Pomegranate juice consumption increases GSH levels and reduces lipid and protein oxidation in human blood

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ABSTRACT

Although several studies have been devoted for the exploitation of the antioxidant properties of pomegranates, only few concern the assessment of the antioxidant effects of pomegranate juice consumption in humans. In this study, 14 healthy volunteers consumed half a liter of pomegranate juice daily for a period of 15 days and the changes of oxidative stress markers in their blood were assessed at four different time points. In particular, their blood samples were taken immediately before the experiment and after 15 days of juice administration, as well as one and three weeks after the interruption of juice administration. The markers studied were the total antioxidant capacity (TAC), the levels of malondialdehyde (MDA), a lipid peroxidation marker and protein carbonyls (CARB) in plasma. Moreover, reduced glutathione (GSH), one of the most important antioxidant molecules and catalase activity (CAT) were also measured in erythrocytes. The results herein showed that the GSH level was significantly increased (22.6%) just after the end of juice administration, the MDA level was reduced by 24.4% a week after stopping the juice consumption and CARB were reduced by 19.6% and 17.7% immediately and one week after stopping juice administration respectively. TAC and catalase activity were not affected. The results of this study further support the evidence that pomegranate juice consumption enhances the antioxidant status in humans by decreasing lipid peroxidation and protein oxidation. Moreover, it was shown for the first time that the pomegranate juice consumption improves the antioxidant mechanisms in erythrocytes by increasing GSH levels. Finally, it was shown for the first time that even a week after stopping the pomegranate juice consumption some of its beneficial effects on antioxidant status still remained in the organism. The aforementioned data support the potential use of pomegranate juice for the prevention of diseases associated with the oxidative stress.
Introduction

Pomegranate (Punica granatum L.) constitutes one of the first recorded cultivated trees by humans. Tough is a native plant of Himalayas (northern India), currently is cultivated in many regions including the Mediterranean basin countries, Iran, Afghanistan, India, China, Japan, Russia and some parts of the United States. The pomegranate fruit has been used in folk medicine from ancient times as antimicrobial (Gurib-Fakim, 2006) and as natural astringent for the treatment of diarrhea and harmful internal parasites (Das et al., 1999). Nowadays, the research interest on pomegranate fruit is increased as a consequence of reports establishing its benefits on human health (Faria and Calhau 2011). In this respect, pomegranates have been studied as protective means of the cardiovascular system, the treatment of the acquired immune deficiency syndrome, in hormone replacement therapy, in oral hygiene (Faria and Calhau 2011), in chemoprevention (Afaq et al., 2005; Lansky et al., 2005), as microbicidal (Neurath et al., 2004) and as antihyperlipidemic (Fuhrman et al., 2005). The significant biological properties of pomegranate fruit are attributed to its chemical composition and especially to the presence of polyphenols, such as hydrolyzable tannins, anthocyanins and ellagitannins (e.g. punicalagin), condensed tannins (proanthocyanidins), flavonoids and phenolic acids (e.g. punic acid) (Seeram et al., 2006). Other phytochemicals found in pomegranates are organic acids, sterols, triterpenoids, fatty acids, triglycerides, and alkaloids (Seeram et al., 2006). The fruit is divided into arils (composed by juice and seeds) and peels which include the interior network of membranes (Lansky and Newman, 2007).

Pomegranate juice (PJ) is the greatest contributor for pomegranate ingestion which contains 85% water, 10% total sugars, 1.5% pectin, ascorbic acid, and polyphenols (Aviram et al., 2000). Several studies have reported a series of results for
its clinical benefits, such as reduction of systolic blood pressure in hypertensive patients, decrease of common carotid artery intima-media thickness (IMT) (Aviram et al., 2004), attenuation of myocardial ischemia and the lipid profile improvement of diabetic patients (Rosenblat et. al., 2006). In addition, its chemopreventive, chemotherapeutic, antiatherosclerotic and antiinflammatory (Aviram and Dornfeld, 2001; Kaplan 2001; Rozenberg et al., 2006; Adams et al., 2006; Malik et al., 2005) were also been investigated. All the aforementioned beneficial effects of PJ on human health have been mainly attributed to its strong antioxidant properties (de Nigris et al., 2005; Balasundram et al., 2006; Rosenblat et al., 2006), since PJ is rich in free radical scavenging compounds such as anthocyanins (3-glucosides and 3.5-glucosides of delphinidin, cyanidin, and pelargonidin), ellagitannins (e.g. punicalagin, the most abundant polyphenol reaching levels of over 2 g/L juice), flavonoids (e.g. quercetin, kaempferol and luteolin glycosides) and polyphenolic acids (e.g. ellagic and gallic acid) (Gil et al., 2000; Seeram et al., 2005; Lansky, 2006). Moreover, the in vitro antioxidant activity of PJ is determined as three folds higher in comparison to those of red wines and green teas and two to six-fold more potent from other natural beverages (Castilla et al., 2008).

It must be noted however, that most of these studies concern the evaluation of the in vitro antioxidant properties of PJ and there are only scarce reports on their effects in humans. Thus, we were intrigued to investigate herein the in vivo antioxidant potency of PJ through the assessment of their total antioxidant capacity (TAC), MDA levels (a biomarker of lipid peroxidation) and the levels of protein carbonyls (CARB), which constitutes a biomarker of protein oxidation in human plasma. Finally, the levels of glutathione (GSH) and catalase activity (CAT) were also evaluated in human erythrocytes.
Materials and methods

Human subjects

Fourteen volunteers (age, 33.5 ± 3.2 yr; height, 172 ± 2.2 cm; weight, 73.3 ± 4.5 kg; body fat, 22.5 ± 2.7%; body mass index, 25.4 ± 1.3 kg/m$^2$) participated in the present study. All were nonsmokers and not receiving anti-inflammatory medication or nutritional supplements. A written informed consent to participate in the study was provided to all participants after they were informed of all risks, discomforts and benefits involved in the study. The procedures were in accordance with the Helsinki declaration of 1975 and an approval was received by the human subjects committee of the University of Thessaly.

The subjects visited the laboratory for the first time for a screening of their anthropometric parameters and completed a health and activity questionnaire. Each participant reported to the laboratory in the morning after an overnight fast and abstained from alcohol and caffeine for 24 h. Body mass was measured to the nearest 0.5 kg (Beam Balance 710, Seca, UK) with the subjects lightly dressed and barefoot. Standing height was measured to the nearest 0.5 cm (Stadiometer 208, Seca), while the percentage of their body fat was calculated from seven skinfold measures (an average of two measurements of each site) using a Harpenden caliper (John Bull, UK), in accordance to published guidelines (American College of Sports Medicine, 2000). Their body mass index was calculated as the ratio of body weight (kg)/height ($m^2$).

Diet and activity before the experiment

The subjects were instructed to follow their usual eating habits during the days prior to the experiment. They were also asked to record their diet for 3 days before the blood collections on a dietary record sheet. The subjects received a copy of their
dietary record sheets and were asked to exactly follow the same food intake patterns (as recorded in their dietary record sheets) before all blood collections.

Study design

The subjects visited the laboratory (08:00–09:00 in the morning) the first day (T1) for anthropometry measurements. Each subject received 30 packs of 250 ml of PJ. The PJ was kindly provided by VITOM Bro Christodoulou Co Ltd, Greece. The antioxidant polyphenolic content of juices was determined using the HPLC method and their quantization was performed by comparing with calibration curves constructed using various concentrations of authentic samples. The results are included in Table 1 and the concentration of each polyphenol is expressed as mg/g of lyophilized juice. In addition, the total polyphenolic content of juice was determined using the Folin-Ciocalteau as mg/L of gallic acid equivalents, while the total flavonoids content was determined using the Gunes method as mg/L of quercetin equivalents. Blood samples were obtained immediately prior the experiment (T1). Then, for the next 15 days the subjects were consumed 0.5 L (2 packs a day) of pomegranate juice. After 15 days the subjects visited the laboratory again at the same time (08:00–09:00 h in the morning) and blood samples were collected (T2). The same procedure was implemented performed after 7 (T3) and 21 (T4) days following the end of the PJ consumption.

Blood collection and handling

Blood samples (10 mL) were drawn from a forearm vein with subjects in a seated position. Blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes, centrifuged immediately at 1370 g for 10 min at 4 °C and the plasma was collected and used for the measurement of TAC and the determination of MDA and protein carbonyl concentrations. The packed erythrocytes were lysed with distilled
water (1:1 v/v), inverted vigorously, centrifuged at 4020 g for 15 min at 4 °C and the erythrocyte lysate was collected for measurement of catalase activity. A portion of erythrocyte lysate (500 μL) was treated with 5% trichloroacetic acid (TCA) (1:1 v/v), vortexed vigorously, and centrifuged at 28,000g for 5 min at 4 °C. The supernatants were removed, treated again with 5% TCA (1.3:1 v/v) and centrifuged again at 28,000g for 5 min at 4 °C. The clear supernatants were transferred to eppendorf tubes and were used for the determination of GSH concentration. A blood aliquot (1 mL) was immediately mixed with EDTA to prevent clotting for hematology. Plasma and erythrocyte lysate were then stored at −80 °C until biochemical analyses.

Assessment of MDA, protein carbonyl levels, GSH levels, CAT activity, and TAC

For MDA determination, a slightly modified assay of Keles et al. (2001) was used. According to this method, 40 μL of plasma was mixed with 934 μL of 5% TCA and 576 μL of BHT 0.8%. The samples were centrifuged at 3,000 g for 3 min at 4 °C. Then the hexane was removed, which is the upper layer of supernatant and 900 μL TCA 5% was added. Then 625 μL was mixed with 375 μL of TBA 0.8%. The samples were placed in a water bath at 70 °C for 30 min and then transferred on ice for 10 min and centrifuged at 3000g for 3 min at 4 °C. The absorbance of the supernatant was read at 521 nm. A baseline absorbance was taken into account by running a blank along with all samples during the measurement. Calculation of MDA concentration was based on the molar extinction coefficient of malondialdehyde. The intra- and inter-assay coefficients of variation (CV) for TBARS were 3.9% and 5.9%, respectively.

Protein carbonyls were determined based on the method of Patsoukis et al. (2004). In this assay, 50 μL of 20% TCA was added to 50 μL of plasma and this
mixture was incubated in an ice bath for 15 min and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded and 500 μL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) [in 2.5 N hydrochloride (HCl)] for the sample, or 500 μL of 2.5 N HCl for the blank, was added to the pellet. The samples were incubated in the dark at room temperature for 1 h, with intermittent vortexing every 15 min and were centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded and 1 mL of 10% TCA was added, vortexed and centrifuged at 15,000 g for 5 min at 4 °C. The supernatant was discarded and 1 mL of ethanol–ethyl acetate (1:1 v/v) was added, vortexed and centrifuged at 15,000g for 5 min at 4 °C. This washing step was repeated twice. The supernatant was discarded and 1 mL of 5 M urea (pH 2.3) was added, vortexed and incubated at 37 °C for 15 min. The samples were centrifuged at 15,000g for 3 min at 4 °C and the absorbance was read at 375 nm. Calculation of protein carbonyl concentration was based on the molar extinction coefficient of DNPH. The intra- and inter-assay CV for protein carbonyls were 4.3% and 7.0%, respectively. Total plasma protein was assayed using a Bradford reagent from Sigma–Aldrich.

GSH was measured according to Reddy et al. (2004). Twenty microliters of erythrocyte lysate treated with 5% TCA were mixed with 660 μL of 67 mM sodium potassium phosphate (pH 8) and 330 μL of 1 mM 5,5′-dithiobis-2 nitrobenzoate (DTNB). The samples were incubated in the dark at room temperature for 45 min and the absorbance was read at 412 nm. GSH concentration was calculated on the basis of a calibration curve made using commercial standards. The intra- and inter-assay CV for GSH were 3.1% and 4.5%, respectively.

Catalase activity was determined using the method of Aebi (1984). Briefly, 4 μL of erythrocyte lysate (diluted 1:10) was added to 2991 μL of 67 mM sodium
potassium phosphate (pH 7.4) and the samples were incubated at 37 °C for 10 min. Five microliters of 30% hydrogen peroxide (H₂O₂) were added to the samples and the change in absorbance was immediately read at 240 nm for 130 s. Calculation of catalase activity was based on the molar extinction coefficient of H₂O₂. The intra- and inter-assay CV for catalase were 6.2% and 10.0%, respectively.

The determination of TAC was based on the method of Janaszewska and Bartosz (2002). Briefly, 20 μL of plasma was added to 480 μL of 10 mM sodium potassium phosphate (pH 7.4) and 500 μL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radical and the samples were incubated in the dark for 30 min at room temperature. The samples were centrifuged for 3 min at 20,000 g and the absorbance was read at 520 nm. The intra- and inter-assay CV for TAC were 2.9% and 5.4%, respectively. TAC is presented as mmol of DPPH• reduced to 2,2-diphenyl-1-picrylhydrazine (DPPH:H) by the antioxidants of plasma.

Statistical analysis

Oxidative stress, hematological and anthropometric data were analyzed by one-way analysis of variance (ANOVA) with repeated measures on time. Pairwise comparisons were performed through simple main-effect analysis. The level of statistical significance was set at \( p < 0.05 \). For all statistical analyses SPSS, version 13.0 (SPSS Inc., Chicago, Ill.) was used. Data are presented as mean ± SEM.

Results

3.2. Polyphenolic composition of the pomegranate juice

Since, the antioxidant properties of pomegranate juice is mainly attributed to its polyphenolic content, the polyphenolic composition, the TPC and the total amount of flavonoids were estimated. The results showed that the TPC was 405.00 mg/L of
equivalent gallic acid and the total amount of flavonoid was 12.67 mg/L of equivalent quercetin. Moreover, the analysis of the polyphenolic composition exhibited that the pomegranate juice contained different classes of polyphenols as flavonoids, phenolic acids, and stilbenes. In particular, the phenolic acids gallic acid, chlorogenic acid, \( p \)-coumaric acid, ellagic acid, protocatechuic acid and ferulic acid were identified with values ranging from 0.63 to 2.00 mg/g of lyophilized juice (\( p \)-coumaric acid, and ellagic acid were identified but it was not possible their separate quantification and their amount was estimated as a whole) (Table 1). In addition, the flavonol quercetin (0.50 mg/g of lyophilized juice) and its glycosylated form rutin (1.37 mg/g of lyophilized juice) were identified (Table 1). The flavanol (\(-\))-epicatechin (0.62 mg/g of lyophilized juice) and the glycosylated dihydrochalcone phlorizin (0.48 mg/g of lyophilized juice) were also contained in the juice (Table 1). Finally, one of the most bioactive polyphenol, the stilbene \textit{trans}-resveratrol was found at a concentration of 0.66 mg/g of lyophilized juice (Table 1).

3.1. Oxidative stress markers

For assessing the effects of PJ consumption on the redox status of subjects, a series of oxidative stress biomarkers, such as MDA, protein carbonyl levels and TAC, were measured in blood plasma. The respective results indicate that the levels of protein carbonyls, a marker of protein oxidation, were significantly \( (P < 0.05) \) reduced by 19.6% and 17.7% respectively, immediately (T2) and 7 days (T3) after stopping the PJ consumption as compared to control (i.e. blood samples received before juice consumption). In addition, 14 days after the PJ consumption end (T4) they returned to control levels (Fig. 1A). The MDA levels, a biomarker of lipid peroxidation, start declining immediately after stopping the PJ consumption (T2) and decreased significantly \( (P < 0.05) \) by 24.4% 7 days after PJ consumption (T3) stopped
and remained lower as compared to control levels even for 14 days after stopping PJ consumption (T4) (Fig. 1C). TAC was not significantly affected at any time point compared in respect the control values (Fig. 1B). On the other hand, the GSH levels measured in erythrocytes were significantly increased ($P < 0.05$) by 22.6% immediately after stopping the PJ consumption (T2). Though the GSH levels remained higher from the control levels for 7 (T3) and 14 (T4) days after stopping the PJ consumption, their differences were not statistically significant (Fig. 2A). Finally, the catalase activity assessed in erythrocytes increased immediately (T2) after stopping the juice consumption compared never reaching statistically significant levels (Fig. 2B).

**Discussion**

Antioxidant is 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 by free radicals’ (Halliwell & Gutteridge, 1999). Free radicals such as reactive oxygen species (ROS) are constantly formed in the human body and considered as necessary at low concentrations for various physiological processes, such as cell signaling. However, overproduction of free radicals may result in oxidative stress, an imbalance between the production of reactive oxygen species and a biological system's ability to detoxify them (Halliwell & Gutteridge, 1999). Oxidative stress has been implicated in the pathology of several human diseases, including cancer, atherosclerosis and neurodegenerative diseases. In recent years there is a great interest on the antioxidant properties of pomegranates (de Nigris et al., 2005; Balasundram et al., 2006; Rosenblat et al., 2006). Although numerous studies have been implemented on the antioxidant activity of pomegranates, only few concern the evaluation of PJ consumption on health using human subjects.
Thus, we were prompted to investigate herein the effects of PJ consumption on redox status in human blood. The oxidative stress markers examined were TAC, MDA and protein carbonyls in plasma and catalase activity and GSH levels in erythrocytes.

The results showed that pomegranate juice consumption enhanced the antioxidant mechanisms of the subjects participated in the study and more importantly the effect lasted for a week after stopping its intake. In particular, the levels of protein carbonyls, a marker of protein oxidation, decreased significantly both immediately and one week after stopping PJ consumption by 19.6% and 17.7% respectively as compared to those before starting PJ consumption. Even two weeks after stopping PJ consumption, the protein carbonyl levels were lower (although not statistically significant) than those before starting juice administration. Similar to our results, other studies have shown that pomegranate juice consumption for four weeks (Guo et al., 2008) or three times a week for 1 year (Shema-Didi et al., 2012) decreased the levels of protein carbonyls in plasma. The observed herein protection of PJ against ROS-induced protein oxidation is critical, since the oxidative modification of proteins by ROS has been implicated in the etiology and/or progression of numerous diseases (Stadtman and Levine 2000), since the oxidatively modified proteins are not repaired and must be removed by proteolytic degradation. Thus, a decrease in the efficiency of proteolysis consequences a respective increase in the cellular content of the oxidatively modified proteins, which in turn may disrupt the cellular function either through the loss of catalytic and structural integrity or by interrupting the regulatory pathways.

Apart from protein oxidation, PJ consumption decreased lipid peroxidation. Specifically, the MDA levels that constitute a biomarker of lipid peroxidation, were statistically significantly lower one week after stopping the PJ consumption as
compared to those before starting PJ consumption. MDA levels were also lower -but not in a statistically significant manner- immediately and two weeks after stopping the PJ consumption. Other studies have also shown that PJ consumption for two weeks (Aviram et al., 2000), four weeks (Guo et al., 2008), three months (Rosenblat et al., 2006 literature) or three times a week for 1 year (Shema-Didi et al., 2012) decreased lipid peroxidation in plasma. Two of these studies have also demonstrated that pomegranate consumption increase the activity of serum paraoxonase, a high density lipoprotein (HDL)-associated esterase that can protect against lipid peroxidation (Aviram et al., 2000; Rosenblat et al., 2006). Lipid peroxidation is a process generated naturally, when reactive oxygen species (ROS) readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction (Mylonas and Kouretas 1999). The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, and even tissues. Moreover, lipid peroxidation in plasma is considered one of the main etiological factors for atherogenesis. Thus, the protective effect of pomegranate juice against lipid peroxidation in plasma may prevent the pathogenesis of several diseases such as cardiovascular diseases (Aviram et al., 2000).

Furthermore, PJ consumption significantly increased the GSH levels in erythrocytes immediately after stopping PJ consumption. GSH levels were also higher but not statistically significant one and two weeks after stopping PJ consumption compared to those before starting juice administration. Another study has also reported that PJ consumption for three months increased GSH levels in serum (Rosenblat et al., 2006). Moreover, the same study demonstrated that PJ consumption increased GSH in monocytes-derived macrophages (Rosenblat et al., 2006). However, Guo et al., (2008) reported that PJ consumption does not affect GSH levels in plasma.
The difference between the results of that study and ours may be explained by the fact that most of the plasma GSH comes from liver and not from erythrocytes (Abbas et al., 2011). The GSH increase induced by PJ consumption is important for the antioxidant status of the human organism, since GSH, a tripeptide consisting of cysteine, glycine and glutamate, is one of the most important intracellular antioxidant molecules that protects cellular components from damage caused by ROS. Specifically, the sulfhydryl group (SH) of cysteine serves as a proton donor and is responsible for the antioxidant activity of GSH (Schulz et al., 2000). The observed increase in erythrocyte GSH levels may be due to the induction of expression or the catalytic activity of enzymes involved in GSH biosynthesis such as glutamate cysteine ligase (GCL) and GSH synthetase (GS). For example, it has been shown that plant polyphenols increase expression of GCL (Moskaug et al., 2005). Moreover, compounds such as polyphenols contained in PJ may exert an antioxidant activity per se resulting in the rescue of GSH and thus increasing its concentration.

Furthermore, the observed increase in the antioxidant status of subjects receiving PJ is not attributed to the increase of catalase activity in erythrocytes, one of the most important antioxidant enzymes. This may be rationalized considering that the results indicated that the PJ intake caused only a non-significant increase in catalase activity immediately after stopping consumption compared to those before starting PJ administration. However, Guo et al., (2008) found that PJ consumption for four weeks increased the catalase activity, but they assessed the enzyme activity in plasma.

In a similar line as the catalase activity, the PJ consumption did not affect the TAC levels in plasma at any time point of blood collection. This was an intriguing result, since PJ administration seems to improve the antioxidant status in plasma as shown by the decrease in levels of both lipid and protein oxidation. Other studies have
also shown an increase in plasma TAC after PJ consumption for two weeks (Aviram et al., 2000) or four weeks (Guo et al., 2008). The difference between these studies and our study may be due to the different methods used for TAC assessment. Additionally, Aviram et al., (2004) have shown that PJ consumption increased TAC activity in serum by 130% but in their experiment the length of PJ administration (one year) was much longer as compared to our study.

The antioxidant activity of pomegranate juice is attributed to a large extent to its high polyphenolic content (Gil et al., 2000; Seeram et al., 2005; Lansky, 2006). Thus, the polyphenolic composition of the pomegranate juice was assessed. Different classes of polyphenols as flavonoids, phenolic acids, and stilbenes were identified in the pomegranate juice and their amounts were comparable to those found in other studies (Elfalleh et al., 2011). All of these polyphenols identified in the pomegranate juice exhibit antioxidant activity (Li et al., 2014; Rice-Evans et al., 1996). However, it has been reported that the major polyphenolic compounds accounting for the antioxidant capacity of pomegranate juice are the hydroxybenzoic acids such as gallic acid and ellagic acid identified at high concentrations in the used pomegranate juice. Moreover, it has been shown that the derivatives of the gallic acid and ellagic acid such as gallotannins and ellagittannins (eg. punicalagin) respectively, and the anthocyanins (3-glucosides and 3.5-glucosides of delphinidin, cyanidin, and pelargonidin), are also responsible for the high antioxidant activity of pomegranate juice (Elfalleh et al., 2011; Mena et al., 2011; Gil et al., 2000; Seeram et al., 2005; Lansky, 2006).

In conclusion, the results herein support further the evidence that the consumption of PJ enhances the antioxidant status in humans by decreasing the oxidative damage in lipids and proteins. Moreover, it was shown for the first time that
PJ consumption improves the antioxidant mechanisms in erythrocytes by increasing GSH levels. In addition, this study demonstrates for the first time that even a week after stopping PJ consumption some of the beneficial effects on antioxidant status still remain in the organism. Thus, PJ may be used for the prevention of diseases associated with oxidative stress, although further research is needed to identify the molecular mechanisms responsible for that activity.

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12. Seeram NP, Adams LS, Henning SM (2005) In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem, 16, pp. 360–367


superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. Clin. Nutr, 87, pp. 1053–1061


Figure legends

Figure 1. The effects of PJ administration on plasma protein carbonyls (A), total antioxidant capacity (TAC) (B) and MDA levels (C). *Significantly different from samples collected before PJ consumption ($P < 0.05$).

Figure 2. The effects of PJ administration on erythrocyte glutathione (GSH) (A) and catalase (B). *Significantly different from samples collected before PJ consumption ($P < 0.05$).