

Clinical investigation of the acute effects of pomegranate juice on blood pressure and endothelial function in hypertensive individuals

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Original Article

Abstract

BACKGROUND: Pomegranate juice (PJ) is rich in bioactive phytochemicals with antioxidant, and anti-inflammatory and cardioprotective functions. The present trial investigated the acute effects of PJ consumption on blood pressure and markers of endothelial function.

METHODS: In this single-arm study, thirteen hypertensive men aged 39–68 years were recruited. Included subjects were assigned to natural PJ (150 ml/day) following a 12 hour fast. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and flow-mediated dilation (FMD), along with serum concentrations of C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin and interleukin-6 (IL-6) were measured at baseline and 4-6 hours after PJ consumption.

RESULTS: Comparison of pre- vs. post-trial values revealed a significant reduction in both SBP (7%; $P = 0.013$) and DBP (6%; $P < 0.010$). However, changes in FMD (20%) as well as circulating levels of CRP, ICAM-1, VCAM-1, E-selectin, and IL-6 did not reach statistical significance ($P = 0.172$).

CONCLUSION: PJ has promising acute hypotensive properties. Consumption of PJ could be considered in the context of both dietary and pharmacological interventions for hypertension.

Keywords: Punica Granatum L., Cardiovascular Disease, Hypertension, Inflammation, Endothelium-Dependent Dilation

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Introduction

Cardiovascular disorders are among the leading causes of death and disability in the world.^{1,2} Hypertension is a major risk factor for cardiovascular and cerebrovascular disease, end stage renal disease, type 2 diabetes, and metabolic syndrome. Hypertension has a high global prevalence of about 15% that is estimated to reach as high as 30% by 2025.^{3,4} Controlled studies have indicated that each 5 mmHg decrease in diastolic blood pressure (DBP) is associated with 15% and 40% reductions in the risk of cardiovascular disease and stroke, respectively.⁵ In spite the introduction of several classes of anti-hypertensive agents with different mechanisms of action, uncontrolled hypertension resistant to drug therapy still remains a frequent medical problem.

Pomegranate (*Punica granatum* L.; Family Punicaceae) is a popular edible fruit with wide applications in traditional medicine.^{6,7} Several lines of modern scientific evidence have also indicated the therapeutic efficacy of pomegranate against different types of disorders.⁸⁻¹¹ The pomegranate is characterized by considerable amounts of biologically active phytochemicals including flavonoids (e.g. anthocyanins, catechins, quercetin, and rutin), other types of polyphenols, ellagitannins, and antioxidant vitamins.¹²⁻¹⁵ Many of these phytochemicals have been shown to possess antioxidant and anti-inflammatory properties plus additional biological activities such as inhibition of angiotensin converting enzyme.¹⁶⁻²⁰ All these activities of the pomegranate are potentially beneficial for the treatment of hypertension and improvement of

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endothelial function.¹¹ However, clinical studies investigating the hypotensive and cardioprotective effects of pomegranate have been scarce.²⁰⁻²⁶

According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7), prehypertension is defined as 120 mmHg \leq systolic blood pressure (SBP) $<$ 140 mmHg and/or 80 mmHg \leq DBP $<$ 90 mmHg.²⁷ Prehypertension is a clinical stage where subjects are at increased risk (2 folds higher) of developing hypertension in the near future.²⁸ Emerging findings suggest that interventions at the prehypertension stage can prevent or delay the progression of disease into established hypertension and subsequent detrimental outcomes.²⁹ The present trial investigated the acute effects of pomegranate juice (PJ) on blood pressure and endothelial function in subjects with diagnosed prehypertension.

Materials and Methods

Subjects

Thirteen hypertensive men aged 39–68 years were recruited for this trial.³⁰ The Ethics Committee at the Shahrekord University of Medical Sciences (Iran) approved the study protocol (code: 92-3-16) and written informed consents were obtained from all participants.

The inclusion criteria were body mass index (BMI) \leq 30, and diagnosed hypertension defined as SBP $>$ 120 mmHg and/or DBP $>$ 80 mmHg. Exclusion criteria were type 1 or 2 diabetes, chronic pancreatitis, liver cirrhosis, kidney stones, renal failure, use of non-steroidal anti-inflammatory drugs, use of antioxidant or vitamin supplements, intense physical activity ($>$ 5 h/week), smoking habit, being vegetarian or having any restrictive dietary requirements, and pregnancy.

Study design

The present study was designed as a single-arm clinical trial. The included subjects were assigned to natural PJ (150 ml/day) following a 12-h fast. SBP, DBP, and flow-mediated dilation (FMD), along with serum concentrations of C-reactive protein (CRP), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and interleukin-6 (IL-6) were measured at baseline and 4-6 h after PJ consumption. Participants were asked not to eat or drink anything during the interval between PJ consumption and final measurements.

Total anthocyanin assay

A pH differential method was carried out to

determine the total anthocyanin content of PJ. In this method, the difference in the absorbance of sample at pHs 1.0 and 4.5 is proportional to the total anthocyanin content. Details of this method have been published previously.^{24,31}

Anthropometric and BP measurements

Measurement of weight and height was carried out using a standard procedure as described previously.^{24,32} Body mass index (BMI) was calculated as weight in kg divided by height in meters squared (m^2). BP was measured by a single operator at baseline and 4-6 h after intake of PJ, according to a standard protocol. BP recordings were performed after rest, employing a stethoscope and calibrated sphygmomanometer (Accurtoorr 1A; Datascope, Japan). Systolic blood pressure was defined as the appearance of the first sound (Korotkoff phase 1) and diastolic blood pressure was defined as the disappearance of the sound (Korotkoff phase 5) during deflating of the cuff.^{24,32}

FMD measurement

Endothelium-dependent FMD was measured on the right brachial artery as described previously.³³ All measurements were carried out by a single operator following a 5-min rest and employing a GE vivid 3 ultrasound apparatus (AtCor Medical, Solingen, Germany). A BP cuff was inflated around the forearm to 200 mmHg for 5 min. Images were recorded at baseline (before inflation), 30 s before cuff release, and then every 15 sec after cuff release for 3 min. Arterial diameter was measured at the end of end-diastolic phase, coinciding with R-wave on the electrocardiogram (ECG). Brachial FMD was expressed as the percentage change in arterial diameter from baseline.³⁴

Blood sampling and biochemical analyses

Twelve-hour fasted blood samples were taken from the left antecubital vein. After being allowed to clot for 2-3 h, serum was isolated by centrifugation at 3500-4000 rpm for 10 min. Serum samples were kept at $-80^{\circ}C$ prior to biochemical analyses. Biochemical analyses were performed using an automated enzymatic assay (Pars Azmoon, Tehran, Iran) on a Hitachi 902 autoanalyzer (for CRP), or enzyme-linked immunosorbent assay (ELISA) with commercial kits (Boster Biological Technology Ltd., Wuhan, China) (for ICAM-1, VCAM-1, E-selectin, and IL-6). Inter-assay coefficients of variation for ICAM-1, VCAM-1, E-selectin and IL-6 were 4.1-6.4%, 6.1-7.7%, 6.6-8.1%, and 3.1-5.5%, respectively. Intra-assay coefficients of variation for ICAM-1, VCAM-1, E-selectin, and IL-6 were 3.4-5.1%, 2.3-3.7%, 5.2-6.9%, and 2.3-4.9%, respectively.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 17; SPSS Inc., Chicago, IL, USA). Data were expressed as mean \pm SD. Group comparisons were made using paired t-test (in case of normally distributed data) or Wilcoxon signed-ranks test (in case of non-normally distributed data). A two-sided P-value of < 0.05 was considered to be statistically significant.

Results

This trial was comprised of 13 hypertensive male adolescents with a mean age, weight, and BMI of 55.92 ± 7.92 yrs, 80.42 ± 11.01 kg, and 27.34 ± 3.82 kg/m², respectively. All 13 subjects completed the study. Total anthocyanin content of PJ was determined to be 5.8 mg per 100 ml of the administered juice.

Comparison of pre- vs. post-trial values revealed a significant reduction in both SBP ($P = 0.013$) and DBP ($P = 0.010$), amounting to an approximate reduction by 7% and 6%, respectively. However, percentage changes in FMD (20%) was not found to be statistically significant ($P = 0.172$). In the same manner, there was no significant difference in the circulating concentrations of inflammatory biomarkers namely hsCRP ($P = 0.263$), ICAM-1 ($P = 0.248$), VCAM-1 ($P = 0.657$), E-selectin ($P = 0.182$), and IL-6 ($P = 0.763$) following consumption of PJ. Baseline and post-trial values for the evaluated parameters are summarized in table 1.

Discussion

The present pilot trial is one of the few clinical evidences on the acute hypotensive and vascular effects of PJ. The results indicated amelioration of both SBP and DBP following consumption of a single dose of PJ. In a previous study, Aviram and Dornfeld investigated the effects of 2-week

supplementation with PJ (50 ml/day) on the SBP of hypertensive patients.²⁰ The findings revealed a significant decrease in SBP amounting to 5%. In the same study, a 36% decrement in the activity of serum angiotensin converting enzyme (ACE) was reported from PJ.²⁰

The same group also investigated the effect of chronic supplementation with PJ (50 ml/day) in patients with carotid artery stenosis. Their results indicated a significant reduction in SBP, but not DBP, starting from 1 month after starting supplementation and generally increasing up to month 12 (equivalent to 12% decrement). However, no further reduction was observed when supplementation was continued for another 2 years.²¹ Another study conducted by Lynn et al. indicated a significant reduction in both SBP and DBP following consumption of PJ (330 ml/day) by healthy subjects for 4 weeks.²² Mathew et al. reported that consumption of pomegranate extract, either during or 15 min before a high-fat meal, can effectively prevent postprandial SBP rise at 2 and 4 h postprandial. Nevertheless, no significant effect was found on DBP.²³ In a recent trial conducted by our group, the effects of a 2-week intake of PJ (from the same source as that used in the present study) were evaluated on BP of hypertensive subjects. Our results implied a significant reduction of both SBP (5%) and DBP (4.5%) compared to the control group who consumed water instead of PJ.²⁴ Although most of the findings by previous trials infer the hypotensive impact of PJ consumption, there are some contrasting findings that are worth attention. Kelishadi et al. investigated the acute (4 h) and chronic (1 month) effects of PJ (240 ml/day) on BP of adolescents with metabolic syndrome and could not find any significant effect.²⁵ In another trial, administration of PJ (240 ml/day) for 3 months was not found to significantly affect SBP or DBP in patients with stable coronary heart disease (CHD), as reported by Sumner et al.²⁶ These negative

Table 1. Acute effects of pomegranate juice on blood pressure, flow-mediated dilation, and biochemical parameters

Parameters	Before	After	P
SBP (mmHg)	125.38 \pm 11.80	116.15 \pm 7.94	0.013
DBP (mmHg)	82.69 \pm 5.25	78.08 \pm 3.25	0.010
FMD (%)	0.30 \pm 0.17	0.36 \pm 0.17	0.172
Hs-CRP (mg/L)	1.39 \pm 1.14	1.20 \pm 0.94	0.263
ICAM-1 (ng/mL)	297.00 \pm 122.30	265.23 \pm 125.35	0.248
VCAM-1 (ng/mL)	1008.31 \pm 450.99	993.92 \pm 447.42	0.657
E-Selectin (ng/mL)	27.77 \pm 15.89	25.49 \pm 14.19	0.182
IL-6 (ng/mL)	0.45 \pm 0.16	0.46 \pm 0.15	0.763

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FMD: Flow-mediated dilation; hsCRP: High-sensitivity C-reactive protein; ICAM-1: Intracellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; IL-6: Interleukin-6; Group comparisons were made using paired t-test (in case of normally distributed data) or Wilcoxon signed-ranks test (in case of non-normally distributed data).

findings might be attributed to the difference in the inclusion criteria applied by these two latter trials. It appears that hypotensive effects of PJ are more likely to be elicited in hypertensive patients, rather than patients with established CHD or metabolic syndrome. Moreover, the overall findings of the trials conducted so far weigh in favor of the beneficial effect of PJ consumption on BP.

The hypotensive properties of PJ could be ascribed to the promising antioxidant properties of phytochemicals present in this complex juice. Oxidative stress is known to play a key role in the pathogenesis of hypertension.³⁵ Increased levels of oxidants have been shown in different experimental models of hypertension.³⁶⁻³⁹ Detrimental effects of oxidative stress are mainly due to the interaction of reactive oxygen species (ROS) (in particular superoxide anion) with vital cellular components, lipids, and proteins, which leads to endothelial dysfunction and vascular resistance. In addition, ROS interfere with the production and vasodilatory actions of endothelium-derived nitric oxide (NO) via attenuating NO synthase (NOS) activity and enhancing NO breakdown.^{40,41} Epidemiological evidence has indicated that diets rich in natural antioxidants are associated with a reduced risk of developing hypertension and cardiovascular events.^{42,43} A plethora of studies have confirmed the considerable antioxidant and radical scavenging effects of PJ, which are mainly due to the anthocyanins and hydrolysable tannins present in the fruit. Interestingly, it has been suggested that some 50% of the total antioxidant activity of PJ is exerted by a specific ellagitannin, named punicalagin.¹⁶ Intestinal hydrolysis of punicalagin yields ellagic acid; the latter being a strong antioxidant compound.⁴⁴

Apart from antioxidant properties, PJ may lower BP through a direct interaction with ACE. As referred above, a significant reduction in the activity of serum ACE has been observed in hypertensive patients following PJ consumption.²⁰ Besides, in an animal study by Mohan et al. PJ administration attenuated angiotensin II-induced hypertension in diabetic rats, and also blocked the effects of different catecholamines on arterial BP and vasoreactivity. Furthermore, chronic administration of PJ counterbalanced the increased ACE activity in diabetic hypertensive rats.⁴⁵ In spite of these positive reports, the ACE inhibitory effect of PJ needs to be further explored as Lynn et al. failed to report any change in serum ACE concentration following PJ consumption for 4 weeks.²²

Another primary outcome measure that was evaluated by the present trial was changes in endothelium-dependent flow-mediated dilation. Our results did not indicate any improvement in FMD and this is in agreement with our recent report on the effects of 2-week PJ intake.²⁴ However, findings from another trial indicated significant improvement in both endothelium-dependent and -independent (nitroglycerin-induced) dilation after 4 h of PJ consumption. In addition, this increased vasodilation was persisted until the end of supplementation period (1 month).²⁵ In the present study, biomarkers of endothelial function, namely ICAM-1, VCAM-1, and E-selectin, remained statistically unaltered compared to baseline levels. It should be noticed that alterations in the circulating levels of these biomarkers is more likely to be exerted by chronic, rather than single dose, consumption of PJ and needs a longer term evaluation to allow the turnover of previously released proteins and observation of possible changes in the expression and subsequent release of these markers due to PJ consumption. This notion is corroborated by our previous study which showed a decreasing trend in serum levels of the aforementioned biomarkers following consumption of PJ.²⁴

The present study has certain limitations that need to be acknowledged. This study did not include a control group. Therefore, our findings might have been confounded by bias. In addition, the present trial was conducted in pilot scale and with a small population size. This small size could potentially account for lack of detecting significant difference despite the increasing trend in FMD and decreasing trends in serum CRP, ICAM-1, VCAM-1, and E-selectin levels. With respect to these limitations, findings of the present trial may not be generalizable to the general population and should be interpreted with caution.

In conclusion, the key finding to emerge from the present study is the acute hypotensive effect of PJ in hypertensive patients. While this trial is not a substitution for well-designed randomized controlled trials, it generates a hypothesis and motivates further research on this topic. Future large-scale investigations are indeed warranted in order to obtain a mechanistic understanding on this observed hypotensive activity.

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Conflict of Interests

Authors have no conflict of interests.

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