.46b	1.60d	25.6a	0.063c
Pomegranate juice 2 ml	275.24a	282.65a	7.41b	23.8b	0.311b
Pomegranate juice 4 ml	272.37a	283.19a	10.82a	23.4b	0.463ab
Pomegranate molasses 0.5ml	274.76a	280.52a	5.76c	22.9bc	0.252b
Pomegranate peel 10%	271.38a	282.46a	11.08a	21.5c	0.550a
LSD	4.23	5.74	1.06	1.47	0.097

PJ: pomegranate juice; PM: pomegranate molasses and PP: pomegranate peel

Enzyme antioxidant

Glutathione is a major, non-protein thiol in living organisms which performs a key role in co-coordinating the innate antioxidant defense mechanisms. It is involved in the maintenance of the normal structure and function of cells, probably by its redox and detoxification reactions (**Gueeri, 1995**). The activity of glutathione peroxidase (GSH-px) enzyme in blood of different groups of was measured and the results are

Table (3). The lowest value was found to be with control aged rats, which recorded only 31.51 U/ml. Moreover, the group of aged rats supplemented by pomegranate juice (2 and 4 ml), pomegranate molasses and pomegranate peel (10%) showed higher GSH-px activates than aged rats control. Furthermore, aged rats supplemented by PJ, PM and PP induced a significant increase in the levels of reduced glutathione by 47.22, 65.25, 61.96 and 84,96%, respectively.

MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage **(Ohkawa *et al.*, 1979)**. An increase in the mean MDA level, a measure of lipid peroxidation, was found in the serum of aged rats (Tables 3). Treatment of aged rats supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) showed lower the mean MDA concentration. aged rats supplemented by 10% PP showed more reduction in MDA level by value 20.56 IU/L (28%) which was a significant different compared with aged rats. In addition, insufficient levels of antioxidants to scavenge peroxy radicals during ageing **(Wei, 1998)** could also have contributed to the elevated level of MDA in the aged rats.

Significantly lower activities of SOD enzyme were noted in the aged rats control when compared to the values of other groups. In aged rats that had been administered of by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP), the activities of these enzymes were significant increase compared to control aged rats.

From the same table could be reported feed on pomegranate products (PJ, PM and PP) improvement on the functionality of enzyme antioxidant in aged rats. Polyphenols have been shown to be important antioxidants for brain tissues, being able to improve short-term memory and Alzheimer's disease, reducing the oxidative stress and inflammation that could lead to motor and cognitive deficits (**Joseph et al., 2005**). These data taken together showed that the use of could be pomegranate products

important to retard or prevent the development of diseases associated with oxidative stress, such as the neurodegenerative diseases.

Table (3): Effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on enzyme antioxidant in aged rats.

Groups	Glutathione (GSH)		Malondialdehyde (MDA)		Superoxide dismutase (SOD)	
	After treatment	Change %	After treatment	Change %	After treatment	Change %
Aged rats (control)	25.58^{d}	---	31.46^{a}	---	31.51^{c}	----
Pomegranate juice 2 ml	37,66^{c}	+47.22	25.54^{b}	-18.82	35.87^{ab}	+13.84
Pomegranate juice 4 ml	42,27^{b}	+65.25	23.28^{b}	-26,00	39.32^{a}	+24.78
Pomegranate molasses 0.5ml	41.43^{b}	+61.96	22.46^{b}	-28.61	34.43^{b}	+9.27
Pomegranate peel 10%	47.27^{a}	+84.79	20.56^{b}	-28.29	38.56^{a}	+22.37
LSD	3.18		3.26		3.01	

Total Cholesterol and triglyceride

Data shown in Table (4) illustrated the effect of supplemented with pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on total cholesterol, and triglyceride in aged rats. Results from table (4) showed the concentration of Total Cholesterol in the aged control rats with value (126.73 mg/dl). The supplemented by PJ with 2 and 4 ml, PM (0.5ml) and PP (10%) induced significant decrease in serum total cholesterol and the values were 123.67, 109.19, 115.94 and 99.36 mg/dl, respectively. Furthermore, the higher reduction percentage of total cholesterol belonged to the aged rats group supplemented with PP (10%) with value 32.61%. From the same table showed the concentration of Triglyceride in the aged control rats with value (155.62 mg/dl). The supplemented by PJ with 2 and 4 mL and PM induced significant decrease in triglyceride compared with aged control and the values were

136.97, 123.65 and 135.48 mg/dl, respectively. Furthermore, the higher reduction percentage of triglyceride belonged to the aged rats group supplemented with 10% PP with value 36.37%. These data agree with **Hossin, 2009** supplementation with pomegranate peel powder at a concentration of 5, 10 and 15 g/100 g for a period of four weeks significantly reduced serum total cholesterol, triglycerides, LDL and lipid peroxidation levels in hypercholesterolemic rats (**Hossin, 2009**).

Table (4): Effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on Total Cholesterol and triglyceride in aged rats.

Groups	Total Cholesterol		Triglyceride	
	After treatment	Change %	After treatment	Change %
Aged rats (control)	134.85a	---	155.62a	---
Pomegranate juice 2 ml	123.67b	-8.29	136.97b	-11.98
Pomegranate juice 4 ml	109.19d	-19.03	123.65e	-20.54
Pomegranate molasses 0.5ml	115.74c	-14.17	135.48d	-12.94
Pomegranate peel 10%	99.36e	-26.31	119.25c	-36.37
LSD	3.58		3.67	

Lipoproteins fraction:

Data in Table (5) illustrated the effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on lipoproteins fractions including high density lipoproteins (HDL-C), low density lipoproteins (LDL-C) and very low density lipoproteins (VLDL-C) of experimental rats. Aged rats resulted a significant decreased in serum HDL-C level (31.16 mg/dl). Treating rats with pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) showed significantly increased HDL-C level. From data in Table (7), it could be observed that LDL-C and VLDL-C level increased significant in aged rats. When

aged rats fed with supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) the level of LDL-C and VLDL-C were significantly decreased as compared with aged control rats. The highest reduction of LDL-C and VLDL-C were achieved by using Pomegranate peel (10%), the reduction values were 51.43 % from LDL-C and 23.36% from VLDL-C, respectively. This data agree well those reported by **Hossin, 2009** Dietary supplementation with peel powder for a period of four weeks significantly reduced serum LDL and lipid peroxidation levels in hypercholesterolemic rats.

Table (5): Effect of supplemented by pomegranate juice (PJ), Pomegranate molasses (PM) and Pomegranate peel (PP) on lipoproteins fractions in aged rats.

Groups	HDL-C		LDL-C		VLDL-C	
	After treatment	Change %	After treatment	Change %	After treatment	Change %
Aged rats (control)	31.16c	--	72.57a	---	31.12a	----
Pomegranate juice 2 ml	34.13b	+9.53	65.15b	-10.22	27.39b	-11.99
Pomegranate juice 4 ml	39.48a	+26.70	41.62d	-42.65	24.73c	-20.53
Pomegranate molasses 0.5ml	34.59b	+11.01	54.05c	-25.52	27.10b	-12.92
Pomegranate peel 10%	40.26a	+29.20	35.25e	-51.43	23.85d	-23.36
LSD	2.96		3.27		0.74	

Conclusion:

The results of this study showed the protective effects of pomegranate juice, pomegranate molasses and pomegranate peel on Symptoms of aging-induced serum oxidative stress (SOD and GPx) and lipid profile (TC, HDL, and LDL) changes in aging rats. The findings of this study also showed considerable antioxidant activity, total phenolic, and total flavonoid contents. However, further studies are needed to

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investigate the mechanisms of oxidative stress induction and protection, some un-expected results.

Recommendation:

Finally based on results of this work the following points could de recommended:

1) More studies are needed to know the natural sources which rich in polyphenols and flavonoids contents**.**

2) Use of pomegranate juice, pomegranate molasses, and pomegranate peel extracts which is a natural sources of polyphenols and flavonoids contents, caused a significant increased in antioxidant enzymes in aged stage.

3) Use pomegranate juice, pomegranate molasses, and pomegranate peel which are a natural sources of polyphenols and flavonoids contents caused a significant decreased for malondialdehyde (MDA) and a significant increased in antioxidant enzymes in aging rats.

4) Isolate the antioxidant from pomegranate peel to use for supplementation food. Eat natural foods which rich in polyphenols and flavonoids contents.

5) pomegranate juice, pomegranate molasses, and pomegranate peel can be used in many products to reduce the cholesterol level, glucose level and enhance the immune system to avoid a lot of diseases in aging stage .

6) Serum total lipids and triglyceride values were decreased significantly by pomegranate juice, pomegranate molasses, and pomegranate peel

7) we recommended to study the effect of pomegranate juice, pomegranate molasses, and pomegranate peel these extracts on others diseases on experimental rats.

8) Research on how to take advantage of food waste pomegranate peel in food fortification.

9) This research is considered to be the beginning of the start of another research to learn a lot about the benefits of pomegranate and its extracts.

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