International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-2, 2017, pp806-812
http://www.bipublication.com

Research Article

Investigating the Protective Effects of Pomegranate Seed Oil on Sperm Parameters and Spermatogenesis Quality in Rats Exposed to Lead

Narjeskhaton Dadkhah[1], Mahran Mesgari Abbasi[3,] Mahnaz Shahnazi[*2], Bita Abdollahi[4] and Mohammad Asghari Jafarabadi[5].

[1] Community- Oriented Nursing & Midwifery Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2] Faculty Member of Nursing and Midwifery Department, Tabriz University of Medical Sciences, Tabriz, IR Iran
[3] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[4]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, IR Iran
[5] Faculty Member of Health And Nutrition Department, Tabriz University of Medical Sciences, Tabriz, IR Iran
*Corresponding Author: Mahnaz Shahnazi, Faculty Member of Nursing and Midwifery Department, Tabriz University of Medical Sciences, Tabriz, IR Iran, E-mail:mshahnazi@tbzmed.ac.ir.

ABSTRACT

Objective:Fruits are rich source of antioxidant with significant potentials inneutralizing adverse impacts of lead on sperm parameters.The present study aimed to determine the effects of pomegranate seed oil on sperm parameters and spermatogenesis qualityin adult rats exposed to lead

Methods:This was an experimental study conducted on male Wistar rats(n=45) weighing180 ± 20gr.The animals were randomly divided into 5groups (n=9):distilled water gavage(control);intraperitoneal distilled water; pomegranate seed oil; lead;and lead plus pomegranate seed oil. The animals received 30day treatment and then were sacrificed for the sperm parameters assessments. Sperm count, motility and morphology assessment, chromatins, epididymis and testis weights were performed. The collected data were analyzed byKruskal-Wallis, Wilcoxon, Mann-Whitney U-test,and Chi-square using SPSS (version13)software. Statistical significance was set as$P < 0.05$.

Results: The results showed statistically significant differences on weight gain, epididymis weight, sperm inviability rate, and high sperm motility between the groups($P < 0.05$).The control and PSO groups showed the highest weight gain, whereas the LPSO group showed the lowest values. The L group showed the lowest epididy misweight. The L group showed the lowest sperm motility, whereas the IP control and PSO groups showed the highest values. Testicular weights and normal morphologic and DNA percentages showed no statistical significant differences among the study groups ($P > 0.05$).

discussion:PSO has positive effects on weight gain, epididymis weight,and viable sperm percentage in rats and reducing the lead induced toxic effects on sperm viability.

Conclusion: PSO has positive effect on spermatogenesis and Sperm Parameters.

Keywords: spermatogenesis, lead acetate, antioxidant, pomegranate seed oil

INTRODUCTION

Human is exposed to a variety of harmful environmental pollutants at different stages of their life. In recent years, concerns about the effects of chemical components on male reproductive system have been increased(1).Several factors such as stress, pollution, inadequate intake of vitamins, pesticides, toxins, and chemotherapy can cause

insufficient production of sperm in testicular tissues, infertility, and reduction of sperm concentration by producing free radicals and oxidizing germ cells (2).Sperms are sensitive to Reactive Oxygen Species (ROS) entered through seminal leukocytes that cause in fertility (3). On the other hand, they produce and consume a certain amount of ROS such as $\bar{O}_2$, H_2O_2 to meet their biological needs (4). Toxic metals are able to disrupt testicular biological functions and Lead is one of them (5), which cause hypothalamic-pituitary axis and spermatogenesis disorder (6).Lead exposure may have a negative effect on sperm production through the activation of ROS production. Human spermatozoa are especially susceptible to peroxidative damages through its high levels of unsaturated fatty acids with multiple double bonds ability to generate ROS, mainly superoxide anion and hydrogen peroxide (7).

Antioxidants are biological and biochemical components consist of a group of organic food such as minerals, vitamins, and unsaturated fatty acids that can reduce oxidative damage. Melatonin, N-acetylcysteine, zinc, vitamins A, C, and E, folic acid, myo-inositol, and selenium arethe most frequent antioxidants that are effective on male infertility (8). Researches show that antioxidants and vitamins(B, C, and E), play an effective role in the treatment of male infertility through strengthening the blood-testis barrier, reducingthe destructive effects of free radicals, and protectingand repairing sperm'sDNA (9-11).

Additionally, polyphenols including dense tannins, hydrolysable tannins, and flavonoids which found in pomegranate (12) have powerful antioxidant properties (13). Moreover, pomegranate seed oil is a rich source of 11-trans-linolenic acid (14), sugar, unsaturated fatty acids, vitamins, polysaccharides, and minerals (15). Pomegranate Juice is efficient in reducing oxidative stress, inhibiting protein kinaseP38 pathways activated by mitogen (MAPK-P38), and preventing the activation of transcription NF-KB factor (16). Pomegranate has been used for medicinal purposes for centuries. Today, Pomegranate is known as a tropical and subtropical fruit originating from the Middle East and has been recognized as a rich source of flavonoids, vitamins (A, B, and C), and tannins with antioxidant properties (17).

Pomegranate seed is as a waste product and its oil often consume as food, color additives, or fuel formulation. In recent years, pomegranate seed oil has been considerably applied due to its high concentrations of bioactive hydrophilic and lipophilic components; especially its hydrophilic component consist of a wide range of biological activities, that imposing antioxidant properties and binding affinity to eicosanoid receptors. This product has high potential for nutritional, pharmaceutical, cosmetic, and health uses (18).

Although the effects of pomegranate seed oil are predictable due to its antioxidant properties and acceptable reducing the effects of oxidative stress, thus fruit effectiveness on fertility parameters must be proven at trial laboratories. However databases represent lack of any reports based on simultaneous effects of lead and pomegranate seed oil on spermatogenesis and sperm parameters. Therefore, the present study aimed to determine the protective effects of pomegranate seed oil on spermatogenesis and sperm parameters (sperm count, mobility, and morphology) in mature male rats treated with lead.

MATERIAL & METHODS:

This experimental study was conducted to investigate the protective effects of pomegranate seed oil on sperm parameters and spermatogenesis quality in rats exposed to lead. The study protocol was approved atApplied Pharmaceutical Research Center, Tabriz University of Medical Sciences in 2014, and researcher considered the national law on the care and use of laboratory animals.

A total of 45 male Wistar rats,aging 8-10 weeks and weighing 180 ± 20 gr, were purchased from Tehran Pasteur Institute andused in this study.To accommodate the animals,they were treated in the animal house of Pharmaceutical Research Center, Tabriz University of Medical Sciences for 1 week under 12 hours of light and 12 hours of darkness at 25°C and humidity of 40-70% based on the principles of Laboratory Animal Care. Then the rats were weighed and randomly

divided into 5 groups with the similar average weights.Interventions werecarried out3 days for 4 weeks:

1. **Control group(C1) :**0.5ml of distilled water orally by gavagedaily
2. **Intraperitoneal control group(C2):**0.5 ml of distilled water intraperitoneally daily
3. **Lead acetate (L):**10 mg / kg/body weight (bw) Intraperitoneally
4. **Lead acetate**+ pomegranate seed oil (L + PSO)**:**10 mg / kg/bw intraperitoneally + pomegranate seed oil 100 mg / kg/bw
5. **Pomegranate seed oil (PSO):**100 mg / kg/bwby gavage

To prepare PSO, pomegranate samples were dehydrated and the seeds were dried at ambient temperature (30°C) in shade. Then, they were ground (40 mesh) and their oils were extracted by cold compression method. Twenty-fourhours after the end of intervention, all the animals were weighed and anesthetized with ether and were sacrificed. The irright reproductive organs containing epididymis and testes were removed, weighed, and dissolved in phosphate-buffered saline (PBS).Subsequently, sperm samples were collected from distal part of epididym and examined based on sperm count, motility, and morphology (15).This sperms were released in the medium provided by GibcoHam's F10 Company. The medium osmolarity and pH were set at 285mmoland7.2-7.4 respectively. Sperm motility was examined by placing a drop of the medium on the slide using an optical microscope with a magnification of × 40.Then, a drop of sperm-containing medium was placed on the glass slides foreosin-nigrosin staining (first stained by eosin and then nigrosin).

Another smear of specimens were fixed on the slides by Carnoy's solution and the stained with acridine orange for the evaluation of sperms with normal DNA based on Tejada (17). The percentage of sperms with normal and denaturated DNA was reported in green and red colors respectively(17). The ratio of motile to non-motile sperms (Motility) was expressed in percentage determined by direct microscopic examination. For this purpose, a drop of medium containing-sperm was placed on a 20 × 20 mm glass slide and then a coverslip was placed on it. To assess the sperm condition, the prepared slides were observed under an optical microscope with the object lens of ×40 and the numbers of motile and non-motile sperms were counted. Percentage of the motile sperms was determined by counting 200 spermatozoa per slide. According to the cellular membrane permeability properties of vital sperms, the numbers of live and dead sperms were counted using eosin-nigrosin staining. Eosin does not penetrate in the membrane of living cells before fixation. Therefore, viable cells remained uncolored and the dead sperms were seen in pink color. At least 300 spermatozoa were evaluated per slide and the percentage of live sperm cells was calculated (16). All stages of experiment were blind and after the interventions, laboratory expert was unaware about the study groups. To determine data normality, descriptive indices (skewness, kurtosis) were used. All the data in study have been reported as IQR ± median and frequency. Data were analyzed by Kruskal-Wallis, Wilcoxon, Mann-Whitney U, and*Chi* squaretestsSPSS13 Inc, Chicago software.

RESULTS

Table 1: Weight Gain Frequency and Changes in Body Weights in Study Groups

Group	Weight befor intervention(gr)	Weight after intervention(gr)	weight gain (gr)	P(2)
Control group(IP)	215/00(265/00162/00)	237/50(195/00,262/75)	31/00 (-,45/004/75)	**0/05**
Control group(gavage)	170/50(25/274,146/25)	213/00(196/25,312/25)	42/00(51/50,37/00)	**0/007**
Lead	(245/00,167/50) 210/00	227/00(171/50,278/50)	14/22(-6/50ر29/50)	**0/06**
Pomegranate seed oil+lead	(285/00,196/50) 228/00	219/77(176/50,250/00)	-8/22(-27/50,16/50)	**0/012**
Pomegranate seed oil	(222/00,188/00) 262/00	231/00(216/00,253/25)	20/87(7/00,36/25)	**0/008**
P(1)	0/076	0/589	0/003	

**Based on Kruskal-Wallis test (P1 < 0.05) there was a significant differencebetween the study groups*

The results showed a statistically significant weight gain in the 5 groups (p = 0.003), C1 and PSO groups had better weight gain compared to the other groups(Table 1). Statistically significant differences were found between the weight gains of C1,(L), and (L+ PSO) (P = 0.02, P = 0.02,and P= 0.006 respectively).The statistical results displayed no significant difference between the study groups based on testis weight (p = 0.55), so that the epididymic weights in the group treated with PSO and the C2werehigher than other groups. In comparison pairs of groups using Mann-Whitney U-test, a significant difference was observed between the C2,L (p = 0.017), and (L + PSO) (p = 0.004) (Table 2).

Table 2: Table of testis andepididymic weights frequency in rat groups.

group	testis weights	epididymic weights
Control group(IP)	1/22 (1/17,1/41)	0/53 (0/45,0/59)
Control group(gavage)	1/37 (1/21,1/48)	0/41(0/23 ,0/62)
lead	1/28 (1/13,1/49)	0/32(0/47,0/21)
Pomegranate seed oil+lead	0/98 (0/46,1/39)	0/34 (0/31,0/63)
Pomegranate seed oil	1/34 (1/28,1/363)	0/49(0/31,0/63)
(P)	0/55	0/006

P < 0.05 was considered significant between the study groups based on Kruskal-Wallis test.

The results of thestudy indicated that the high sperm motility (equal and more than 50%)was in C2and PSO groups compared to the other groups (P < 0.001).In comparing the pair groups,astatistically significant difference were found between the C2 and (L+ PSO) (P= 0.009 and P = 0.008 respectively) (Table 3).

Table 3: Spermatography within the study groups

Group variable	Control group(IP)	Control (group(gavage	lead	Pomegranate seed oil+lead	Pomegranate seed oil	(P)
sperm motility of 50%	9(100)	7(77/6)	3(33/2)	8(88/8)	9(100)	**p<0/001**

According to the finding of the study, there were no significant differences between percentages of normal morphology and normal DNA (P = 0.47 and P = 0.24respectively) (Table 4).

Table 4: Frequency of sperm viability, abnormal morphology, and DNA variations within the study groups

Group	percentages of non-viable sperm	percentages of normal morphology	percentages of normal DNA
Control group(IP)	10/00(16/00, 10/00)	100/00(,100/0099/00)	12/00(10/00,19/00)
Control group(gavage)	10/00 (5/50,10/00)	100/00(,100/0099/00)	48/00(3/50,50/00)
Lead	50/00 (12/50,93/50)	99/00(98/50,100/00)	25/00(13/00,45/00)
Pomegranate seed oil+lead	10/00(5/00,10/00)	99/33(,100/0098/50)	12/00(1/50,20/00)
Pomegranate seed oil	10/00(,19/506/00)	99/75 (,100/00100/00)	26/00(2/75,51/00)
(P)	**0/003**	**0/47**	**0/24**

P < 0.05 was considered significant between the study groups based on Kruskal-Wallis test.

The non-viable sperms percentage had significantly differences among study groups (p = 0.003); the maximum number of dead sperms was found in L, moreover, there was a significant difference between the C1 and C2 and (L + PSO) groups (p = 0.002).

DISCUSSION:

Nowadays, herbal compounds are mostly used in the forms of plant or fruit extracts containing a complex mixture of different materials, while in most cases, it is not clear which combination has beneficial effects (19), however, the evidence shows that most of them reinforce each other's biological effects (20).In the West, health-promoting effects of plants are being increasingly studied (21, 22). Due to the presence of several useful components in pomegranate fruit, there is a growing interest for its use in preventive medicine.

For example, pomegranate is rich in ellagic acid which has toxic effect on cancer cells (22) and is effective on treatment of certain cancers such as colon (Seeram et al.2005) and prostate cancers (23, 24). In addition, this fruit has strong antioxidant properties attributed to polyphenols, anthocyanins, etc. Pomegranate polyphenols include flavonoids (flavonols, anthocyanins, etc.), concentrated tannins (pro-anthocyanidins), and tannins (e.g. hydrolysable ellagitannins andgallotannins), whichall of them are capable to deactivating the products of oxidative catabolism. Also, PSO contains vitamin E, ellagic acid, sterols, fatty acids, and conjugated fatty acids such as punicic acid (25).

The present study assessed the effects of PSO on spermatogenesis quality and sperm parameters in rats exposed to lead. The results showed the sperms motility rates in the C2 and PSO differed significantly from Lgroup. Present study was also consistent with the study conducted by Al-attare et al,. revealing exposure to high doses of lead can cause decrease sperm motility (26). Furthermore, (L+ PSO) displayed more than twice the mobility rate compared toL group. The results showed intraperitoneal injection of 10 mg / kg / bw lead acetate for 30 days were increased the percentage of non-viable sperms in L group, while(L+ PSO) group showed lower percentage of non-viable sperms than lead alone. The study of Paulo and Liwa et al,.(2011)confirmed the reduction of sperm counts after intraperitoneal injection of lead acetate 8 mg / kg / mouse for 35 days (21). Other studies indicate that multiple factors affect spermatozoa viability, sperm motility percentage, and fertility rate (27- 30).

Sperm sensitivity to oxidative damage and its high ability to produce ROS (31) and environmental or physiological factors may be activating this pathway and decreasing motility and viability of sperms. The positive effects of PSO on the percentage of viable sperms in lead-exposed rats were witnessed due to the presence of flavonoids and polyphenols (12, 13),impose their antioxidant effects probably by reducing or removing oxidative stress or damages(32, 33).The minimum and maximum epididym weights were seen in the L group and PSO group respectively. Epididym weight loss might be due to the decreased testosterone levels reducing sperm counts, in turn (34).Furthermore, lower weights of reproductive organs in the L group can be associated with a decrease in germ cells and spermatids (35).

The evidence of this study suggests that . consumption of pomegranate seed oil may reduce the adverse effects of oxidative stress on sperm parameters such as epididymic weight, percentage of viable sperm, and sperm motility and considerably decrease the toxic effects of lead on sperms caused by lead acetate. However, no positive effects of pomegranate seed oil on other fertility parameters were seen in present research. Thus, in future studies, it is recommended that preventive effects of other dosages of this oil be investigated in longer terms.

REFERENCES:

1. Bustos-Obregón E. Adverse effects of exposure to agropesticides on male reproduction. APMIS Denmark. 2011.709: 233-242.,
2. Amin A, Hamza AA. Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. Asian J Androl. 2006;8(5): 607-12.
3. Imai H, Suzuki K, Ishizaka K, Ichinose S, Oshima H, Okayasu I.Failure of the expression of phospholipid hydroperoxide glutathione peroxidase in the spermatozoa of human infertile males. J Biol Reprod.200; 64: 674- 683. :
4. Baker M.A, Aitken R.J.The importance of redox regulated pathways in sperm cell Endocrinol. Mol Cell Endocrinol. 2004; 216(1-2):47-54.
5. 5.Winder C. Reproductive and chromosomal effect of occupational exposure to lead on the male. Reprod Toxicol.1989;3: 221-233.
6. Sokol RZ. Hormonal effects of lead acetate in the male rat. Biol Reprod. 1987; 37; 1135-38.
7. Reglero MM, Taggart MA, Castellanos P, Mateo R. Reduced sperm quality in relation to oxidative stress in red deer from a lead

mining area. Environ Pollut. 2009;157(8-9):2209-15.
8. ShowellMG, BrownJ, ClarkeJ, Hart RJ. Antioxidants for female subfertility (Review). 2013.
9. Mosher WD, Pratt WF. Fecundity and infertility inthe United States: incidence and trends. J FertilSteril. 1991;56(2): 192-3
10. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge University Press, New York.1999.
11. Brooks DE. Activity of androgenic control of glycolytic enzymes in the epididymal spermatozoa of the rats. J Biochem. 1976;156 (3): 527–37.
12. Rosenblat M, Hayek T & Aviram M . Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. Atherosclerosis.2006;187(2):363-371.
13. Gil MI, Tomas- Barberan FA, Hess-Pierce B, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem. 2000; 48(10): 4581-4589.
14. Schubert S.Y, Lansky E.P, Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. J Ethnopharmacol.1999;66(1):11-7.
15. Kohno H, Suzuki R, Yasui Y. Pomegranate seed oil rich in conjugated linoleic acid suppresses chemically induced colon carcinogenesis in rats. Cancer Sci.2004;95(6): 481–486.
16. Afaq F, Saleem M, Krueger CG, Reed JD, Mukhtar H. Anthocyaninandhydrolyzable tannin-rich pomegranate fruit extractmodulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice. Int J Cancer. 2005;113 (3):423-433.
17. Hayden MS, Ghosh S. Siganling to NF-κB. Genes Develop.2004; 18(18):2195-2224.
18. Wang Z, Pan Z, Mal H, Griffiths G. Extract of phenolics from pomegranate peels. *Open Food* Sci. J. 2011;5:17–25.
19. Straccia M.C, Siano F, Coppola R, laCara F, Volpe M.G. Extraction and characterization ofvegetable oils from cherry seed by different extractionprocesses. *C*hem. Eng Trans. 2012;27(3): 91–96.
20. Leiva K, Rubio J, Peralta F. Effect of Punica granatum (pomegranate) on sperm production in male rats treated with lead acetate. Toxicology Mechanisms and Methods. 2011; 21(6): 495-502.
21. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006; 71:1397-1421.,
22. Schafer A, Chovanova Z, Muchova J, Sumegova K, Liptakova A, Durackova Z, Hogger P. Inhibition of COX-1 and COX-2 activity by plasma of human volunteers after ingestion of French maritime pine bark extract (Pycnogenol). Biomed Pharmacother. 2005;60(1):5-9.
23. Rasheed Z, Akhtar N, Anbazhagan N.A, Ramamurthy S, Shukla M, HaqqiT.M. Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI induced expression of pro-inflammatory cytokines by inhibiting the activation of MAP Kinases and NF-κB in human KU812 cells. *J*Inflam. 2009; 6 (1): 1476-1489.
24. Fadavi A, Barzegar M, Azizi M.H. Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. J. Food Compos. Anal. 2006;19(6-7): 676–680.
25. Albrecht M, Jiang W, Kumi- Diaka J, Lansky E.P, Gommersall L.M. Pomegranate extracts potently suppress proliferation Xenograft growth and invasion of human prostate cancer cells. JMed Food. 2004; 7(3): 274–283
26. Schubert S.Y, Lansky E.P, Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J.* Ethnopharmacol.1999; 66(1): 11–17.
27. Al-Attar AM. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuriesin male mice.

Saudi Journal of Biological. Saudi J Biol Sci.2011;18(1):63-72.
28. Jalili A, Jamzad Z. Red data book of plant species of iran. Research institute of forests and rangelands. Iran. 1999; pp: 663.
29. Murray MT. The healing power of herbs Rocklin. California, Prima Publishing. 1995; pp: 173-83.
30. Garg S, Doncel G, Chabra S, Upadhyay SN, Talwar GP. Synergistic spermicidal activity of neem seed extract, reetha saponins and quinine hydrochloride. Contraception. 1994;50(2):185-90.
31. Leiva K, Rubio J, Peralta F, Gustavo F. Effect of Punica granatum (pomegranate) on sperm production in male rats treated with lead acetate. Toxicology Mechanisms and Methods. 2011;21(6): 495-502.
32. Anjum MR, Sainath SB, Suneetha Y, Reddy PS. Lead acetate inducedreproductive and paternal mediated developmental toxicity in rats. Ecotoxicol Environ Saf. 2011;74(4): 793-9.
33. ElfallehW,Tlili N, Nasri N, Yahia Y,Hannachi H,Chaira N, Ying M, Ferchichi A. Antioxidant capacities of phenolic compounds and tocopherols from tunisian pomegranate (Punica granatum) fruits. JFood Sci.2011;76 (5), 707–713.
34. Schubert, S.Y; Lansky E.P.; Neeman, I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J.* Ethnopharmacol.1999;66(1): 11–17.
35. Chapin R, Harris M, Davis B, Ward S, Wilson R. The effects of perinatal juvenile methoxychlor exposure on adult rat nervous, immune and reproductive system function. Fundam Appl Toxicol.1997;40(1):138-157.