



## Pomegranate juice is potentially better than orange juice in improving antioxidant function in elderly subjects

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### ABSTRACT

In the present study, 26 elderly subjects were recruited and randomly divided into 2 groups, that is, orange (low in antioxidant capacity) and pomegranate (high in antioxidant capacity) groups, and 250 mL of juice was consumed daily for 4 weeks. Changes in plasma antioxidant capacity, activity of antioxidant enzymes, contents of ascorbic acid, vitamin E, reduced glutathione, malondialdehyde, oxidized low-density lipoprotein and carbonyls, and the degree of DNA damage in mononuclear blood cells were measured. Urine samples were collected for determination of 8-hydroxy-2'-deoxyguanosine content. Increased plasma antioxidant capacity and decreased plasma carbonyl content were demonstrated after daily consumption of pomegranate juice. In comparison, orange juice consumption presented a less significant effect on antioxidant function in elderly subjects. It is concluded that daily consumption of pomegranate juices is potentially better than orange juice in improving antioxidant function in the elderly. Because the plasma ascorbic acid, vitamin E, and reduced glutathione contents did not differ significantly between the 2 groups in this study, the phenolics may be the functional components contained in pomegranate juice that accounted for the observations.

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### Introduction

A growing body of evidence indicates that accumulation of oxidative damage to macromolecules is increased progressively in the aging process. Over expression of antioxidant enzymes or supplementation of some antioxidants appears to be effective in extending the life span in some nematode and *Drosophila* strains and even in mouse models [Harman D (2001), Mockett RJ, (2002), Chaudhuri A, (2004)]. Energy restriction, which can extend life span, is also effective in reducing oxidative stress [Weindruch R (1996)]. However, inconsistent data have been generated from animal and human studies in the attempt to demonstrate the antioxidant and antiaging effects of antioxidant supplementation [Priemé H, et al (1997), Zandi P, et al (2004), Petersen RC, et al (2005), Parashar, A (2010)]. Therefore, more well-controlled studies are needed to confirm the beneficial effects of antioxidants occurring naturally in various food sources and to elucidate the possible underlying mechanisms.

Fruits and vegetables are important sources of various vitamins, minerals, and fibers for humans. However, they are different in many aspects, including the contents of vitamins, minerals, and fibers as well as their antioxidant capacity.

We reported more than a 90-fold difference in antioxidant capacity among 30 fruits commonly consumed in India as determined by the ferric reducing/antioxidant power (FRAP) assay [Parashar, A. (2010)]. It is well known that fruits are rich in various antioxidants, including ascorbic acid, carotenoids, and phenolics. By using high-performance liquid chromatography coupled with coulometric array detection, we found that some fruits with strong antioxidant capacity eluted more than 70 antioxidant peaks [Parashar, A. et al. (2009)]. Some studies have demonstrated that the antioxidants contained in certain

fruits and vegetables are bioavailable [Parashar, A., et al., (2009), Parashar, A., et al. (2008), Parashar, A., et al. (2008)]. Therefore, these fruits and vegetables can be considered an ideal source of natural antioxidants. It is plausible to speculate that increasing consumption of these fruits intentionally will increase the intake of natural antioxidants, which may provide an alternative in the intervention of the aging process by protecting against oxidative damage.

Various fruits may provide protection differently against oxidative stress because they are different in antioxidant capacity. We hypothesize that fruits with high antioxidant capacity are more effective than those with low antioxidant capacity in reducing oxidative damage associated with the aging process. It is rather difficult and time consuming to purify all natural antioxidants from fruits and study their biologic properties individually.

From a practical point of view, some fruits can be considered a good source for natural antioxidants. Previously, we found that pomegranate juice, which is high in antioxidant capacity, could improve antioxidant function and reduce oxidative damage to macromolecules significantly in aged rats. In comparison, orange juice, which is low in antioxidant capacity, was less effective as compared with pomegranate juice [Manach C, et al (1995)].

In the present study, 26 elderly subjects were recruited, and the effects of daily consumption of pomegranate juice or orange juice on antioxidant function were further investigated in elderly subjects over a 4-week period.

The purpose of this study was to compare the efficacy of pomegranate juice and orange juice in improving antioxidant function in elderly subjects.

### Material And Methods / Experimental Details / Methodology

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### Human subjects and study design

Twenty men and 6 women older than 60 years, living in a district of Mathura, People's Republic of India, were recruited. They were nonsmokers and basically healthy (without cancer; hypertension; or active conditions of the heart, liver, kidney, and lung diseases) and volunteered to participate in the study. Their general physical characteristics are presented in Table 1. Approval for this human study was granted by the Department of Scientific Program Management of the institute, and written informed consent was obtained from each subject.

The subjects were randomly assigned to 2 groups, that is, pomegranate group and orange group. The pomegranate juice was freshly made from pomegranate pulps kindly provided by Shandong Red Pomegranate Co Ltd, India. The orange juice was purchased from the Great Lake Co Ltd, Mathura, People's Republic of India.

Because fruits are important in human nutrition and highly recommended for regular consumption, it is not ethical to ask participants to exclude fruits or fruit juices completely from their diet in the intervention period. Therefore, we did not set up a control group having placebo or plain water in the present study.

The antioxidant capacity and major antioxidant contents of the 2 fruit juices were measured, and the results are listed in Table 2.

During the 4-week intervention period, a total of 250 mL of pomegranate juice or orange juice was distributed to each participant every morning and usually consumed within 2 hours after breakfast as instructed. All participants were asked to abstain from any antioxidant supplements and fruits or fruit juices except the fruit juices used in the study.

### Dietary nutrient intake

Before the intervention started, all subjects were given instructions for a balanced diet based on the dietary guidelines recommended by the Indian Nutrition Society. During the second to third week of the study, a 7-day dietary survey was carried out using a 24-hour recall method to compare daily nutrient intakes between the 2 groups. The food composition data were cited from the India Food Composition 2006, which

was compiled by the Institute of Nutrition and Food Safety, Indian Center for Disease Control and Prevention.

### Blood collection

At the beginning and end of the study, blood samples were drawn from the antecubital vein after overnight fasting and treated with heparin. Whole blood was centrifuged to obtain plasma, and the mononuclear blood cells were obtained by density gradient centrifugation on Ficoll-Paque solution. Plasma was used for measurement of antioxidant capacity; activity of antioxidant enzymes; and contents of ascorbic acid, vitamin E (VE), reduced glutathione (GSH), malondialdehyde (MDA), oxidized low-density lipoprotein (ox-LDL), and carbonyls. The mononuclear blood cells were used for measurement of DNA damage.

### Plasma antioxidant capacity and contents of ascorbic acid, VE, and GSH

The FRAP assay described by Benzie and Strain [Parashar, A., et. al. (2008).] was followed to analyze plasma antioxidant capacity. Plasma content of ascorbic acid was quantified spectrophotometrically based on the reaction with 2,4-dinitrophenylhydrazine [Parashar, A., et. al. (2007)]. An improved fluorometric method was used to determine plasma VE content [Etminan M, et. al. (2005)]. Plasma GSH was assayed spectrophotometrically by the reaction of 5,5'-dithiobis-2-nitrobenzoic acid with thiols. The kit was purchased from the lovely Bioengineering Institute, People's Republic of India.

### Plasma activities of antioxidant enzymes

Plasma activity of superoxide dismutase (SOD) was measured through the inhibition of nitroblue tetrazolium reduction by the superoxide radicals generated by the xanthine/xanthine oxidase system. One unit of SOD activity is defined as the enzyme amount causing 50% inhibition in 1.0 mL reaction solution. Plasma activity of glutathione peroxidase (GSH-Px) was determined by measuring the reduction of GSH per minute on the base of its catalysis.

The final result was expressed as a decrease of 1.0  $\mu\text{mol/L}$  GSH per 5 minutes for 0.1 mL plasma at 37°C after the nonenzymatic reaction is subtracted. Plasma activity of catalase (CAT) was determined by measuring the intensity of a yellow complex formed by molybdate and  $\text{H}_2\text{O}_2$  at 405 nm after ammonium molybdate was added to terminate  $\text{H}_2\text{O}_2$  degradation, which is catalyzed by CAT. One unit of CAT activity is expressed as the degradation of 1.0  $\mu\text{mol/L}$   $\text{H}_2\text{O}_2$  per second per 1.0 mL plasma. Detailed procedures for these measurements followed the instructions for the commercial assay kits obtained from Lovely Bioengineering Institute.

### Plasma contents of MDA, ox-LDL, and carbonyls

The content of plasma MDA was determined by thiobarbituric acid-reactive species assay [Guo CJ, et. al. (2003)]. A commercial enzyme-linked immunosorbent assay kit purchased from TPI Inc was used to measure plasma ox-LDL content. Plasma carbonyl content was detected by the reaction with 2, 4-dinitrophenylhydrazine as reported by Levine et al [Guo CJ, et. al. (1997)].

### Urinary 8-hydroxy-2'-deoxyguanosine content and blood mononuclear cell DNA damage

At the end of the study, morning urine samples were collected after overnight fasting for determination of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and creatine contents. The 8-OH-dG in urine was quantified by an enzymelinked immunosorbent assay procedure. The kit was purchased from the Japan Institute

for the Control of Aging. Blood mononuclear DNA damage was analyzed using the single-cell microgel electrophoresis assay, also known as Comet assay [Cao GH, et. al. (1998)]. The results were expressed as the percentage of cells with DNA damage (with comet-like images) and the comet tail length.

#### Statistical analysis

All data were expressed as means  $\pm$  SD and checked for normality before being subjected to further analysis. Student t test was used for normally distributed data and Wilcoxon rank sum test for nonnormally distributed data in analyzing the statistical significance for the difference between the 2 groups. The level of significance was set at  $P < 0.05$  for all statistical tests.

#### Results And Discussion

Pomegranate (*Punica granatum* L) is a celebrated medicinal food and has been chosen as the symbol of medicine for the 2000 UK Millennial Festival of Medicine [Verhagen H, et. al. (1995)]. Pomegranate juice was found to be a powerful antioxidant and rich in phenolics [Ko SH, et. al. (2005)]. In the present study, the pomegranate juice used was higher in antioxidant capacity and contained more antioxidants than the orange juice, except for ascorbic acid, as indicated in Table 2. The studies by Aviram et al [Xu J, et al (2005)] and Kaplan et al [Benzie IFF, Strain JJ (1996)] showed that pomegranate juice consumption reduced oxidative stress, atherogenic modification to LDL, and platelet aggregation in humans and atherosclerotic apolipoprotein E-deficient mice. The phenolics contained in pomegranate juice had been considered the possible functional components [Xu J, et al (2005), Benzie IFF, Strain JJ (1996)]. In a previous study, we demonstrated that daily intragastric administration of pomegranate juice was effective in decreasing serum contents of carbonyls and ox-LDL and protecting against blood mononuclear cell DNA damage in aged rats [Parashar, A. (2010), Parashar, A et al. (2009)]. By comparison, orange juice administration only displayed significantly protective action against blood mononuclear cell DNA damage [Manach C, et al (1995)]. In the current study, daily consumption of 250 mL pomegranate juice for 4 weeks improved antioxidant function significantly in elderly subjects, as shown by increased plasma FRAP value and decreased plasma carbonyl content ( $P < 0.05$ ). Orange juice consumption, however, did not affect plasma FRAP value and plasma carbonyl content (Table 3). Although plasma GSH-Px and CAT activities were significantly increased in both groups at the end of the study ( $P < 0.01$ ), there was no significant difference between the 2 groups in plasma SOD, GSH-Px, and CAT activities. This indicates that the differences in antioxidant effects displayed after consumption of the 2 fruit juices in elderly subjects were not due to increased activity of these antioxidant enzymes.

There is no significant difference between 2 groups as analyzed by Student t test. \*  $P < 0.05$  compared with the initial, analyzed by Student paired t test. \*\*  $P < 0.01$  compared with the initial, analyzed by Student paired t test.

We measured fasting plasma levels of ascorbic acid, VE, and GSH and found that there was no significant difference between the 2 groups at the end of the study (Table 4). This is in agreement with the results we obtained in a previous animal experiment [Manach C, et al (1995)]. Therefore, it is reasonable to speculate that antioxidants other than ascorbic acid, VE, and GSH may contribute to the antioxidant activity of pomegranate juice after consumption, as shown in the present study. It seems

more likely that the diverse types of phenolic compounds contained in pomegranate juice may act as important components in improving the antioxidant function in elderly subjects. Gil et al [Ko SH, et. al. (2005).] identified several phenolic compounds in pomegranate juice, such as anthocyanins, punicalagins, ellagic acids, and hydrolysable tannins.

Data are expressed as means  $\pm$  SD ( $n = 13$  subjects in both groups). The difference in MDA content was analyzed by Student paired t test. The difference in carbonyls content was analyzed by Wilcoxon rank sum test. There is no significant difference between 2 groups in ox-LDL content as analyzed by Wilcoxon rank sum test. \*  $P < 0.05$  compared with orange. \*\*  $P < 0.01$  compared with the initial.

Noda et al [Brewster MA, Turley CP (1987)] reported that 3 major anthocyanins found in pomegranate juice were delphinidin, cyanidin, and pelargonidin. We hydrolyzed pomegranate pulp with hydrochloric acid and identified 4 flavonoids, that is, quercetin, kaempferol, luteolin, and myricetin, by high-performance liquid chromatography, in which the content of quercetin was as high as 16.78 mg% (data not shown). These phenolic compounds are well known for their properties in scavenging free radicals and inhibiting lipid oxidation in vitro. The intestinal absorption of quercetin, one of the widely occurring polyphenols in the plant kingdom, has been characterized in animal and human studies [Warwich WJ (1978), Kunio Y (1979), Levine RL, et al (1990) and Singh NP, et al (1988)]. We also found that oral pretreatment with quercetin could protect significantly against hepatic ischemia-reperfusion injury in rats [Langley P (2000)]. Anthocyanins, another important group of polyphenolic compounds, are reported to be partly absorbed in elderly women and adult men [Gil MI, et. al. (2000), Aviram M, et al (2000) and Kaplan M, et al (2001)]. Therefore, we consider it a possibility that the increased plasma antioxidant capacity in elderly subjects shown in the current study may be attributed to the phenolics contained in pomegranate juice.

Blood and urine samples were collected after overnight fasting on the final day of the study. Data are expressed as means  $\pm$  SD ( $n = 13$  subjects in both groups). Wilcoxon rank sum test was used in analyzing the differences in urinary content of 8-OH-dG and blood mononuclear cells with DNA damage and Student t test for blood mononuclear cell DNA tail length. No significant difference was found in either urinary content of 8-OH-dG or blood mononuclear cell DNA damage between 2 groups.

#### Conclusion

Pomegranate juice is a mixture of diverse antioxidants, it is also possible that the antioxidant action of pomegranate juice is generated from a concerted action of a combination of these antioxidants. Proteins are major components of biologic systems and play an important role in a variety of cellular functions. An age-related increase in oxidative damage to proteins has been well documented, which may lead to enzymatic inactivation and enhance the likelihood of proteolysis. The most widely studied oxidative stress-induced modification to proteins is the formation of carbonyl derivatives on lysine, arginine, proline, histidine, cysteine, and threonine residues [Parashar, A., Gupta, S.K., (2008)]. In the present study, we found that pomegranate juice consumption decreased plasma carbonyl content significantly in elderly subjects ( $P < 0.05$ ), whereas orange juice consumption resulted in no significant change, as shown in Table 5. It is known that some polyphenols, such as quercetin,

have a great affinity for proteins such as albumin, which may be an experimental basis for the proteinprotecting action of pomegranate juice [Levine RL, et al (1990), ]. However, there is no significant difference in the changes in plasma MDA and ox-LDL contents between the 2 groups studied (Table 5). This observation may suggest that phenolic compounds are less effective in reducing oxidative damage occurring in lipophilic phases.

The results of the dietary survey showed that the daily intake of most nutrients, including ascorbic acid and VE, is not significantly different between the 2 groups.

However, the mean intake of  $\beta$ -carotene is higher in the orange group than in the pomegranate group (3.2 vs 2.4 mg) [Etminan M, et. al. (2005)]. Because  $\beta$ -carotene is an important lipid-soluble antioxidant, this difference may weaken the antioxidant activity of pomegranate juice after consumption when compared with the orange juice [Etminan M, et. al. (2005)]. Although the urinary 8-OH-dG excretion was about 21% less in the pomegranate group than in the orange group (Table 6).

A longer term study may be necessary to demonstrate more significant changes in these oxidative damage markers after pomegranate juice consumption in elderly subjects. In addition, a larger study group that would include a proper control group is necessary. Pomegranate juice is more effective in improving antioxidant function and reducing oxidative damage than orange juice after consumption in elderly subjects.

Therefore, the consumption of fruit juices high in antioxidant capacity may provide more benefits to the healthy than those low in antioxidant capacity. Because there was limited subject participation in this short-term intervention, a large-scale and long-term study is warranted to confirm the results obtained in this study.

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**Table 1**

#### Baseline characteristics of the study subjects

	Orange	Pomegranate
Age (y)	62.8 ± 4.7	64.1 ± 4.3
Sex (male/female)	11/3	10/3
Height (m)	1.66 ± 0.05	1.66 ± 0.04
Body weight (kg)	66.8 ± 8.7	63.4 ± 7.0
Body mass index (kg/m <sup>2</sup> )	24.2 ± 2.5	23.0 ± 2.5

All data, except sex, are expressed as means ± SD.

**Table 2**

#### Antioxidant capacity and major antioxidant contents of 2 fruit juices

	Orange	Pomegranate
FRAP (mmol/L)	3.06 ± 0.3	5.41 ± 0.4
Ascorbic acid (mg%)	10.98 ± 0.8	7.19 ± 1.0
Total phenolics (mg%)	22.0 ± 2.8	112.3 ± 6.4
Flavonoids (mg%)	9.2 ± 0.8	17.4 ± 1.8
Proanthocyanins (mg%)	3.4 ± 0.1	18.2 ± 1.2

**Table 3**  
**Plasma antioxidant capacity and activity of antioxidant enzymes in elderly subjects at the initial and final days of the study**

	Orange	Pomegranate
FRAP (mmol/L)		
Initial	1.37 ± 0.16	1.33 ± 0.18
Final	1.36 ± 0.14	1.46 ± 0.26 *
SOD (U)		
Initial	91.34 ± 14.77	89.10 ± 12.08
Final	81.26 ± 21.42	85.8 ± 13.32
GSH-Px (U)		
Initial	62.45 ± 4.29	65.26 ± 5.04
Final	70.05 ± 5.03 **	70.39 ± 5.55 **
CAT (U/mL)		
Initial	1.44 ± 0.44	1.34 ± 0.43
Final	2.74 ± 0.27 **	2.87 ± 0.39**

**Table 4**  
**Plasma contents of ascorbic acid, VE, and GSH in elderly subjects at the initial and final days of the study**

	Orange	Pomegranate
Ascorbic acid (mg%)		
Initial	0.90 ± 0.20	0.91 ± 0.16
Final	0.53 ± 0.13 *	0.51 ± 0.12 *
VE (µg/mL)		
Initial	14.06 ± 2.34	15.50 ± 2.67
Final	12.20 ± 2.05	14.67 ± 3.73
GSH (mg/L)		
Initial	189.45 ± 26.91	208.87 ± 35.44
Final	199.96 ± 27.78	208.27 ± 46.45

**Table 5**  
**Plasma contents of MDA, ox-LDL, and carbonyls in elderly subjects at the initial and final days of the study**

	Orange	Pomegranate
MDA (µmol/L)		
Initial	10.58 ± 2.90	9.10 ± 2.30
Final	7.33 ± 1.22 **	6.78 ± 1.98 **
ox-LDL (µmol/L)		
Initial	16.19 ± 10.24	19.17 ± 9.85
Final	19.62 ± 10.58	19.97 ± 13.26
Carbonyls (µmol/mL)		
Initial	84.62 ± 62.72	89.61 ± 83.42
Final	121.50 ± 84.45	76.75 ± 43.81 *

**Table 6**  
**Urinary 8-OH-dG content and blood mononuclear cell DNA damage in elderly subjects on the final day of the study**

	Orange	Pomegranate
8-OH-dG (ng/mmol creatine)	824.41 ± 343.66	651.57 ± 332.44
Mononuclear cell DNA damage		
Cells with DNA damage (%)	21.8 ± 13.8	20.7 ± 14.3
Tail length (µm)	9.8 ± 1.1	9.5 ± 1.2