



Antioxidant and Anti Infertility Potency of Pomegranate (*Punica granatum* L.) in γ Irradiated Rats

Gh.A. El-Tawill, N.M. El-Fatih, S.A. Montaser[#], M.M. Ahmed

Radiation Biology Department, National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, P. O. Box; 29 Nasr City, Egypt.



THIS study aims at examining the radiation protection activity of pomegranate extract against oxidative stress, lipid profile escalation (T. cholesterol and Triglycerides) and infertility (sex hormone profile and sperm abnormalities) progressed in male rats after ionizing irradiation. Rats were divided into four groups, six adult male rats each as follows: 1) control group, 2) pomegranate group treated with a concentration of 22mg pome extract/kg of animal body weight (day after day for two weeks), 3) irradiated group exposed to 5 Gy single dose, and 4) pomegranate and irradiated group treated with the same previous concentration of pomegranate extract before being exposed to a 5 Gy single dose of gamma rays. Measurements in irradiated group recorded disturbances in oxidative stress markers manifested by significant increases in the content of the lipid peroxidation product malondialdehyde (MDA) accompanied by decreases in both glutathione (GSH) contents and superoxide dismutase (SOD) activities. Lipids were also affected by irradiation where significant elevations for both cholesterol and triglycerides were observed. The data obtained for sex hormones levels recorded a significant decline in testosterone level. This decline was associated with abnormalities in sperms (abnormalities of the head, tail and both head & tail). However, the level of FSH and LH recorded significant elevations. Furthermore, in the group of rats that received pome extract before exposure to radiation, the results revealed that pomegranate appear to have a strong ability to cause a significant guard for sex hormones, preventing sperm abnormalities and adjustment of oxidative stress markers & lipid profile.

Keywords: Antioxidant, Infertility, Ionizing irradiation, Pomegranate.

Introduction

The exposure of organisms to radiation hazards causes formation and accumulation of reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$) consequently. These are the main cause of the irreversible oxidative damages to biological macromolecules, such as lipids, proteins, and DNA in the cells (Phaniendra et al., 2015).

Many studies dealt with investigating the side effects of radiation exposure in the course of both radiotherapy and occupational exposure,

especially its effects on somatic and germ cells.

The estimation of antioxidant levels and infertility factors provide a good guide for health hazards after irradiation. Accordingly, many researches and attempts have been executed to dispose these deleterious effects using natural sources of radiation protectors (Hassan et al., 2015; Abdallah et al., 2019).

In general, antioxidants are compounds, which can weed out, scavenge, and suppress the formation of ROS and reduce lipid peroxidation. The biological antioxidants, such as GSH, and glutathione peroxidase (GSH-Px), catalase (CAT)

[#]Corresponding author email: sherien_morsy@yahoo.com

Received 6/12/2020; Accepted 30/3/2021

DOI: 10.21608/ejrsa.2021.52462.1109

©2020 National Information and Documentation Center (NIDOC)

and SOD enzymes, have a significant role as suppressors or scavengers of free radicals (Birben et al., 2012).

Vidal et al. (2003) and Mirdehghan & Rahemi (2007) stated that the pomegranate fruit cultivated for centuries is rich in natural antioxidants and possess high nutritional and therapeutic values which attenuate oxidative stress. Li et al. (2006) and Matthaïou et al. (2014) attributed the biological and therapeutic properties of pome extract to the presence of polyphenols (ellagitannins, flavonoids, phenolic acids, stilbenes, tannins and anthocyanins), minerals and vitamins which are effective free radical scavenging compounds. In addition, Adams et al. (2010) found that ellagic acid in pomegranates inhibits aromatase, the enzyme responsible for converting androgen to estrogen.

The moderate expenditure of pomegranate fruits reduces the susceptibility to free radical lipid peroxidation as well as increases resistance to LDL and high-density lipoprotein (HDL)-cholesterol oxidation (Aviram et al., 2002). Furthermore, pome supplementation displays inhibition of lipid peroxidation in type II diabetics and healthy individuals (Ahmed et al., 2015).

Oxidative stress has been identified as an important factor that influences male fertility status (Ilacqua et al., 2018).

Acute stress might impair testicular function; testicular tissue of stressed rats showed higher levels of cortisol and displayed apoptosis for both germ cells and Leydig cells (Chen et al., 2012). However, chronic stress might be determined by the presence of glucocorticoid receptors in Leydig (Maeda et al., 2015), Sertoli (Hazra et al., 2014) and germ cells (Yazawa et al., 2000). Many researches indicated that radiotherapy (46–50 Gy) has a significant impact on testicular function. Significant changes in testosterone levels were observed after a testicular dose of only 1.5–5.5 Gy. Radiation treatment of prostate cancer patients with a testicular exposure to less than 10 Gy resulted in increased FSH and LH levels in serum (Dueland et al., 2003).

Most of male infertility (abnormal sperms) causes are attributed to physical or local damage of Leydig and seminiferous tubules, while sterility is almost hormonal. That to say, the

hormonal production that takes place in the Leydig cells of the testicles is more resistant to radiation therapy than the sperm production. Giwerzman et al. (1991) confirmed that testicular carcinoma patients treated with radiation therapy (20 Gy) exerts insignificant changes in the serum testosterone levels, suggesting that the production of male sex hormones is relatively resistant to irradiation.

Rao et al. (2016) demonstrated that treatment of mice with pome juice for several days could prevent oxidative stress induced damage in testes. Moreover, other studies revealed that the usage of pome extract ameliorates the testicular damage and reduce infertility level (Sherif et al., 2013).

Based on all above, the present study comprises several experimental measurements in order to verify the study goal. Determination of oxidative stress markers (MDA and GSH concentrations and SOD activity), assessment of lipid profile (T. Cholesterol and total triglycerides) and sex hormone levels (testosterone, FSH and LH) in addition to monitoring sperm abnormalities were done in the 4 rat groups encompassed in this work to evaluate the antioxidant, anti-infertility capabilities of pomegranate extract against the deleterious effects of radiation exposure.

Materials and Methods

Experimental animals

Adult male Swiss Albino rats (weighing 150 ± 2.5 g) were obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA); Cairo, Egypt. All animals were allowed one week to acclimatize in animal housing conditions before being used for the study. The rats were housed in plastic cages (6 rats in each) under standard laboratory conditions that include all hygienic measures with constant illumination and ventilation, temperature and humidity. The experiment was conducted at the National Center for Radiation Research and Technology (NCRRT), the Egyptian Atomic Energy Authority, Egypt. The animals were fed standard diet and provided with water *ad libitum*.

Pomegranate (Pome) extract

Pome extract was obtained from Meijer Distribution, INC. as antioxidant support (Herbal supplement) from USA. 250 mg/tablet.

Irradiation facility

A whole-body gamma-irradiation process was performed with a Canadian Gamma Cell, Caesium-137 (¹³⁷Cs) unit at the NCRRT, Egyptian Atomic Energy Authority, Cairo, Egypt. Animals were exposed to a single dose level of 5Gy γ -rays with a dose rate of 0.4Gy/min at the time of experiment.

Experimental design

Adult male albino rats were randomly categorized into four equal groups (n= 6). These groups were classified as: Control group: rats were neither irradiated nor treated. Pome group: group of rats received *pome G.* extract administrated orally (22.5 mg / kg b. wt.) dissolved in 0.5 ml of distilled water for the first fourteen days of the experiment. This dose was chosen according to Turk et al. (2008). Irradiated group (Irrad. group): rats were exposed to a single dose (5 Gy) of whole body γ -radiation with a dose rate of 0.4 Gy/min at day fourteen of the experiment. Pome and Irradiated group (Pome and Irrad. group): rats received Pome extract for fourteen days before being irradiated.

Oxidative stress markers measurements: (MDA, SOD and GSH)

The extent of lipid peroxidation was assayed according to Ohkawa et al. (1979). The method is based on the determination of MDA, an end product of lipid peroxidation, which reacts with thiobarbituric acid in acidic medium to yield a pink-colored trimethine complex exhibiting maximum absorption at 532 nm. GSH content, in blood, was measured using spectrophotometry at 412 nm, based on the development of yellow coloration when 5,5 -dithiol-bis (2-nitrobenzoic acid) is added to sulfhydryl compounds according to Beutler et al. (1963). SOD activity determination was carried out according to Minami & Yoshikawa (1979). Xanthine-xanthine oxidase was used to generate O₂•- and nitroblue tetrazolium (NBT) decline was used as an indicator of O₂•- production. SOD activity was measured using spectrophotometry at 540 nm.

Lipids assay (cholesterol and triglycerides)

Total cholesterol content in blood serum was determined according to Allain et al. (1974). Triglycerides were measured according to Fossati & Principe (1982).

Hormones assay (Testosterone, FSH and LH)

The plasma testosterone, FSH and LH levels

were measured by the ELISA method using a DRG Elisa kits.

Sperm abnormalities

Epididymides of each rat were minced finely with small scissors in 3 ml phosphate buffers pH (7.8). Evaluation of sperm head, tail and head and tail abnormalities and amorphism were made according to the criteria of Wyrobek et al. (1984). Smears were prepared on clean dry slides, which stained in 1% Eosin-Y (1 gm Eosin Y dissolved in 100 ml distilled water). Sperm smears were examined at 40X magnification by a light microscopy. For each dose, about 1000 sperms were assessed for morphological abnormalities of the sperm shape.

Mutation factor and mutation index were calculated as follows: Mutation factor= frequency of abnormal sperm head, tail and head and tail (treated)/ frequency of abnormal sperm head, tail and head and tail (control). Mutation index= frequency of abnormal sperm head, tail and head and tail (treated- control)/ frequency of abnormal sperm head, tail and head and tail (control) according to Ekaluo et al. (2008).

Statistical analysis

Results were expressed as Mean \pm S.E. Data were subjected to one-way analysis of variance (ANOVA- test) to determine the statistical significance of the differences according to Snedecor & Cochran (1989). Differences were considered statistically significant at P< 0.05.

Results

Table 1 displays that the recorded increase in lipidperoxidation (high MDA levels) of the irradiated rats was more than two folds over the level in control group and pome group, while a significant decrease was observed in the group of rats treated with pome extract before irradiation compared to the irradiated group.

Pome extract group showed a significant enhancement in both antioxidant enzyme SOD and GSH content when compared with the control group. However, SOD & GSH content showed its drastic depletion in the irradiated group when compared with the control and pome treated groups. On the other hand, in Pome and irradiated groups, a significant improvement was recorded in SOD and GSH values when

compared with the irradiated group.

Table 2 shows that, administration of Pome extract was competent to reduce total cholesterol levels and triglycerides significantly in plasma when compared with the control group.

Lipids were also affected by ionizing radiation exposure and the recorded increase was 2.7 folds in cholesterol & 2.8 folds in triglycerides over their values in control group. However, pome extract treated groups recorded a significant improvement in their levels when compared with the irradiated group.

Table 3 presents an insignificant difference between the control and Pome group for hormones levels (FSH, LH and Testosterone). However, in the irradiated group the level of FSH and LH recorded significant increases accompanied by significant decline in testosterone level. Administration of Pome extract ameliorated the effects of radiation exposure on FSH, LH and Testosterone levels when compared with the irradiated group.

Table 4 reveals that the administration of Pome extract before radiation exposure leads to reduction in the values of mutation factor and mutation indices. A significant decrease in the percentage of abnormalities were recorded in Pome and the irradiated group as compared to the irradiated group despite the fact that its average

number did not reach the control values.

Discussion

The inevitable usage of radiation therapy to cure a wide range of malignancies or even the occupational exposure to different levels of ionizing radiation resulted in many undesirable side effects. In many cases, these bad effects are related to the increased of oxidative burdens in tissue and organs subjected to radiation exposure which is confirmed in various studies. Targeting the amendment of oxidative unbalances by different means could be a good achievement to control different biological issues evolved after exposure to ionizing radiation.

This might take place by consuming nontoxic and safe natural antioxidants found in many edible fruits. This study is interested in pomegranate that represents a phytochemical reservoir of heuristic medicinal value and its juice contains a diverse array of phytochemicals, including polyphenolic constituents (anthocyanins), hydrolyzable tannins (ellagitannins and gallotannins) and condensed tannins (proanthocyanidins) (Lansky & Newman, 2007). Faria & Calhau (2011) described that some of these compounds might exhibit potent antioxidant and anti-inflammatory properties that prevent and interfere several inflammation-driven diseases, including cancer.

TABLE 1. Effect of Pome extract on MDA, SOD and GSH levels in blood of irradiated and treated rats

Groups	MDA (U/ml) (mean ± S.E)	SOD (U/ml) (mean ± S.E)	GSH (mg/dl) (mean ± S.E)
Control group	20.22±0.48	96.45±1.02	42.92±0.62
Pome. group	19.68±0.57	100.33±1.33 ^(a)	45.47±0.60 ^(a)
Irrad. group	46.53±0.89 ^(a,b)	59.33±0.41 ^(a,b)	23.97±0.56 ^(a,b)
Pome and Irrad group	39.68±0.82 ^(a,b,c)	74.33±2.26 ^(a,b,c)	32.97±0.77 ^(a,b,c)

a: Significant with control

b: Significant with Pome

c: Significant with irradiated group.

TABLE 2. Effect of Pome extract on serum cholesterol and triglycerides levels (mg/dl) in irradiated and treated rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control group	103.34±1.44	66.4±2.01
Pome. group	98.15±1.05 ^(a)	61.28±2.11 ^(a)
Irrad. group	284.83±1.16 ^(a,b)	185±0.52 ^(a,b)
Pome and Irrad group	220.67±1.11 ^(a,b,c)	143.83±1.92 ^(a,b,c)

Legends as in Table 1

TABLE 3. Effect of Pome extract on FSH, LH and testosterone levels in blood of irradiated and treated rats

Groups	FSH (ng/ml) (mean ± S.E)	LH (ng/ml) (mean ± S.E)	Testosterone (ng/ml) (mean ± S.E)
Control group	6.58±0.151	0.72±0.049	5.50±0.19
Pome. group	6.60±0.163	0.75±0.04	5.72±0.16
Irrad. group	8.78±0.23 ^(a,b)	0.78±0.03 ^(a)	2.60±0.13 ^(a,b)
Pome and Irrad group	7.73±0.26 ^(a,b,c)	0.72±0.05 ^(b,c)	3.03±0.07 ^(a,b,c)

Legends as in Table 1

TABLE 4. Mutation factors and indices of sperm morphology abnormalities in irradiated and treated rats (1000 sperm/ rat)

Groups		Control group	Pome group	Irrad. group	Pome + irradi. group
	%	0.80	0.76	13.70	10.40
Head abnormalities	Mutation factor	1.00	0.93	16.85	12.78
	Mutation index	0.00	0.07	15.85	11.73
	%	0.81	0.80	15.00	13.10
Tail abnormalities	Mutation factor	1.00	0.98	18.40	16.10
	Mutation index	0.00	0.02	17.42	15.10
	%	0.16	0.20	2.50	2.10
H & T abnormalities	Mutation factor	1.00	1.20	15.30	13.10
	Mutation index	0.00	0.20	14.10	12.10
	%	1.80	1.76	31.37	16.56
Total abnormalities	Mutation factor	1.00	0.98	17.40	9.20
	Mutation index	0.00	0.02	16.42	8.20

Legends as in Table 1

In the present study, increases in the level of MDA parallel with decreases in the concentration of the antioxidant molecule (GSH) and decline in the SOD activity were recorded (Table 1). This could suggest that it is a concern of redox tone twists in the tissues of the irradiated rats. Gao et al. (2018) reported that radiation could induce different biological effects. Radiolysis inside the cells increases the release of ROS, especially hydroxyl radicals which attack and damage DNA and also responsible for other radiation-induced injuries (Chen & Kuo, 2017).

MDA increases observed after exposure to gamma radiation might be attributed to the increment of ROS formation, which attack the cellular and membranous lipid structures. Kurata et al. (1993) stated that increased ROS react with polyunsaturated fatty acids and release toxic and reactive aldehyde metabolites, which are the end products of lipid peroxidation process such as MDA. In the same context, the antioxidants respond to the damage effects of oxidation and

terminate oxidative chain reactions by removing free radical intermediates, and inhibiting other oxidation reactions by donating electrons to the free radicals and becoming oxidized by themselves (Aprioku, 2013).

Among the antioxidants, the non-enzymatic antioxidant molecules (reduced glutathione; GSH) which is a water-soluble tripeptide thiol found in the cell cytosol and other aqueous phases (Mannervik, 1987) contain thiol group which readily interacts with free radicals, especially the ·OH, by donating a hydrogen atom (Pastore et al., 2003). Also, the enzymatic antioxidant SOD which represents a family of metalloenzymes was found in every oxygen-based organism function to catalyze the body's primary antioxidant defense reaction (dismutation of O₂⁻ to H₂O₂) (Zelko et al., 2002; Nozik-Grayck & Suliman, 2005).

Therefore, the decreases observed in non-enzymatic antioxidant constituents (GSH) and enzymatic antioxidant activities (SOD) are related

directly to excessive ROS production where, the GSH contents are consumed in the process of ROS and free radicals' neutralization while, SOD works as a possible target to ROS and free radicals' outbreak. Accordingly, the failure of these antioxidant defense mechanisms in controlling the over production of ROS evokes oxidative stress status which brought injuring of membrane lipids, proteins and nucleic acids (Ahmed, 2005). Ahmed et al. (2018) indicated that the inactivation of SOD enzyme, is possibly due to increased superoxide radical production out of its capacity to dispense with or inhibition of enzyme active protein by the plentiful H_2O_2 .

In contrary, rats received oral supplements of pomegranate extract before exposure to gamma radiation showed a significant decrease in MDA concentration and a significant amelioration in the antioxidant activities (GSH and SOD) as compared to the irradiated rats (Table 1). This could be due to the antioxidant activities of pome extract phenolic compounds, which are found in excess. These phenolic antioxidant compounds might decrease the consumption of natural antioxidant enzymes and molecules and supporting them during their effort to detoxify radiation-generated free radicals via screening this antioxidant from being attacked by free radicals (Krishna & Kumar, 2005).

In addition, The obtained data pointed out to significant elevation in plasma cholesterol and triglycerides in the irradiated rats as compared to the control rats. Conversely, the administration of Pome extract reduces the total cholesterol levels and triglycerides significantly when compared with the irradiated rats (Table, 2). These results could be interpreted in the view that Pome juice has the ability to control hyperlipidemia recorded after radiation exposure. Taheri et al. (2017) and Les et al. (2018) reported the inhibitory effect of Pome extract on lipid profile and triglycerides. Moreover, Esmailzadeh et al. (2006) and Esmaeilinezhad et al. (2018) proved that pome juice consumption modifies heart disease risk factors in hyperlipidemia cases. The consumption of concentrated Pome juice significantly reduces total cholesterol, low-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol /high-density lipoprotein-cholesterol and total cholesterol/ high-density lipoprotein-cholesterol.

Furthermore, the rise of the oxidative stress

and the emerging of hyperlipidemia after radiation exposure are accompanied with hormonal alteration evident by a significant decrease in testosterone concentration of the irradiated rats whereas, FSH and LH levels were more resistant (Table 3). In addition, sperms deformity was observed in head, tail, and head & tail parallel to the hormonal disturbances (Table 4).

After radiation exposure, the oxidative stress bangs all body organs in a relative manner. Male testis and germinal epithelium including spermatogonia are radiosensitive organs, which are more susceptible to radiation exposure than other organs. Moreover, testosterone is the most sensitive male sex hormone affected by gamma radiation exposure (Xu et al., 2008). The impairment of normal spermatogenesis and sperm deformation are the most common causes of male factor infertility (Brugh et al., 2003). The oxidative stress raised in male reproductive system post radiation exposure induced sperm abnormalities and hormonal disturbances (Agarwal et al., 2005; Sikka, 2001). Turk et al. (2008) stated that ionizing radiation produces free radicals causing oxidative breakdown of polyunsaturated fatty acids of cell membrane. Polyunsaturated fatty acids found in high concentrations in spermatozoa are responsible for regulation of sperm maturation, spermatogenesis, capacitating, acrosome reaction and eventually in membrane fusion, and low antioxidant capacity. Thus, spermatozoa are especially susceptible to peroxidative damage because of these high fatty acid contents. Furthermore, peroxidation of sperm lipids, which destroys the structure of the lipid matrix in the membranes of spermatozoa 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.

Additionally, significant improvement of both sex hormones levels and sperm abnormalities were observed in group received pome extract before irradiation when compared to irradiated rats (Tables 3 and 4). This observation can be discussed in the light of the remarkable amelioration occurrence for antioxidants, (the GSH concentration and SOD activity) as well as the reduction of MDA level (Table 1). This improvement causes substantial adjustment of cellular redox tone, leading to the regulation of

oxidative stress and finally protect sperms and the hormonal production process from being damaged by ionizing radiation. Turk et al. (2008) indicated that the enhancement of the biological antioxidants, GSH, glutathione peroxidase (GSH-Px), catalase (CAT) and SOD have a significant role in reducing complications of ROS scavengers which likely improve the sperm function (Turk et al., 2008).

Moreover, DNA damage of the sperm causes the presence of low quality sperm driving to fertilization problems as spermatozoa requires integral DNA during fertilization (Duran et al., 2002; Meseguer et al., 2008). Because DNA is a favourite target of free radicals released after radiation exposure, the sperms are largely influenced and hereafter sperm deformities are severely existing. The phenolic compounds found in pome extract such as ellagic acid and punicalagin can armour the DNA molecules from mutagens including free radicals and ROS. Accordingly, pome extract could be able to prevent sperm deformity (Zahin et al., 2014).

Conclusion

According to the obtained results, it could be suggested that Pome extract rich in phenolic compounds has considerable radiation protection effects against radiation-induced oxidation deleterious effects, male sex hormone disturbances and sperm abnormalities (infertility). As a recommendation, the presence of Pome fruit and juice in daily nutritional regimen could be beneficial to improve health and to upgrade the quality of life.

Ethical consideration: All procedures that involved animals and their care including feeding, extract administration, blood sampling, gastric intubation, anesthesia, and euthanasia were taken under the close supervision of the Research Ethics Committee for the National Center for Radiation Research & Technology (REC-NCRRT), Egyptian Atomic Energy Authority. Serial number: 13A/20

Conflict of interest: The authors declare that there is no conflict of interest.

Funding sources: The present research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

- Abdallah, I.Z.A., Ahmed, M.M., Montaser, S.A., Hafez, S.S. (2019) Efficiency of Kumquat fruit (*Fortunella margarita*) extract against hepatotoxicity and infertility induced by Gamma irradiation in male albino rats. *Egypt. J. Rad. Sci. Applic.* **32**(2), 175-187.
- Adams, L.S., Zhang, Y., Seeram, N.P., Heber, D., Chen, S. (2010) Pomegranate ellagitannin-derived compounds exhibit anti-proliferative and anti-aromatase activity in breast cancer cells. *In Vitro Cancer Prev. Res. (Phila)*, **3**(1), 108-113.
- Agarwal, A., Prabakaran, S.A., Said, T.M. (2005) Prevention of oxidative stress injury to sperm. *J. Androl.* **26**(6), 654-60.
- Ahmed, R.G. (2005) Is there a balance between oxidative stress and antioxidant defense system during development? *Med. J. Islam World Acad. Sci.* **15**(2), 55-63.
- Ahmed, M.M., Said, Z.S., Montaser, S.A., El-Tawil, G.A. (2018) Antioxidant and antimutagenic properties of calcium sennasoides in γ -irradiated human blood culture. *Int.J. Radiat. Res.* **16**(3), 323-332.
- Ahmed, S.T., Islam, M., Bostami, A.B.M.R., Mun, H., Kim, Y., Yang, C. (2015) Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by products. *Food Chemistry*, **188**, 481-488.
- Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., Fu, P.C. (1974) Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**, 470-475.
- Aprioku, J.S. (2013) Pharmacology of free radicals and the impact of reactive oxygen species on the testis. *J. Reprod. Infertil.* **14**(4), 158-172.
- Aviram, M., Dornfeld, L., Kaplan, M., Coleman, R., Gaitini, D., Nitecki, S., Hofman, A., Rosenblat, M., Volkova, N., Presser, D., et al. (2002) Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs Exp. Clin. Res.* **28**, 49-62.
- Beutler, E., Duron, O., Kelly, B. (1963) Improved *Egypt. J. Rad. Sci. Applic.* **33**, No.2 (2020)

- method for determination of blood glutathione. *J. Lab. Clin. Med.* **61**, 882–888.
- Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O. (2012) Oxidative stress and antioxidant defense. *World Allergy Organ J.* **5**(1), 9–19.
- Brugh, V.M., Matschke, H.M., Lipshultz, L.I. (2003) Male factor infertility. *Endocrinol. Metab. Clin. North. Am.* **32**(3), 689-707.
- Chen, H.H., Kuo, M.T. (2017) Improving radiotherapy in cancer treatment: Promises and challenges. *Oncotarget*, **8**, 62742–62758.
- Chen, Y., Wang, Q., Wang, F.F., Gao, H.B., Zhang, P. (2012) Stress induces glucocorticoid-mediated apoptosis of rat Leydig cells *in vivo*. *Stress*, **15**, 74–84.
- Dueland, S., Guren, M., Olsen, D., Poulsen, J., Tveit, K. (2003) Radiation therapy induced changes in male sex hormone levels in rectal cancer patients. *Radiotherapy and Oncology*, **68**, 249–253.
- Duran, E.H., Morshedi, M., Taylor, S., Oehninger, S. (2002) Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. *Hum Reprod.* **17**(12), 3122-8.
- Ekaluo, U.B., Ikpeme, E., Udokpoh, A. (2008) Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. *The Internet Journal of Toxicology*, **7**(1), 1-5.
- Esmailzadeh, A., Tahbaz, F., Gaieni, I., Alavi-Majd, H., Azadbakht, L. (2006) Cholesterol-lowering effect of concentrated pomegranate juice consumption in type II diabetic patients with hyperlipidemia. *Int. J. Vitam. Nutr. Res.* **76**(3), 147-51.
- Esmailinezhad, Z., Babajafari, S., Sohrabi, Z., Eskandari, M.H., Amooee, S., Barati-Boldaji, R. (2018) Effect of synbiotic pomegranate juice on glycemic, sex hormone profile and anthropometric indices in PCOS: A randomized, triple blind, controlled trial. *Nutr. Metab. Cardiovasc. Dis.* **2**, 201–208.
- Faria, A., Calhau, C. (2011) The bioactivity of pomegranate: impact on health and disease. *Crit. Rev. Food Sci. Nutr.* **51**, 626–34.
- Fossati, P., Principe, L. (1982) Serum triglycerides determined calorimetrically fragmentation in lymphocyte. *J. Immunol.* **139**, 3199-3206.
- Gao, H., Dong, Z., Gong, X., Dong, J., Zhang, Y., Wei, W., Jin, S. (2018) Effects of various radiation doses on induced T-helper cell differentiation and related cytokine secretion. *Journal of Radiation Research*, **59**, 395–403.
- Giwerzman, A., von-der-Maase, H., Berthelsen, J.G., Skakkebaek, N.E. (1991) Localized irradiation of testes with carcinoma in situ: effects on Leydig cell function and eradication of malignant germ cells in 20 patients. *J. Clin. Endocrinol. Metab.* **73**(3), 596–603.
- Hassan, N.H., El Dawy, H.A.S, Ahmed, M.M., Montaser, S.A., Harb, S.M. (2015) The possible radio-protective and anti-infertility roles of *Spirulina* in adult male mice exposed to whole body γ -irradiation. *Isotope & Rad. Res.* **47**(3), 315-330.
- Hazra, R., Upton, D., Jimenez, M., Desai, R., Handelsman, D.J., Allan, C.M. (2014) *In vivo* actions of the Sertoli cell glucocorticoid receptor. *Endocrinology*, **155**, 1120–1130.
- Ilaqua, A., Izzo, G., Emerenziani, G.P., Baldari, C., Aversa, A. (2018) Lifestyle and fertility: the influence of stress and quality of life on male fertility. *Reprod. Biol. Endocrinol.* **16**, 115.
- Krishna, A., Kumar, A. (2005) Evaluation of radioprotective effect of Rajira (*Amaranthus paniculatus*) extract in Swiss albino mice. *J. Radiat. Res.* **46**, 233–239.
- Kurata, M., Suzuki, M., Agar, N.S. (1993) Antioxidant systems and erythrocyte lifespan in mammals. *Comp. Biochem. Physiol.* **106**(3), 477-87.
- Lansky, E.P., Newman, R.A. (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, **109**, 177–206.
- Les, F., Arbone´s-Mainar, J.M., Valero, M.S., Lo´pez, V. (2018) Pomegranate polyphenols and urolithin A inhibit α -glucosidase, dipeptidyl peptidase-4, lipase, triglyceride accumulation and adipogenesis related genes in 3T3-L1 adipocyte-like cells. *J. Ethnopharmacol.* **220**, 67–74.
- Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. (2020) Serum triglycerides determined calorimetrically fragmentation in lymphocyte. *J. Immunol.* **139**, 3199-3206.
- Egypt. J. Rad. Sci. Applic. **33**, No.2 (2020)

- (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem.* **96**, 254-260.
- Maeda, N., Tahata, S., Yagi, T., Tanaka, E., Masu, K., Sato, M., Haeno, S., Onaga, T., Yokota, H. (2015) Assessment of testicular corticosterone biosynthesis in adult male rats. *PLoS One*, **10**(2), e0117795.
- Mannervik, B. (1987) The enzymes of glutathione metabolism: an overview. *Biochem. Soc. Trans.* **15**(4), 717-8.
- Matthaiou, C.M., Goutzourelas, N., Stagos, D., Sarafoglou, E., Jamurtas, A., Koulocheri, S.D., Haroutounian, S.A., Tsatsakis, A.M., Kouretas, D. (2014) Pomegranate juice consumption increases GSH levels and reduces lipid and protein oxidation in human blood. *Food Chem. Toxicol.* **73**, 1-6.
- Meseguer, M., Martinez-Conejero, J.A., O'Connor, J.E., Pellicer, A., Remohi, J., Garrido, N. (2008) The significance of sperm DNA oxidation in embryo development and reproductive outcome in an oocyte donation program: a new model to study a male infertility prognostic factor. *Fertil. Steril.* **89**(5), 1191-9.
- Minami, M., Yoshikawa, H. (1979) A simplified assay method of superoxide dismutase. *Clin. Chem. Acta*, **92**, 337-342.
- Mirdehghan, S.H., Rahemi, M. (2007) Seasonal changes of mineral nutrients and phenolics in pomegranate (*Punica granatum* L.) fruit. *Sci. Hort.* **111**, 120-127.
- Nozik-Grayck, E., Suliman, H.B. (2005) Piantadosi CA. extracellular superoxide dismutase. *Int. J. Biochem. Cell Biol.* **37**(12), 2466-71.
- Ohkawa, H., Ohishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**(2), 351-358.
- Pastore, A., Federici, G., Bertini, E., Piemonte, F. (2003) Analysis of glutathione: implication in redox and detoxification. *Clin. Chim. Acta*, **333**(1), 19-39.
- Phaniendra, A., Jestadi, D.B., Periyasamy, L. (2015) Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J. Clin. Biochem.* **30**(1), 11-26.
- Rao, F., Tian, H., Li, W., Hung, H., Sun, F. (2016) Potential role of punicalagin against oxidative stress induced testicular damage. *Asian Journal of Andrology*, **18**, 627-632.
- Sherif, W.M., Sibghatullah, S., Sree, H., Mueen, A. K., Ibrahim, A.R. (2013) Sensibility of male rats fertility against olive oil, Nigella sativa oil and pomegranate extract. *Asian Pac. J. Trop. Biomed.* **3**, 563-8.
- Sikka, S.C. (2001) Relative impact of oxidative stress on male reproductive function. *Curr. Med. Chem.* **8**(7), 851-62.
- Snedecor, G.W., Cochran, W.G. (1989) "Statistical Methods" 8th ed., Iowa State Univ. Press, Ames, Iowa, USA.
- Taheri, S.Z., Sarker, M.M.R., Rahmat, A., Alkahtani, S. A., Othman, F. (2007) The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of Langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague-Dawley rats. *BMC Complement Altern. Med.* **17**, 156.
- Turk, G., Sonmez, M., Aydin, M., Yuce, A., Seyfettin Gur, Murat, Y. Emrah, H., Aksu, H., Aksoy, H. (2008) Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical Nutrition*, **27**, 289-296.
- Vidal, A., Fallarero, A., Peña, B.R., Medina, M.E., Gra, B., Rivera, F., Gutierrez, Y., Vuorela, P.M. (2003) Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. *J. Ethnopharmacol.* **89**, 295-300.
- Wyrobek, A., Watchmaker, C., Cordon, I. (1984) Sperm morphology testing in mice. In: "Hand Book of Mutagenicity Test Procedures", Kilbeg, B.J., Legator, M. and Ramel, C. (Eds.), pp. 739-750. El Sevier Science Publishers, Amsterdam.
- Xu, G., Intano, G.W., McCarrey, J.R., Walter, R.B., McMahan, C.A., Walter, C.A. (2008) Recovery of a low mutant frequency after ionizing radiation-induced mutagenesis during spermatogenesis. *Mutat Res.* **654**, 150-7.
- Yazawa, H., Sasagawa, I., Nakada, T. (2000) Apoptosis
Egypt. J. Rad. Sci. Applic. **33**, No.2 (2020)

of testicular germ cells induced by exogenous glucocorticoid in rats. *Hum Reprod.* **15**, 1917–1920.

Zahin, M., Ahmad, I., Gupta, R.C., Aqil, F. (2014) Punicalagin and ellagic acid demonstrate antimutagenic activity and inhibition of benzo[a]pyrene induced DNA adducts. *Biomed. Res. Int.* **2014**, 467465. doi: 10.1155/2014/467465.

Zelko, I.N., Mariani, T.J., Folz, R.J. (2002) Superoxide dismutase multigene family: a comparison of the CuZnSOD (SOD1), Mn-SOD (SOD2), and ECSOD(SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* **33**(3), 337-49.