

# Effect of Pomegranate Juice on Paraoxonase Enzyme Activity in Patients with Type 2 Diabetes

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## ABSTRACT

**OBJECTIVE:** The aim of this study was to investigate the effect of pomegranate juice (PJ) on the paraoxonase and arylesterase activity of human serum paraoxonase( PON1).

**MATERIAL AND METHOD:** Fifty patients with type 2 diabetes mellitus consumed 200 ml of PJ daily for a period of 6 weeks. Blood was collected from the patients before and after PJ consumption after 12 h of fasting. Blood sugar, total cholesterol, triglyceride, and HDL-C were measured by enzymatic kit method and autoanalyzer. The concentration of LDL-C was calculated by using Friedwald formula. The malondialdehyde concentration ( $\mu\text{mol/L}$ ) was determined by thiobarbituric acid (TBA) assay. Paraoxonase and arylesterase activity of PON1 enzyme were measured using paraoxone and phenylacetate as the substrates.

**RESULTS:** The concentration of fasting blood sugar, total cholesterol, LDL-C and malondialdehyde significantly ( $p<0.001$ ) decreased after the intervention. Paraoxonase and arylesterase activity of PON1 significantly ( $p<0.001$ ) increased after the intervention. However, there were no significant changes in serum triglyceride and HDL-C. There was a significant positive correlation between paraoxonase and arylesterase activity of PON1 and serum HDL-C concentration. A significant negative correlation was detected between paraoxonase and arylesterase activity of PON1 and FBS.

**CONCLUSION:** It can be concluded that PJ consumption as an antioxidant may have a contribution in changing fasting blood sugar, lipid profiles, lipoprotein oxidation, and PON1 activity.

**KEYWORDS:** Pomegranate Juice, PON1 Activity, Diabetes Mellitus.

## INTRODUCTION

Paraoxonase(EC.3.1.8.1,aryldialkylphosphatas e) has been extensively studied in the field of toxicology (1). Paraoxonase hydrolyzes organophosphate compounds are widely used as insecticides and nerve gases (2, 3). Human serum paraoxonase (PON1) is synthesized in the liver and is physically associated with HDL, on which it is almost exclusively

located. Several studies have indicated that PON1 can prevent lipid peroxide accumulation on LDL both in vitro and in vivo (4,5). Some studies have shown that serum PON1 activity is reduced in diabetes (6-13). Recently, studies in PON1 demonstrated that PON1 has a protective role against diabetes development, secondary to its unique antioxidant properties (14). Serum paraoxonase activity is inversely

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correlated with blood glucose levels in diabetic patients (15). The high concentrations of glucose in diabetic serum could account for PON1 dissociation from HDL (15). Paraoxonase activity is under both genetic and environmental influences and varies widely among individuals (12). Among the main risk factors responsible for coronary heart disease (CHD), diet has an important role in patients with diabetes as well, because it regulates the levels of plasma lipids and lipoproteins, blood pressure, energy balance, thrombogenesis, and the oxidative modification or protection of plasma lipids and lipoproteins (16). Pomegranate (*Punica granatum*) fruit has been consumed extensively in the form of fresh fruit, concentrate juice and pomegranate sour in salads in Turkey and the Mediterranean region) (16). The pomegranates are found to be a rich source of polyphenolic compounds that include flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallagic acid, and ellagic acid esters of glucose) which account for 92% of their antioxidant activities (17). In recent years, many research results have been published about the beneficial effects of pomegranate fruit. For example, it has been reported that consumption of pomegranate juice may be helpful against coronary heart disease (18) and Alzheimer's disease (19). Also, pomegranate extract significantly improves arteriogenic erectile dysfunction (20) and sperm quality in male patients (21). In addition, the more remarkable scientific articles are available on cancer chemoprevention by pomegranate extracts and active compounds in vitro, as well as in experimental animal models (22). Recent findings have demonstrated that consumption of PJ by patients with diabetes decreases oxidative stress in their serum and contributes to PON1 stabilization, increases PON1 association with HDL, and stimulates the enzyme catalytic activities (23, 24). The aim of the present study was to determine the effect of PJ consumption on paraoxonase

enzyme activity in patients with type 2 diabetes.

## **MATERIALS AND METHODS**

### **Study Design and Participants:**

In this quasi-experimental interventional study, fifty patients with type 2 diabetes mellitus, consumed 200 ml of PJ daily for a period of 6 weeks. The patients served as their own controls because we compared all data after the pomegranate consumption with the baseline values. In the present study, the patients had no hypertension and cardiovascular diseases. None of the participants received any antioxidant supplementations in the past 3 months. They followed their own normal diet and did not change their diet and life style during study. PJ was prepared from the whole fruit after being cut and exposed to arils for the squeezing process. The juice was then filtered, pasteurized, concentrated and stored at  $-18^{\circ}\text{C}$ . The concentrated PJ was diluted 1:4 (v:v) to 16 Brix (Brix is a measurement of soluble solids in fruit juice and represents the sugars and many other soluble substances such as salts, acids, and tannins. Brix is measured in grams per hundred milliliters) with water to obtain a single-strength PJ to be used in the study. Measurements: Blood was collected from the patients before and after PJ consumption after 12 h of fasting. Serum was then separated, and fasting blood sugar (FBS), total cholesterol, triglyceride and HDL-C were measured by enzymatic kit method (Pars Azmon kit) with autoanalyzer (Autolab-AMS). The concentration of LDL-C was calculated by using Friedwald formula. The MDA concentration ( $\mu\text{mol/lit}$ ) was determined by the thiobarbituric acid (TBA) assay. To 0.5 ml plasma, 1 ml of trichloroacetic acid was added and the tube was left to stand for 10 min at room temperature. After centrifugation at 3500 RPM for 10 min, the supernatant was separated. Thereafter 0.5 ml supernatant were added to 0.5 ml thiobarbituric acid (TBA) and carried out in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen absorbance was determined at the

wavelength of 532 nm. Paraoxonase activity (U/l) was measured by Eckerson method (25). To 20 µL of serum 700 µL buffer [2 mmol/L paraoxone, 2 mmol/L Cacl2 in 1 mmol/L tris-Hcl buffer(PH=8)] was added and then Para-nitrophenyl was measured as a product at 412 nm wavelength. Also arylesterase activity of PON1 was measured by using 10 µL serum and mixture of 2 mmol/L phenylacetate and 2 mmol/L Cacl2 in 100 mmol/L tris-Hcl buffer (PH=8). Then hydrolysis rate of phenylacetate at wavelength 270 nm was measured by spectrophotometer (25).

**Statistical Analysis:**

The data were analyzed by the SPSS package Version 11 (SPSS Inc., Chicago, IL, USA). Kolmogro-Smirnov test was applied for determining the distribution of quantitative data. To compare the means of serum data

before and after the study, the paired t-test was utilized. The correlation coefficient also was used. The values (P-value < 0.05) were considered significant.

**RESULTS**

The ratio of male to female patients was nearly the same. The patients' mean age was 45 ± 8y and the mean for their BMI was 30± 3 kg/m<sup>2</sup>. Table 1 shows the comparison of serum lipids, lipoproteins and malondialdehyde before and after PJ consumption. As it is shown in Table 1 mean of FBS, total cholesterol, LDL-C and malondialdehyde decreased significantly (p<0.001) after the intervention. However, there were no significant changes in serum triglyceride and HDL-C concentration. Table 2 shows paraoxonase and arylesterase activity of PON1 and their ratio before and after PJ consumption. Paraoxonase and aryl esterase

**Table 1- Mean Concentration and Standard Deviation of Fasting Blood Sugar (FBS), Lipids , Lipoprotein and Malondialdehyde in Patients with Type 2 Diabetes before and after Pomengranate Juice Consumption**

Biochemical Parameters	Before Mean ± SD	After Mean ± SD	P-Value
FBS(mg/dl)	195.38 ± 31.91	160.64 ± 37.47	<0.001
TC(mg/dl)	179.02 ± 29.16	160.04 ± 11.98	<0.001
TG(mg/dl)	169 .64 ± 10.70	162.08 ± 9.02	0.05
HDL-C(mg/dl)	36.58 ± 4.60	38.36 ± 5.56	0.98
LDL-C(mg/dl)	101.29 ± 15.78	85.12 ± 13.94	<0.001
MDA(µmol/L)	0.073 ± 0.046	0.029±0.021	<0.001

FBS= fasting blood sugar, TC= total cholesterol TG= triglyceride, HDL-C= high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, MDA= malondialdehyde

**Table 2- Mean Concentration and Standard Deviation of the Paraoxonase Activity, Arylesterase Activity of PON1 and their Ratio in Patients with Type 2 Diabetes before And after Pomengranate Juice Consumption**

ytivitca emyznE	cBefore	After	P-Value
	Mean ± SD	Mean ± SD	
Pytitvca esanoxoara (U/l)	135.02 ±104.14	225.18±149.52	<0.001
ytivitca esaretselyrA (U/l)	165.02±56.63	246.36±49.26	<0.001
Arylesterase/Paraoxonase	1.22±0.54	1.94±0.32	<0.001

U/l= one unit of paraoxonase activity is defined as 1 µmol of paranitrophenol formed per minute

**Table 3- Correlation between Paraoxonase Activity and Arylesterase Activity of PON1 and Other Biochemical parameters**

Biochemical parameters	ytivitca esanoxoaraP		tyivitca esaretselyrA	
	r	p-value	r	p-value
FBS(mg/dl)	-0.690	0.000	-0.718	0.000
TC(mg/dl)	-0.234	0.270	-0.090	0.67
TG(mg/dl)	-0.071	0.74	-0.054	0.80
HDL-C(mg/dl)	0.451	0.027	0.622	0.001
LDL-C(mg/dl)	-0.245	0.249	-0.040	0.854
MDA(µmol/L)	-0.432	0.021	-0.502	0.035

activity of PON1 and their ratio increased significantly after the intervention ( $p < 0.001$ ). Also the correlation between paraoxonase activity and arylesterase activity of PON1 and other biochemical parameters revealed a positive correlation between paraoxonase and arylesterase activity of PON1 and serum HDL-C concentration which was statistically significant. A significant negative correlation was detected between paraoxonase and arylesterase activity of PON1 and FBS (Table 3).

## DISCUSSION

Pomegranate is an important source of bioactive compounds and has been used in traditional medicine for centuries. PJ is known to be high in antioxidant activity (24, 26). The present study was designed to evaluate the effect of PJ on FBS, lipid profiles, lipid oxidation PON1 paraoxonase activity, arylesterase activity of PON1 and their correlation in patients with type 2 diabetes. Our results showed that daily consumption of 200 ml PJ decreased the mean of FBS, total cholesterol, LDL-C and malondialdehyde significantly ( $p < 0.001$ ). However, there were no significant changes in serum triglyceride and HDL-C concentration. The results of this study are in line with that of M.I. Gil et al. They reported that consumption of PJ in patients with type 2 diabetes decreases cholesterol and LDL-C concentration (15). Esmailzadeh et al. have shown significant reduction of total cholesterol ( $P < 0.006$ ), LDL-C ( $P < 0.006$ ), LDL-C/HDL-C ( $P < 0.001$ ), and total cholesterol/HDL-C ( $P < 0.001$ ) after 8 weeks of PJ consumption in patients with diabetes. However, there were no significant changes in serum triacylglycerol and HDL-C concentrations (23).

In this study, mean of paraoxonase and arylesterase activity of PON1 and their ratio were increased significantly after the intervention ( $p < 0.001$ ). This indicated that polyphenols compounds in PJ have antioxidant effect. Rosenblat et al. reported that PJ consumption results in a significant reduction of thiobarbituric acid reactive substances

(TABARS) and an increase in PON1 activity; thus, these findings are in line with those of our study (26,27). Yukio Ikeda et al. and other investigators have shown that PON1 levels decrease in patients with diabetes (6-13).

PJ increased PON1 activity because its components (tannins and anthocyanin) have direct effect on enzyme activity (16). In this study, the correlation between paraoxonase activity and arylesterase activity of PON1 and other biochemical parameters showed that there is significant positive correlation between paraoxonase and arylesterase activity of PON1 and serum HDL-C concentration ( $r = 0.451$   $p = 0.027$ ,  $r = 0.622$   $p = 0.001$ ). Also there is a significant negative correlation between paraoxonase and arylesterase activity of PON1 and FBS ( $r = 0.69$   $p < 0.001$ ,  $r = 0.718$   $p < 0.001$ ). Measurement of PON1 enzyme activity, evaluation of changes in its performance in patients with type 2 diabetes and managing PJ supplement were the new and positive points of this research. Measurement of just one antioxidant enzyme was the negative aspect of the study. Therefore more extensive studies have to be carried out on PON1 enzyme for the management of diabetes.

## CONCLUSION

These results demonstrated that PJ consumption for 6 weeks may exert beneficial effects on fasting blood sugar, lipid profiles, lipoprotein oxidation and PON1 activity. Therefore, the juice can have more potential as a health supplement which is rich in normal antioxidant.

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