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Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats

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Summary
Background & aim: Pomegranate fruit is inescapably linked with fertility, birth and eternal life because of its many seeds. The aim of this study was to investigate the effects of pomegranate juice (PJ) consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level of male healthy rats.
Methods: Twenty-eight healthy adult male Wistar rats were divided into four groups; each group containing seven rats. One milliliter distilled water, 0.25 mL PJ plus 0.75 mL distilled water, 0.50 mL PJ plus 0.50 mL distilled water and 1 mL PJ were given daily for seven weeks by gavage to rats in the first, second, third and fourth groups, respectively. Body and reproductive organ weights, spermatogenic cell density, sperm characteristics, levels of antioxidant vitamins, testosterone, and lipid peroxidation and, antioxidant enzyme activities were investigated. All analyses were done only once at the end of the seven week study period. Data were compared by analysis of variance (ANOVA) and the degree of significance was set at $P < 0.05$.
Results: A significant decrease in malondialdehyde (MDA) level and marked increases in glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase (CAT) activities, and vitamin C level were observed in rats treated with different doses of PJ. PJ consumption provided an increase in epididymal sperm concentration, sperm motility, spermatogenic cell density and diameter of seminiferous tubules and germinal cell layer thickness, and it decreased abnormal sperm rate when compared to the control group.

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Conclusion: The results suggest that PJ consumption improves sperm quality and antioxidant activity of rats.

Introduction

Pomegranate (*Punica granatum*) has been used in the folk medicine of many cultures especially in the Middle East.[1] Edible parts of pomegranate fruit represent 52% of total fruit weight, comprising 78% juice and 22% seeds.[2] Fresh juice is rich in vitamin C,[2] and polyphenolic compounds such as anthocyanins, punicalagin, ellagic and gallic acid.[3,4]

Pomegranate has become more popular because of the attribution of important physiological properties, such as anticancer,[5,6] antiproliferative, apoptotic,[3] HIV-I entry inhibitory, topical microbicidal,[7] cardioprotective,[8] antihyperlipidemic,[9] etc. Additionally, many investigators[10–12] have reported that pomegranate and its derivatives have free radical scavenger and potent antioxidant activity.

Reactive oxygen species (ROS) are highly reactive oxidizing agents belonging to the class of free radicals. The production of ROS in various organs including the testis is a normal physiological event; however, the alterations in their synthesis stimulate the oxidation and DNA damage of cells.[13] The plasma membrane of sperms contains a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage. The lipid peroxidation destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the defects of membrane integrity.[14–16]

Antioxidants, in general, are compounds which dispose, scavenge, and suppress the formation of ROS and lipid peroxidation. Among the well known biological antioxidants, glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide-dismutase (SOD) have a significant role as a suppressor or scavenger of free radicals. Hence, the application of ROS scavengers is likely to improve sperm function.[13,17]

Although the ancient forefathers of medicine, Hippocrates, Soranus, Dioscorides, prescribed the seeds and rind of the pomegranate to prevent conception in women,[18] the pomegranate fruit is inescapably linked with fertility, birth and eternal life because of their many seeds.[19] However, there is no evidence about the positive and/or negative effect of pomegranate and/or its extracts on male fertility. In the present study, to see the effects of pomegranate juice (PJ), a potent antioxidant, on male fertility; we examined epididymal sperm characteristics, spermatogenic cell density, and antioxidant enzyme activity, levels of testosterone and lipid peroxidation in rats to which PJ was given orally for seven weeks.

Materials and methods

Pomegranate juice and chemicals

Pasteurized PJ (100% pure, pasteurized pomegranate juice, 250 mL, Elite Natural Beverage Co., Ankara, Turkey) was purchased from a local store. The other chemicals were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and experimental design

Twenty-eight healthy adult male Wistar rats (eight weeks old) were used in this study. The rats were housed in cages specially designed to minimize field perturbation. The walls of the cages consisted of Perspex, and feeding pens and water bottles were mounted outside the cages. The rat had free access to maintenance food and water. Commercially obtained cork flakes were used as bedding material. The cages were washed once a week. Animals were maintained under standard laboratory conditions on a 12 h light/dark cycle in a temperature-controlled room at $21 \pm 3\,^{\circ}C$.

The rats were randomly divided into four groups; each group containing seven rats. Only 1 mL distilled water was administered to rats in the first group, and they served as *control*. 0.25 mL PJ plus 0.75 mL distilled water was given to rats in the second group, and named *PJ-low*. The third group received 0.50 mL PJ plus 0.50 mL distilled water, and named *PJ-middle*. Only 1 mL PJ was given to rats in the fourth group, and also named *PJ-high*. Both distilled water and pomegranate juice were given by gavage daily for seven weeks. This administration period is necessary to determine the effect of PJ on sperm production because the rats need a period of 48–52 days for the exact spermatogenic cycle including spermatocytogenesis, meiosis and spermiogenesis.[20]

Sample collection

The rats were sacrificed using ether anaesthesia at the end of seventh week. Blood samples were collected from V. cava via sterile injector containing heparin and centrifuged at $3000g$ for 5 min. Plasma was separated and then stored at $-20\,^{\circ}C$ until biochemical and hormonal analyses.

Testes, epididymides, seminal vesicles, prostate and Cowper glands were removed, cleared of adhering connective tissue and weighed. Testis tissues were fixed in Bouin's solution for histologic examinations.

Evaluation of sperm parameters

The epididymal sperm concentration was determined with a hemocytometer using a modified method described by Türk et al.[21] and Sönmez et al.[22] The right epididymis was finely minced by anatomical scissors in 1 mL of isotonic saline in a Petri dish. It was completely squashed by a tweezers for 2 min, and then allowed to incubate at room temperature for 4 h to provide the migration of all spermatozoa from epididymal tissue to fluid. After incubation, the epididymal tissue-fluid mixture was filtered via strainer

to separate the supernatant from tissue particles. The supernatant fluid containing all epididymal spermatozoa was drawn into the capillary tube up to 0.5 lines of the pipette designed for counting red blood cells. The solution containing 0.595 M sodium bicarbonate, 1% formalin and 0.025% eosin was pulled into the bulb up to 101 lines of the pipette. The contents of the pipette were mixed by holding the ends of the pipette between the thumb and the index finger and shaking it vigorously in 100 back-and-forth 30 cm movements. The bulb of the pipette contains a small glass bead that makes thorough mixing possible. Sufficient solution was then blown from the pipette to ensure that the diluents containing no sperm were flushed from the capillary. This gave a dilution rate of 1:200 in this solution. Approximately 10 μL of the diluted sperm suspension was transferred to both counting chambers of an Improved Neubauer (Deep 1/10 mm, LABART, Darmstadt, Germany) and allowed to stand for 5 min. The sperm cells in both chambers were counted with the help of a light microscope at 200× magnification. The remainder of the supernatant fluid of each rat was stored at −20 °C to determine the lipid peroxidation level and antioxidant enzyme activities in spermatozoa.

The percentage of forward progressive sperm motility was evaluated using a light microscope with heated stage as described by Sönmez et al.[23] For this process, a slide was placed on a light microscope with a heated stage warmed up to 37 °C, and then several droplets of Tris buffer solution [0.3 M Tris(hydroxymethyl)aminomethane, 0.027 M glucose, 0.1 M citric acid] were dropped on the slide and a very small droplet of fluid obtained from left cauda epididymis with a pipette was added to the Tris buffer solution and mixed by a cover-slip. The percentage of forward progressive sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score. To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400× magnification. A total of 300 sperm cells was examined on each slide (2100 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as a percentage.[21]

Biochemical studies

Lipid peroxidation level
The plasma and sperm lipid peroxidation levels were measured according to the concentration of thiobarbituric acid reactive species (TBARs)[24] and the amount of produced malondialdehyde (MDA) was used as an index of lipid peroxidation. Briefly, one volume of the test sample and two volumes of stock reagent (15%, w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated in boiling water for 15 min. After cooling, the precipitate was removed by centrifugation at 1500g for 10 min, and then absorbance of the supernatant was read at 532 nm against a blank containing all reagents except test sample on a spectrophotometer (Shimadzu 2R/UV–visible, Tokyo, Japan). The MDA level was expressed in nmol/mL.

Glutathione level and glutathione peroxidase activity
The reduced glutathione (GSH) contents in plasma and sperm samples were measured at 412 nm using the method of Sedlak and Lindsay.[25] The samples were precipitated with 50% trichloroacetic acid, and then centrifuged at 1000g for 5 min. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of Tris–EDTA buffer (0.2 mol/L; pH 8.9) and 0.1 mL of 0.01 mol/L 5,5′-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min, and then read at 412 nm on the spectrophotometer. The levels of GSH were expressed in nmol/mL. The glutathione peroxidase (GSH-Px) activity was determined according to the method of Lawrence and Burk.[26] The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide (NaN$_3$), 0.2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 IU/mL oxidized glutathione (GSSG)-reductase, 1 mM GSH, and 0.25 mM H$_2$O$_2$. The enzyme source (0.1 mL) was added to 0.8 mL of the above mixture and incubated at 25 °C for 5 min before initiation of the reaction with the addition of 0.1 mL of peroxide solution. The absorbance at 340 nm was recorded for 5 min on the spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. The GSH-Px activity was expressed in IU/L.

Catalase activity
The plasma and sperm catalase (CAT) activity was measured as previously described by Góth.[27] Briefly, 0.2 mL of samples was incubated in 1.0 mL substrate (65 μmol per mL hydrogen peroxide in 60 mmol/L sodium–potassium phosphate buffer; pH 7.0) at 37 °C for 60 s. Sample catalase activity is linear up to 100 kU/L. If the catalase activity exceeded 100 kU/L the samples were diluted with the phosphate buffer (2- to 10-fold) and the assay was repeated. One unit catalase decomposes 1 μmol of hydrogen peroxide/min under these conditions. The enzymatic reaction was terminated with 1.0 mL of 32.4 mmol/L ammonium molybdate [(NH4)$_6$Mo$_7$O$_{24}$·4H$_2$O] and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm on the spectrophotometer against the blank containing all the components except the enzyme and expressed in kU/L, where k is the first-order rate constant.

Determination of vitamin levels
Sample preparation
For the extraction of vitamins A and E from plasma, 100 μL of plasma was deproteinized with 100 μL of ethanol and was extracted with 600 μL of chloroform. The extract was shaken for 5 min before centrifuging at 12 000 rpm for 8 min. The organic layer was extracted and evaporated to dryness under nitrogen.[28]

Vitamin C in plasma was extracted as follows: plasma protein was precipitated with 60% methanol and 1 mM EDTA. One hundred microliters of plasma was mixed with 400 μL of 60% methanol/EDTA, incubated for 10 min at 4 °C before centrifuging. The clear phase was transferred to another polypropylene tube and evaporated to dryness under nitrogen. All reconstituted vitamins were mixed

together before injecting into the High Performance Liquid Chromatographic (HPLC) system.[28]

Chromatography

The levels of plasma vitamins A, E and C were determined using the HPLC method. The HPLC system consisted of Merck−Hitachi (Germany) L-7100 LaChrom pump, a Shimadzu (Japan) RF-551 spectrofluorometric detector equipped with a Merck−Hitachi D-7500 programmable integrator, and a Rheodyne 7725i sample injector fitted with a 20 μL sample loop. Vitamins A, E and C were separated on a 150 mm × 3.3 mm ID Separon SGX NH_2 analytical column, fitted with a 50 mm × 3.3 mm ID precolumn, by isocratic elution with 97:3 (v/v) n-hexane-2-propanol, at a flow rate 0.8 mL/min. Before the use of a mobile phase (methanol−acetonitrile−tetrahydrofuran, 75:20:5, v/v/v) fluorimetric detection was performed at the wavelengths $\lambda_{ex} = 298$ nm, $\lambda_{em} = 325$ nm, with high sensitivity.[29] The amounts of vitamins A, E and C were expressed in mg/L.

Testosterone

The plasma testosterone level was measured by the ELISA method using a DRG Elisa testosterone kit (ELISA EIA-1559, 96 Well kit, DRG Instruments, GmbH, Marburg, Germany) according to the kit manufacturer's instructions. The sensitivity, intra- and inter-assay variation coefficients of kit were 0−16 ng/mL, 3.28−4.16% and 4.73−9.94%, respectively.

Histologic examination

To determine the changes in spermatogenic cell density, testis tissues were fixed in Bouin's solution for 48 h, they were dehydrated through graded concentrations of ethanol, embedded in paraffin wax, sectioned at 5 μm thicknesses and stained with Mayer's hematoxylin and eosin (H&E). Ten seminiferous tubules (ST) were randomly examined per section and, their diameters and germinal cell layer thicknesses (from the basal membrane towards the lumen of the tubule) were measured using an ocular micrometer in a light microscope and, the mean size of ST and germinal cell layer thickness were calculated.

Data analysis

Data are presented as mean ± standard error of means (SEM). The degree of significance was set at $P < 0.05$. The changes in body weights were analyzed by two-way analysis of variance for repeated measures in the General Linear Model (GLM) procedure. One-way analysis of variance (ANOVA) and post hoc Tukey-HSD test were used to determine the differences among the groups in terms of all the sperm characteristics, biochemical parameters and histological findings. All the analyses were carried out using the SPSS/PC (Version 10.0; SPSS, Chicago, IL) software package program.

Results

Body and reproductive organ weights

None of PJ doses had statistically significant effect on body weights of the rats during seven weeks when compared to the control group. The initial and final body weights of control, low dose PJ, middle dose PJ and high dose PJ groups were

Table 1 Reproductive organ weights, epididymal sperm concentration, sperm motility, abnormal sperm rate, diameter of seminiferous tubules and germinal cell layer thickness in each rat group

Variable		Control	Pomegranate juice		
			Low	Middle	High
Testis (mg)	Right	1302.9 ± 33.8	1404.3 ± 55.8	1486.7 ± 60.4	1442.0 ± 67.5
	Left	1344.3 ± 40.2	1415.7 ± 42.6	1500.0 ± 53.7	1506.0 ± 50.2
Epididymis (mg)	Right	505.7 ± 16.0	528.6 ± 9.4	511.7 ± 16.2	514.0 ± 18.1
	Left	510.0 ± 15.7	525.7 ± 13.2	513.3 ± 15.4	510.0 ± 19.0
Seminal vesicles (mg)		1072.0 ± 41.3	1247.1 ± 99.8	1091.7 ± 65.2	1107.1 ± 41.7
Prostate (mg)		604.3 ± 45.2	664.3 ± 51.0	611.7 ± 48.1	626.0 ± 32.0
Cowper (mg)	Right	35.7 ± 3.7	36.7 ± 3.3	40.0 ± 3.7	38.0 ± 3.7
	Left	30.0 ± 0.0	41.4 ± 4.6	45.0 ± 5.6	42.0 ± 3.7
Epididymal sperm concentration (million/g)		223.3 ± 11.1[A]	251.2 ± 12.8[AB]	297.5 ± 14.7[BC]	334.2 ± 20.0[C]
Sperm motility (%)		74.3 ± 5.3[a]	79.1 ± 3.9[ab]	84.0 ± 4.0[ab]	94.2 ± 1.4[b]
Abnormal sperm rate (%)	Head	6.8 ± 0.3	6.9 ± 0.9	5.6 ± 0.3	4.2 ± 0.8
	Tail	5.1 ± 0.9	4.3 ± 0.6	3.0 ± 0.3	2.6 ± 0.6
	Total	11.9 ± 1.1[a]	11.2 ± 1.0[a]	8.6 ± 0.6[ab]	6.8 ± 0.7[b]
Diameter of seminiferous tubules (μm)		241.8 ± 2.5[!]	284.6 ± 3.2[#]	300.4 ± 3.1[*]	300.7 ± 3.0[*]
Germinal cell layer thickness (μm)		64.6 ± 1.1[!]	80.0 ± 1.6[#]	88.7 ± 2.0[*]	89.0 ± 1.6[*]

Data are expressed as mean ± SEM.
The mean differences between the values bearing different upper cases (A, B, C; $P < 0.01$), lower cases (a, b; $P < 0.05$) or icons (!, #, *; $P < 0.001$) within the same row are statistically significant.

Table 2 Plasma and sperm malondialdehyde, glutathione levels and glutathione peroxidase, catalase activities in each rat group

Variable		Control	Pomegranate juice		
			Low	Middle	High
Malondialdehyde (nmol/mL)	Plasma	1.34 ± 0.14^A	0.62 ± 0.06^B	0.85 ± 0.03^B	0.69 ± 0.03^B
	Sperm	8.33 ± 0.59^A	8.27 ± 0.21^A	4.18 ± 0.19^B	3.04 ± 0.28^B
Glutathione (nmol/mL)	Plasma	0.0937 ± 0.0028^a	0.0934 ± 0.0009^a	0.109 ± 0.0047^b	0.108 ± 0.0026^b
	Sperm	1.107 ± 0.056^a	1.158 ± 0.099^b	1.134 ± 0.081^{ab}	1.157 ± 0.057^b
Glutathione peroxidase (IU/L)	Plasma	7.58 ± 0.23^A	8.76 ± 0.14^B	8.71 ± 0.09^B	9.77 ± 0.14^C
	Sperm	25.61 ± 0.56^A	25.82 ± 0.91^A	25.69 ± 0.60^A	28.82 ± 0.37^B
Catalase (kU/L)	Plasma	27.54 ± 1.73^A	33.51 ± 2.73^A	38.60 ± 2.65^A	56.17 ± 3.93^B
	Sperm	35.94 ± 2.04^A	35.34 ± 3.84^A	34.44 ± 1.94^A	54.94 ± 3.30^B

Data are expressed as mean $\pm$ SEM.
The mean differences between the values bearing different upper cases (A, B, C; $P < 0.001$) and lower cases (a, b; $P < 0.01$) within the same row are statistically significant.

271.2 ± 8.0 and 294.3 ± 8.9, 265.6 ± 5.9 and 280.6 ± 8.3, 281.6 ± 4.5 and 290.7 ± 9.7, 267.6 ± 14.1 and 295.0 ± 4.1, respectively. Although the weights of testes, epididymides, seminal vesicles, prostate and Cowper glands of rats, treated with different doses of PJ were numerically higher than those of the control group, the differences between the groups were not statistically significant (Table 1).

Epididymal sperm characteristics

The effects of different doses of PJ on epididymal sperm concentration, sperm motility and abnormal sperm rate are presented in Table 1. While both middle and high dose PJ significantly increased the sperm concentration ($P < 0.01$) when compared to the control group, no significant changes were observed in the low dose PJ group. Furthermore, sperm concentration of rats in high dose PJ group was also significantly higher than those in the low dose PJ group ($P < 0.01$).

Both low and middle dose PJ provided non-significant increases in sperm motility and, non-significant decreases in abnormal sperm rate when compared to the control group. However both increase in sperm motility and decrease in total abnormal sperm rate of rats in high dose PJ group were statistically significant in comparison to the control group ($P < 0.05$).

Plasma and sperm lipid peroxidation level and antioxidant enzyme activities

The MDA and GSH levels, GSH-Px and CAT activities of all the groups are given in Table 2. All doses of PJ caused significant decreases in plasma MDA levels when compared to the control group ($P < 0.001$). Additionally, significant decreases in sperm MDA levels were observed in rats in both middle and high dose PJ groups when compared to the control group ($P < 0.001$).

There were significant differences between the groups in plasma and sperm GSH levels ($P < 0.01$). While all doses of PJ caused significant increases in plasma GSH-Px ($P < 0.001$), only high dose PJ significantly increased sperm GSH-Px activity ($P < 0.001$) in comparison to the control group. Low and middle dose PJ increased both plasma and sperm CAT activities when compared to the control group, but the difference was not significant ($P > 0.05$). However, increases observed in plasma and sperm CAT activities of high dose PJ group were statistically significant when compared to the control and the other groups ($P < 0.001$).

Plasma vitamin and testosterone levels

The levels of plasma vitamins A, E, C and testosterone in all the groups are given in Table 3. Vitamins A, E and

Table 3 Levels of plasma vitamins A, E, C and testosterone in each rat group

Parameters	Control	Pomegranate juice		
		Low	Middle	High
Vitamin A (mg/L)	0.78 ± 0.27	0.81 ± 0.03	0.89 ± 0.24	0.85 ± 0.27
Vitamin E (mg/L)	0.72 ± 0.21	2.39 ± 0.72	1.51 ± 0.88	3.45 ± 1.42
Vitamin C (mg/L)	3.72 ± 0.71	5.39 ± 0.84	$8.91 \pm 0.53^{a,b}$	10.84 ± 0.94^c
Testosterone (ng/mL)	0.84 ± 0.07	0.88 ± 0.07	0.92 ± 0.16	1.07 ± 0.10

Data are expressed as mean $\pm$ SEM.
[a] $P < 0.001$, versus control.
[b] $P < 0.05$, versus low dose pomegranate juice.
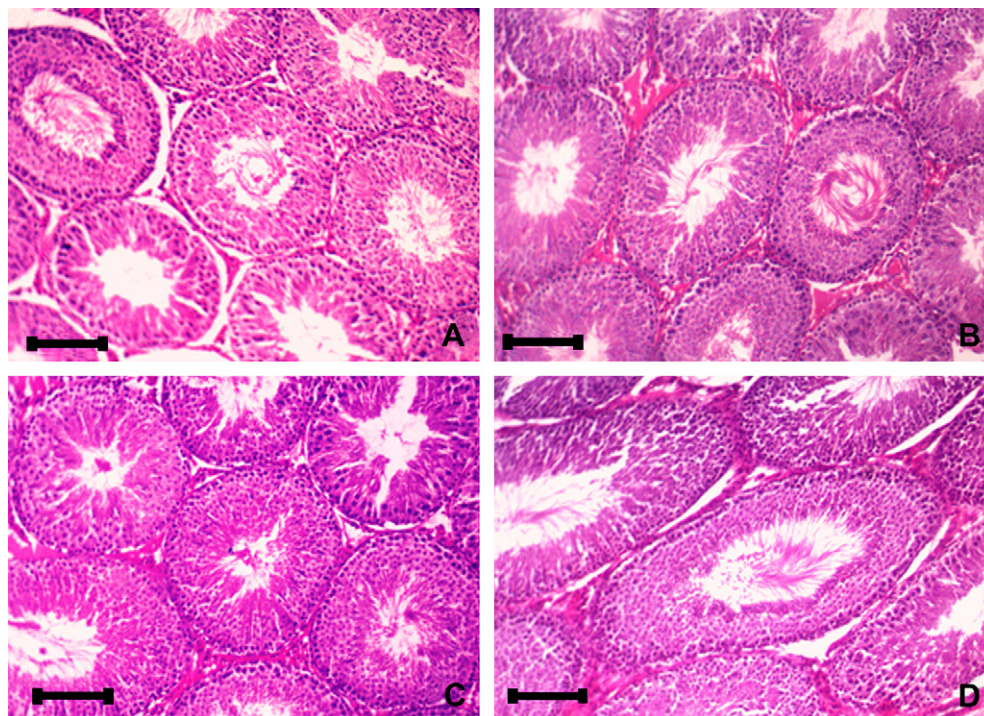[c] $P < 0.001$, versus both control and low dose pomegranate juice.

Figure 1 Photomicrographs of testis tissue from rats that consumed only distilled water (A), low (B), middle (C) and high doses of pomegranate juice (D). Hematoxylin and eosin (H&E); bar = 150 μm. Although a low dose of PJ caused a slight increase in spermatogenic cell density especially spermatozoa, both middle and high doses of PJ provided marked increases in all the spermatogenic cells ranging from spermatogonia, spermatocytes, spermatids to spermatozoa.

testosterone levels in the rats treated with low, middle or high doses of PJ were similar to those of the control group. In contrast, plasma vitamin C level in rats received high dose of PJ was significantly ($P < 0.001$) higher than both control (2.91-fold) and low dose PJ group (2.01-fold). Similarly, middle dose of PJ administration provided a significant increase in plasma vitamin C level when compared to both control ($P < 0.001$) and low dose PJ group ($P < 0.05$).

Spermatogenic cell density

All doses of PJ provided significant increases in the diameter of ST and germinal cell layer thickness when compared to the control group ($P < 0.001$). Increases determined in diameter of ST and germinal cell layer thickness of rats in middle or high dose PJ groups were significantly ($P < 0.001$) higher than those in the low dose PJ group (Table 2). It was observed that although a low dose of PJ caused a slight increase in spermatogenic cell density especially spermatozoa, both middle and high doses of PJ provided marked increases in all the spermatogenic cells ranging from spermatogonia, spermatocytes, spermatids to spermatozoa in comparison to the control group. As a consequence of the increase in diameter of ST and spermatogenic cells of rats in middle and/or high dose PJ groups, both interstitial space and lumen of the ST seemed to be reduced in size. The presence of degenerating spermatogenic cells that can be present in normal testis was less in rats that received different doses of PJ, especially middle and high doses when compared to the control. However, Sertoli and Leydig cell concentrations were not affected by PJ administration (Fig. 1).

Discussion

The results of this study demonstrated, for the first time, that daily consumption of PJ for seven weeks caused increased spermatogenic cell density, epididymal sperm concentration, sperm motility and decreased abnormal sperm rate related with decreased lipid peroxidation in male rats.

PJ is an important source of anthocyanins, hydrolyzable tannins punicalagin and punicalin,[5] ellagic and gallic acids,[3,4] and also contains vitamin C.[2] The antioxidant and free radical scavenging activity of phenolic compounds derived from pomegranates[10—12] and vitamin C[23] have been reported. In this study, it was observed that PJ had no significant effect on body and reproductive organ weights, levels of plasma vitamins A and E, and testosterone except plasma vitamin C levels when compared to the control. The significant increase observed in plasma vitamin C level of rats that received middle and high doses of PJ supported the fact that PJ is a source of vitamin C.

Many compounds, metabolized by cells cause the increase in the levels of electrophilic radicals that can react with oxygen giving rise to ROS, one of the main sources of free radicals like hydrogen peroxide (H_2O_2), singlet-oxygen ($^1O_2$), hydroxyl radical ($\cdot OH$) or peroxynitrite. ROS are normally synthesized in several essential metabolic processes for living cells including the spermatozoa; however, excessive generation of ROS produced by spermatozoa themselves[14,30] or by the combinations of xenobiotics[31,32] and immunosuppressive agents[21] induces the formation of toxic lipid peroxides. When ROS begin to accumulate, cells exhibit a defensive mechanism using various antioxidant

enzymes. The main detoxifying systems for peroxides are CAT and GSH. CAT is an antioxidant enzyme, which destroys H_2O_2 that can form a highly reactive OH in the presence of iron as a catalyst. By participating in the glutathione redox cycle, GSH together with GSH-Px converts H_2O_2 and lipid peroxides to non-toxic products.[13,15,33] Phenolic compounds derived from pomegranate,[8,12] vitamins C,[23] E, melatonin,[22] lycopene,[21,31,32] have been used as antioxidant agents to prevent various lipid-peroxidation-induced damages in different organs. A significant decrease in MDA level, by-product of lipid peroxidation, and marked increases in GSH, GSH-Px and CAT activities of plasma and sperm samples of rats received different doses of PJ were observed in the present study. These findings demonstrate that PJ has a potent anti-oxidative effect.

ROS are highly reactive and can react with many intracellular molecules, mainly unsaturated fatty acids (phospholipids, glycolipids, glycerides and sterols) and transmembrane proteins with oxidizable aminoacids. The oxidation of these molecules causes the increase in the cellular membrane permeability. ROS can attack the unsaturated bonds of the membrane lipids in an autocatalytic process, with the genesis of peroxides, alcohol and lipidic aldehydes as by-products of the reaction. Thus, the increase of free radicals in cells can induce the lipid peroxidation by oxidative breakdown of polyunsaturated fatty acids in membranes of cells.[14,16] Spermatozoa are especially susceptible to peroxidative damage because of the high concentration of polyunsaturated fatty acids which are involved in regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion, and low antioxidant capacity. Obviously, peroxidation of sperm lipids destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with the rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability and increased mid-piece morphological defects, and even it completely inhibits spermatogenesis in extreme cases.[13,15,17] In the present study it was observed that epididymal sperm concentration and sperm motility of rats that received different doses of PJ were significantly higher than those of the control. Additionally, only a high dose of PJ significantly decreased total abnormality of sperms when compared to the control. Similarly, while a low dose of PJ caused a slight increase in spermatogenic cell density especially spermatozoa, both middle and high doses of PJ provided marked increases in all the spermatogenic cells ranging from spermatogonia, spermatocytes, spermatids to spermatozoa when compared to the control. Additionally PJ provided an increase in the diameter of ST and germinal cell layer thickness. In this study, improvements observed in spermatogenic cell density and sperm quality may be attributed to prevention of excessive generation of free radicals, produced by spermatozoa themselves, by means of their antioxidant property of PJ.

Conclusion

Spermatogenic cell density and sperm quality increased because increased antioxidant capacity protected spermatozoa against peroxidative damage in healthy rats. Hence,

it can be said that there is a positive relation between PJ consumption and sperm parameters.

Conflicts of interest statement

There is no conflict of interest among any of authors.

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