

THE POSSIBLE PROTECTIVE ROLE OF THE POMEGRANATES JUICE ON THE TESTIS OF AGED MALE ALBINO RATS

YASSIEN, R. I.✉

Department of Histology, Faculty of Medicine, Menoufia University. 6 Abdel-Rehim
Badawy St. from Dr. Mostafa El-Naggar St., ShebinElkom - 32511, Menoufia,
Egypt. E. mail: raniayassien@yahoo.com Cell: +2 01028073793

Received: December 19, 2016; Accepted: January 8, 2017

Abstract: *This study was carried out to investigate effect of Pomegranates juice (PJ) on the testis of aged male albino rat. PJ is a strong antioxidant used for many medical disorders. Animals were randomly divided into 3 groups of ten rats for control group and fifteen rats for each other two groups. In (group I) and (group II) animals were received distilled water by gavage daily for 52 days. Where in (group III) animals were received PJ by gavage at a dose of 10 ml/kg/day daily for 52 days. All animals were sacrificed at the end and testis were separated and processed. Light microscopic studies of aged animals demonstrated loss and degeneration of germ cells, sertoli and leydig cells. Excessive collagen deposition in aged group was detected. Morphometric studies exhibited decreased epithelium height, diameter of STs in aged group. While biochemical studies showed decreased testosterone level and increased FSH& LH. PJ group showed improved pictures nearly similar to control group. Therefore, it is noticed the beneficial effect of pomegranate juice on testis in aged rats that may makes it one of the most important foods for the future.*

Key words: Pomegranates juice, Aging, Rats

INTRODUCTION

Aging is an extremely complex and multifactorial process that proceeds with the gradual deterioration in functions of body organs. It can be described as the gradual, lifelong accumulation of molecular damage to cells and tissues in response to exposure to stress associated with environment and lifestyle. The precise biological and cellular mechanisms responsible for the aging are not known, but a constellation of complex and interrelated factors, including oxidative stress-induced protein and DNA damage in conjunction with insufficient DNA damage repair, as well as genetic instability of mitochondrial and nuclear genomes [1]. In the rat testis, the seminiferous tubules are the site of

continuous spermatogenesis. They are ensheathed by a layer of contractile cells, the peritubular myoid cells. Sertoli cells activate the development and maintain the viability of germ cells by secreting hormonal and nutritive materials. Leydig cells are essentially the only site of testosterone production in the male to form and maintain male secondary sexual structures and characteristics [2].

Male sexual dysfunction composed of several problems associated with sperm concentration, motility, shape and may be due to hormonal imbalance as low testosterone level [3] Specific age-related changes both in function and structure of the seminiferous tubules have been reported in most mammals including man and rat [4].

Sexual dysfunctions increase with aging and the causes degenerative diseases, and stress associated with industrialized lifestyles. 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. The plasma membrane of sperms contains a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage [2]. 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 [5].

By alteration of protein and nucleic acid structure, oxidative stress has a destruction effect on cells. Moreover, oxidative stress increases the intracellular free calcium, destruction membrane ion transport and permeability. Because of its association with some of the abnormal physiological processes, lipid peroxidation has attracted much attention in recent years [6].

Antioxidants, in general, are compounds which scavenge and suppress the formation of reactive oxygen species (ROS) and lipid per-oxidation. [2]. Although efficient, the antioxidant enzymes and compounds do not prevent the oxidative damage completely [1]. Hence, the application of ROS scavengers is likely to improve sperm function [2].

Fruits and dietary sources are a promising source of new therapeutic options. Pomegranate is a nutrient dense antioxidant rich fruit has been revered as a symbol of health and fertility. Pomegranate has been used in traditional medicine as remedy for a lot of symptoms like eye sore, scurvy, blood clotting and diarrhea etc. The recent interest for this fruit is not only because of the pleasant taste, but also due to various medicinal properties such as anti-atherogenic, antiparasitic, antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory and cardioprotective [7].

Pomegranate has become more popular because of the attribution of important physiological properties, such as anticancer, antiproliferative, HIV-I entry inhibitory, antihyperlipidemic [8]. Pomegranate

is rich in antioxidant of polyphenolic class which includes tannins and flavonoids. These antioxidant molecules have been shown to be bioavailable and safe [2]. In addition, pomegranate juice (PJ) has been reported to be a rich source of vitamin C and minerals. PJ improve sperm count, motility, and abnormal sperm rate [9]. The current study aims to evaluate the beneficial effect of pomegranate juice on testis in aged rats that may makes it one of the most important foods for the future.

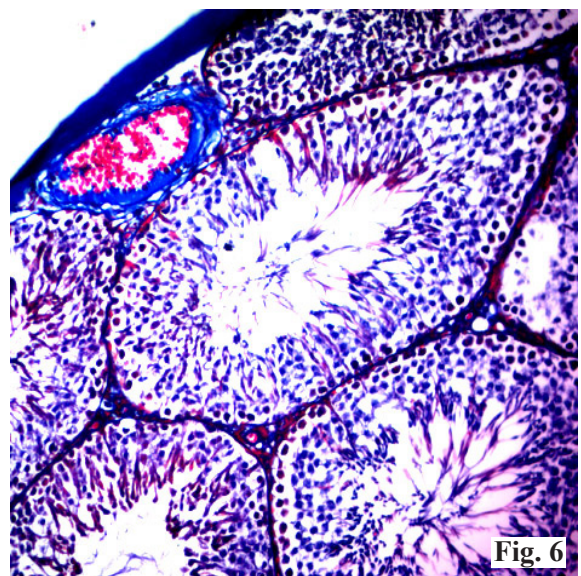
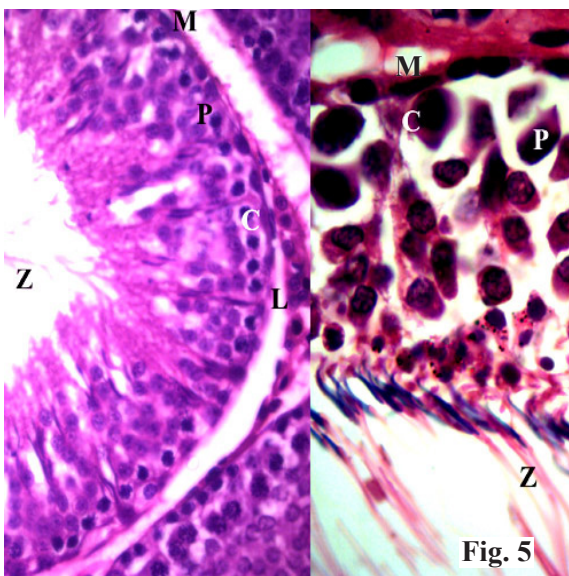
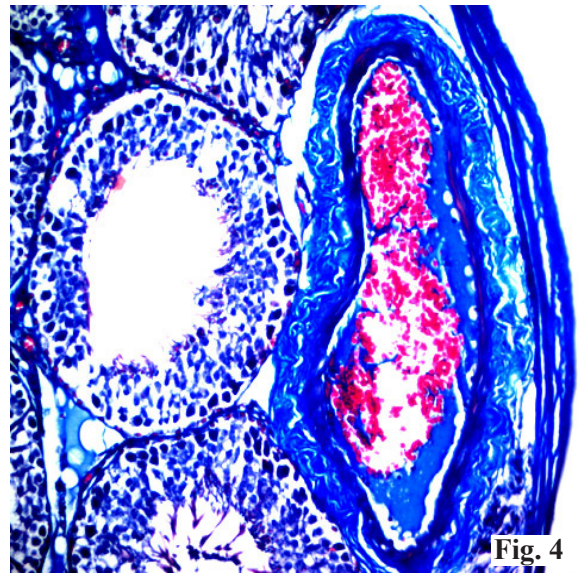
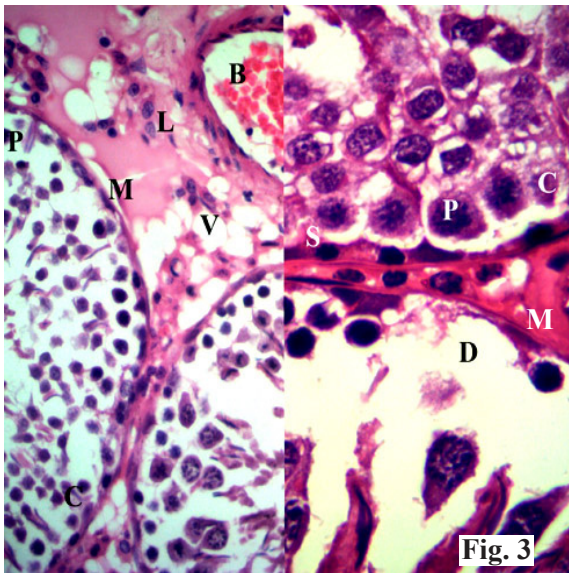
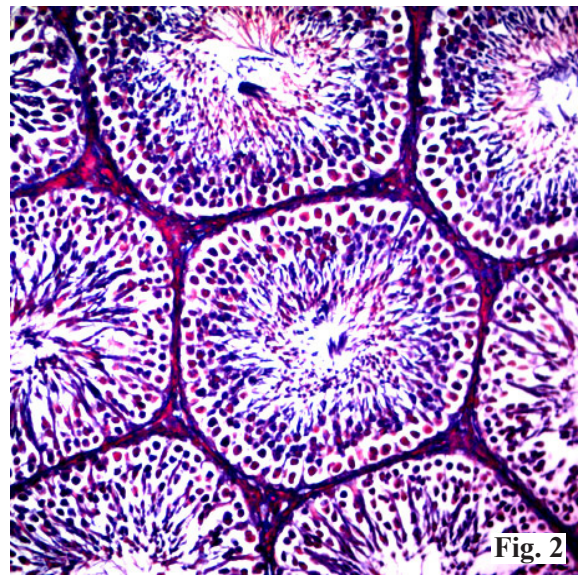
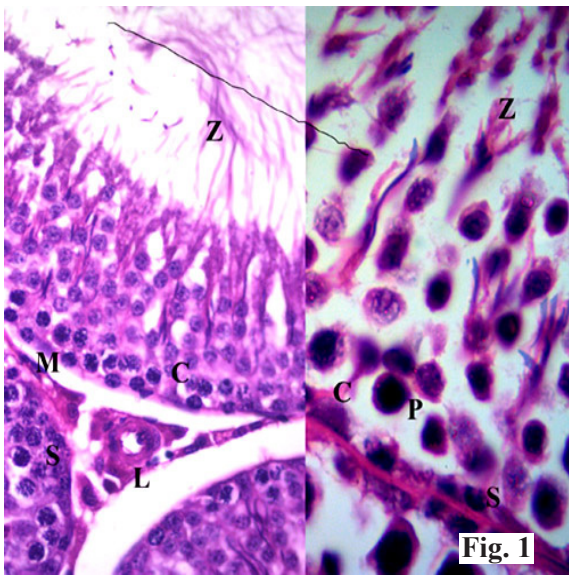
MATERIALS AND METHODS

Experimental animals: Ten adult (6 months old) male albino rats ranging in weights from 120 to 150g and forty aged (24 months old) male albino rats ranging in weights from 300 to 400g, were used in this study. The animals obtained from breeding animal house, Faculty of Medicine, Menoufia University. They were housed in the animal laboratory of Menoufia faculty of medicine. Strict care and cleaning measures were utilized to keep the animal in a normal healthy condition. The animals were put in animal cages under the prevailing atmospheric conditions and also were fed to standard diet and liberal supply of tap water. They were kept under a photoperiod of 12 h light: 12 h darkness, in controlled conditions of temperature (20-24°C). All ethical protocols for animal treatment were followed. The experimental protocol was accepted by the Ethical Committee of Menoufia faculty of medicine.

Pomegranates fruits were purchased freshly from local market. The fruits were peeled and the seeds were squeezed with a squeezing machine. The pomegranates juice (PJ) was filtered to remove any water-insoluble materials and the PJ was immediately placed in dark bottles wrapped with aluminum foil and stored at 4°C to be used fresh daily. Pomegranate juice was given by gavage daily for 52 days. 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 [2].

Experimental procedure: Rats were divided into three groups, as follows:

1. Group I (adult control group): Ten (6 months



old) rats received distilled water by gavage daily for 52 days.

2. **Group II (aged group):** Twenty (24 months old) rats received distilled water by gavage daily for 52 days.
3. **Group III (aged with PJ group):** Twenty (24 months old) rats received PJ by gavage at a dose of 10 ml/kg/day daily for 52 days [10].

At the end of the experiment, blood samples were collected from the retro-orbital venous plexus of all rats for biochemical study. The animals were then scarified. The testes of all rats were collected for light examination of the seminiferous Tubules. Each testis was fixed in 10% formalin for 24 hours. They were then subjected to the normal procedures for paraffin blocks embedding, sectioning, and staining.

Histological study: Paraffin sections of 5 μ m were obtained and stained with hematoxylin and eosin (H&E) to show the histological details & Masson's trichrome stain to detect the collagen fibers [11].

Biochemical study: Quantitative measurement of serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were carried out adopting ELISA technique using kits specific for rats purchased from BioVendor (Gunma, Japan) according to the protocol provided with each kit.

Morphometric study: The measurements were determined using a 'Leica Qwin 500 C' Image analyzer computer system (Leica Imaging System Ltd., Cambridge, UK). Measuring the germ

epithelium height and diameter of STs were carried out in 10 non overlapping fields in each section at a magnification of $\times 400$.

Statistical analysis: Results were expressed as mean \pm SD. Data for multiple variable comparisons were analyzed using the one-way analysis of variance (ANOVA) followed by Turkey-Kramer's Multiple Comparison Test [12], where $p < 0.05$ was considered significant.

RESULTS

Histological Results

Group I (adult Control): Testicular sections revealed that the seminiferous tubules were separated by interstitial tissue. The basal layer of the germinal cells was supported by the lamina propria which contained myoid cells. The tubules were lined by Sertoli and spermatogenic cells. Sertoli cells appeared as elongated cells wedged between spermatogenic cells. They contained basal elongated nuclei. Spermatogenic cells included spermatogonia, spermatocytes, spermatids and spermatozoa. Spermatogonia rested on the basal laminae and had rounded dark nuclei. Inner to the spermatogonia were primary spermatocytes with large nuclei containing coarse clumps of chromatin. Spermatids with vesicular nuclei and prominent nucleoli as well as sperm were observed. The interstitial tissue between the seminiferous tubules contained interstitial cells of leydig. They appeared rounded in shape with rounded vesicular nuclei and prominent nucleoli (Fig. 1). The Masson trichrome

Explanation of figures

Fig. 1: A photomicrograph of a section of albino rat testis of the group I (adult control) showing parts of seminiferous tubules, surrounded by basal laminae contained myoid cells (M) and lined by Sertoli cells (C), spermatogonia (S), primary spermatocytes (P) and spermatozoa (Z). Leydig cells appear rounded in shape (L) in the interstitial tissue. The magnifications are 400 and 1000 respectively (H & E).

Fig. 2: A photomicrograph of a section of albino rat testis of the group I (adult control) showing minimal deposition of collagen between seminiferous tubules. (Masson X 400).

Fig. 3: A photomicrograph of a section of albino rat testis of the group II (aged) showing parts of seminiferous tubules, surrounded by basal laminae contained myoid cells (M) with flat nuclei and lined by Sertoli cells (C), few spermatogonia (S), primary spermatocytes (P). Few Leydig cells (L) can be seen in the interstitial tissue. Notice: degenerated spermatogenic cells (D), congested blood vessels (B) and many vacuoles (V) in the interstitium. The magnifications are 400 and 1000 respectively. (H & E).

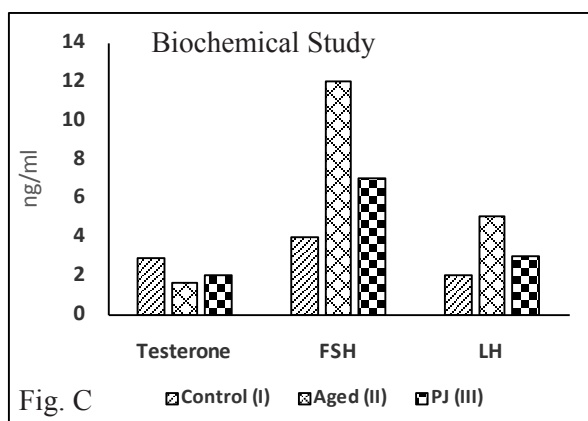
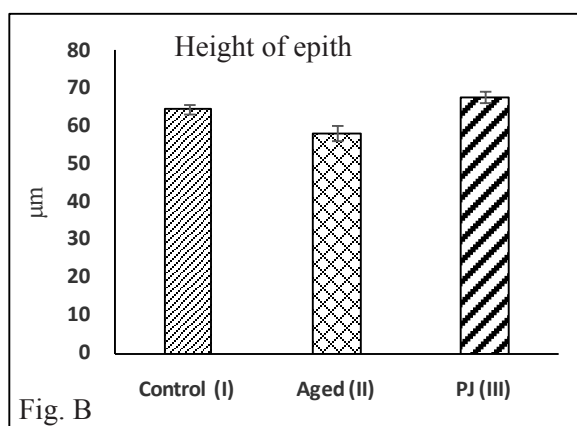
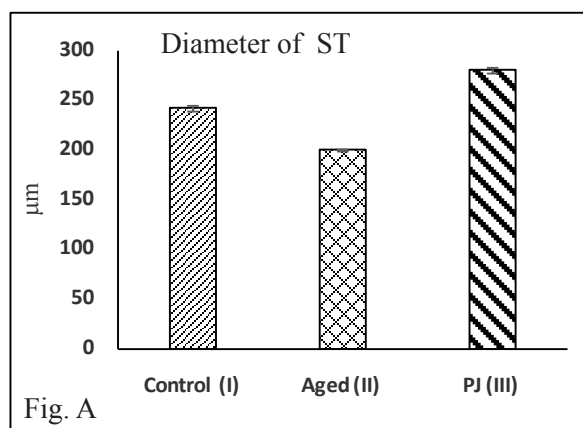
Fig. 4: A photomicrograph of a section of albino rat testis of the group II (aged) showing excessive deposition of collagen between seminiferous tubules and around congested blood vessels. (Masson X 400).

Fig. 5: A photomicrograph of a section of albino rat testis of the group III (aged with PJ) showing parts of seminiferous tubules, surrounded by basal laminae contained myoid cells (M) and lined by Sertoli cells (C), spermatogonia (S), primary spermatocytes (P) and spermatozoa (Z). Leydig cells appear rounded in shape (L). The magnifications are 400 and 1000 respectively. (H & E).

Fig. 6: A photomicrograph of a section of albino rat testis of the group III (aged with PJ) showing mild deposition of collagen between seminiferous tubules and around blood vessels. (Masson X 400)

stained section showed minimal amount of collagen fibers deposition in between STs (Fig. 2).

Group II (aged group): Light microscopic examination revealed testicular sections with loss of histological architecture of some seminiferous tubules. They were surrounded by basal laminae and some tubules showed degeneration and lined by scanty cells. Homogeneous acidophilic material with many vacuoles between the seminiferous tubules was noted. Also, congested blood vessels appeared. Myoid cells were present at the basal laminae. Some interstitial Leydig cells appeared



(Fig. 3). The Masson trichrome stained section showed excessive collagen fibers deposition in between STs and around congested blood vessels (Fig. 4).

Group III (aged group): Light microscopic examination revealed the seminiferous tubules were apparently normal in shape. The tubules were lined by spermatogenic cells and Sertoli cells. spermatocytes, spermatids and sperm could be detected. Sertoli cells appeared wedged between the spermatogenic cells with their indented nuclei. Between the seminiferous tubules, there was interstitial tissue containing Leydig cells, which appeared rounded in shape with rounded nuclei (Fig. 5). The Masson trichrome stained section showed mild amount of collagen fibers deposition in between STs (Fig. 6).

Morphometric results: Statistical analysis of the diameter of seminiferous tubules (STs) and height of germinal epithelium in ten fields of each slide showed a significant decrease in Aged group compared with the control adult group. PJ group showed a significant increase of the diameter of STs and height of germinal epithelium (Figs. A,B).

Biochemical results: Statistical analysis of the FSH, LH, and testosterone levels indicated that FSH & LH level markedly increased in the aged group comparing to the control adult group. The PJ group showed lower levels of the FSH & LH comparing to the aged group, but still higher than the level of the control adult group. Testosterone levels showed decrease in the aged group as compared to the control adult group. The PJ group showed some improvement of the testosterone level. (Fig. C).

DISCUSSION

Several general changes take place in the body as it ages viz.: hearing and vision decline, muscle strength lessens; soft tissues such as skin and blood vessels become less flexible. There is an overall decline in body tone. Although the exact causes of aging remain unknown, scientists are learning a great deal about the aging process and the mechanisms that drive it. Aging is the process by which cell gradually reach to their optimum function then start to decline [13].

In this study at six months old, the animals appeared

in their maximum development then degeneration became progressive and significant at 24 months old. Aged group was manifested by decreasing in seminiferous tubule diameter, lumen, desquamated cells and diminution in the number of cell the same finding was reported by many scientists [4,13]. Degenerative changes involving all types of spermatogenic cells as well as Sertoli cells were revealed. Degeneration of peritubular tissue was also seen [14]. Leyding cell disappeared in from some of the aged testis sections but the fate of the lost cells is unknown, raising the possibility that the cells disappeared by dedifferentiation back into interstitial cells. The interstitial cells might be increased that ended in a complete tubular sclerosis [3,4].

Atrophy of the seminiferous tubules are often observed adjacent to tubules exhibiting normal spermatogenesis. This focal atrophy suggests that aging-related changes in the seminiferous epithelium are intrinsic to the testis [13].

In the present study Masson trichrome stain of aged group showed excessive collagen deposition in the interstitial space. It could be explained that the diminution of seminiferous tubule diameter caused by germ cell loss results in the increase in collagen fibers around the tubules in an attempt to fill the empty space [15]. Others researchers added that, congestion in the blood vessels and abundant collagen fibers could be due to the reduced testosterone secretory capacity per Leydig cell [13]. Oxidative stress is an imbalance between the production of high ROS and their efficient removal by antioxidant systems. ROS can be produced in large amounts by spermatozoa mitochondria, and a variety of enzymes including the xanthine- and nicotinamide adenine dinucleotide phosphate oxidases, and by CYP isozymes in the testis. Free radicals have high affinity to cell membrane lipids, especially PUFAs, leading to tissue damage. Spermatogenesis is an extremely active replicative process. Excessive generation of free radicals-induced DNA damage results in increased testicular apoptotic germ cell [7]. However, the poor vascularization of the testes means that oxygen tensions in this tissue are low and that competition for this vital element is extremely intense. Despite the low oxygen tensions that characterize the testicular microenvironment, spermatozoa and other cells within the testis remain vulnerable to oxidative

stress due to the abundance of high PUFAs and the presence of potential ROS-generating systems [9].

In this study, morphometric results showed height of spermatogenic cells and diameter of ST were significantly reduced compared with the control group. This could be explained by apoptosis due to oxidative stress with age producing an accelerated germ cell loss [14]. More ever, other authors confirmed that fibrosis of the tunica propria hinders the metabolic exchange between the seminiferous epithelium and testicular interstitium, thus leading to germ cell loss and tubular atrophy with decreased diameter [4]. Another explanation, the loss of germ cells and decreased height could result from a decreased ability of Sertoli cells to support germ cell survival and differentiation [13]. Sertoli cells send numerous fine cytoplasmic processes which envelope the associated germ cells. So, degeneration of Sertoli cell could be the cause of germ cell sloughing [2].

The biochemical results in our study revealed that testosterone in aged group were significantly decreased as compared with control. This was in agreement with other scientists who explained that, androgens stimulate the growth by inducing the protein synthesis, but increase in free radicals causes oxidation of proteins together with lipids in tissues. In addition, permanent androgenic stimulation is necessary for normal growth and functions of testes, Therefore, disturbances in the synthesis of androgens and proteins decreased testosterone concentration [14]. Also, it could be explained by degeneration of Leydig cells in aged testis [1]. FSH and LH in aged group show increased levels as compared to control group. It could be owing to a negative feedback due to decreased testosterone level. An increased in stress hormones, such as cortisol, leading to a subsequent disturbance in another hormone called gonadotropin releasing hormone (GnRH). GnRH secreted by hypothalamus played a role in the production of FSH and LH. This stress/fertility link has been fairly well established [1,3]. Also, the oxidative stress was previously suggested to represent a common mechanism in endocrine disruptor-mediated dysfunction [14].

Natural antioxidants are found in various plant products such as fruits, leaves, juices, seeds, and oils. They have attracted special interest because

of their ability to decrease the xenobiotics and the chemicals-induced increase in free radicals, which can cause various degenerative diseases. Pomegranate, in addition to ancient historical uses, has been used in several systems of medicine. PJ (Pomegranate juice) is a polyphenol-rich juice with high antioxidant capacity. Pomegranate has a protective effect due to its active ingredients like tannins, phenolic acids, estrogenic flavonoids and conjugated fatty acids [16].

Light microscopic examination of PJ group revealed most of the STs retained normal appearance. These results were confirmed morphometrically by the significant increase in the germinal epithelial height and diameter of STs. This was in agreement with Mahmoud and Solaiman [10].

PJ group showed significantly increased spermatogenic cell density, sperm concentration, in male rats [2,17]. The ameliorations may possibly be related to antioxidant effect of PJ and also to its inhibitory effect on CYP activity [7,9].

In the present study of PJ group testosterone increased and FSH and LH decreased due to ability of pomegranate to reduce stress hormones, such as cortisol [3]. Although efficient, the antioxidant compounds did not prevent the oxidative damage completely. Also, did not ameliorate the abnormal disturbance up to the normal [1].

In conclusion, pomegranate juice has potent effect on degenerative changes caused by aging due to its potent antioxidant effects. Hence, it can be said that there is a positive effect of PJ consumption on male fertility.

REFERENCES

- [1] Moustafa, M., Alazzounui, A.S. and Gabri, M.S.: *Egyptian J. Hospital Med.*, 57: 598-611 (2014).
- [2] Said Ahmed, M.A.A., Abd El-Hakem, A.H., Ewis, S.H.A. and Eid, R.A.: *Intern. J. Advan. Res.*, 1(9): 831-851 (2013).
- [3] Al-Olayan, E.M., El-Khadragy, M.F., Metwally, D.M. and Abdel Moneim, A.E.: *BMC Complement Altern. Med.*, 14: 164 (2014).
- [4] Morales, E., Horn, R., Pastor, L.M., Santamaría, L., Pallarés, J., Zuasti, A., Ferrer, C. and Canteras, M.: *Histol. Histopathol.*, 19(2): 445-455 (2004).
- [5] Atilgan, D., Parlaktas, B., Uluocak, N., Gencten, Y., Erdemir, F., Ozyurt, H., Erkorkmaz, U. and Aslan, H.: *Exp. Ther. Med.*, 8(2): 478-482 (2014).
- [6] Rehan, M.R., Zedan, A.M.G., El-Hashash, S.A., Farid, M.A. and El-Shafie, G.A.: *African J. Biotechnol.*, 15(36): 1977-1985 (2016).
- [7] Bhargavan, D., Harish, S. and Krishna, A.P.: *Intern. J. Appl. Biol. Pharmaceu. Technol.*, 6(3): 229-236 (2015).
- [8] Karwasra, R., Kalra, P., Gupta, Y.K., Saini, D., Kumar, A. and Singh, S.: *Food Funct.*, 7(7): 3091-3101 (2016).
- [9] Türk, G., Çeribaşı, S., Sönmez, M., Çiftçi, M., Yüce, A., Güvenç, M., Kaya, Ş.Ö., Çay, M. and Aksakal, M.: *Toxicol. Ind. Health*, 32(1): 126-137 (2016).
- [10] Mahmoud, S.A. and Solaiman, A.A.: *Egyptian J. Histol.*, 37(2): 233-247 (2014).
- [11] Suvarna, S.K., Layton, C. and Bancroft, J.D.: *Bancroft's Theory and Practice of Histological Techniques*. Churchill Livingstone, China (2013).
- [12] Armitage, P., Berry, G. and Matthews, J.N.S.: *Statistical Methods in Medical Research*. Blackwell Science, Oxford (2001).
- [13] Mahmood, I.M.: *Tikrit J. Pure Sci.*, 13(3): 36-39 (2008).
- [14] El-Gerbed, M.S.A.: *J. Appl. Pharmaceu. Sci.*, 3(8): S53-S63 (2013).
- [15] Söderström, K.O.: *Andrologia*, 18(1): 97-103 (1986).
- [16] Johanningsmeier, S.D. and Harris, G.K.: *Annu. Rev. Food Sci. Technol.*, 2: 181-201 (2011).
- [17] Türk, G., Sönmez, M., Aydın, M., Yüce, A., Gür, S., Yüksel, M., Aksu, E.H. and Aksoy, H.: *Clin. Nutr.*, 27(2): 289-296 (2008).