Iranian Journal of Medical Physics

ijmp.mums.ac.ir

Effect of Gamma Radiation on Some Sperm Factors of Male Rats

Quasy AL-Dulamey[1], Laith Ahmed Najam[2*], Sheamaa T Al-Dbag[1]

1. Department of Biophysics, College of Science, , University of Mosul, Mosul, IRAQ
2. Department of Physics, College of Science, Mosul University, Mosul, IRAQ

ARTICLE INFO

Article type:
Original Paper

Article history:
Received: Jul 14, 2019
Accepted: Mar 06, 2020

Keywords:
Gamma Radiation
Rat
Epididymis
Dose Rate
Sperm

ABSTRACT

Introduction: The present study investigated the risks of ionizing radiation on sperm counts in chronic doses and compared the findings with previous results in similar and different conditions to minimize oxidant stress on sperm parameters rather than using black seed oil.

Material and Methods: Twenty rats were used in experimental designs 1 and allocated unordered to four groups. Each group included five. The ranges of 2-3 months and 170 -200 g, respectively .The healthy rats were obtained from the University of Mosul ,Iraq .Experimental design 2, was conducted on 50 rats .The rats were exposed to three different doses for 30 days similar to those of experimental design 1. Oral black seed oil was administrated a dose of 20 mg/kg in group 2 .

Results: In experimental design 1, there was a significant decrease in sperm count, live sperm percentage and normal sperm percentages respectively. However a significant increase was observed in dead sperm and abnormal sperm percentages in experimental design 1.The administration of black seed oil in excremental design 2 improved all the parameters with reducing abnormal and dead sperm counts rather than increasing normal and live sperm counts at all doses .

Conclusion: The use of black seed reduce the oxidative stress caused by low dose gamma radiation .Therefore , this substance can be used as a therapeutic option for the treatment of several type of cancer especially those under the treatment of low dose gamma radiation through enhancement of protection for a long time.

► *Please cite this article as:*
AL-Dulamey Q, Najam LA, Al-Dbag S. Effect of Gamma Radiation on Some Sperm Factors of Male Rats. Iran J Med Phys 2021; 18: 1-9. 10.22038/ijmp.2020.39626.1526.

Introduction

It is knows that sperm contains the least amount of non-chromosomal material to be changed and damaged. The irradiation with gamma ray leads to chromosomal deletions indicating either decreasing or increasing the effect .Moreover, Sperm mutagenesis is simple to do with. Reproductive management is actually one of the main strategies of ascendant sheep in the industry with many impacts on the rental positions. [1].The most important target organ in ionizing radiation leading to damage is the testis. Multiple studies reported that irradiation induces some histological sequel in the organs and tissues involving the increase of loose fibrous tissue [2,3] . Moreover, electromagnetic radiation was found in the depletion of more advanced spermatogenic cells as the gamma ray of natural tissue effects in fibrosis, contributing to all the effects of ionizing radiation observed in a study [4] in the testis [5]. Furthermore, the changing spermatogonia are more sensitive to ionizing radiation and are killed by the induction of a dose close to 3 Gy/h in Sprague-Dawley rats, and mice [6] . Before the exposure to gamma ray as ionizing radiation the radioprotective agents as important elements used to decrease the induced damaging including radiation induced lethality [7] .According to evidence [8] the effect of gamma ray at 4, 5, and 6 Gy was observed on the whole body of mice leading to a significant increase in abnormal sperm count .This change in sperm count with rising radiation dose and reached 25 % . In a study carried out by Eissa et al in 2007 [9] , it was also demonstrated that the effect of gamma ray on testicular organs of rats led to the reduction of the value of spermatogenic cells at irradiation groups compared to that reported for the control group . The reduction in the value of spermatogenic cells increased with raising radiation dose, where the maximum change was reported as 68% [10]. The negative outcomes were obtained from another study [11] indicating that ionizing radiation resulted in increasing abnormal sperm count. In addition this change increased with raising radiation dose and time exposure. The combined action of X-ray and nonylphenol in mice sperm showed that the sperm count decreased with increasing the absorbed dose from (0.05 to 0.20 Gy) with a maximum change in the sperm count as 64 %. [12].However, the change was noticed in abnormal sperm count as 17.5 %. Reid et al. in the other study conducted in 1981 determined the effect of radiation dose on the stem cell of mouse testis [13] at 100 rad with a change in the sperm counts of irradiation groups compared to that of control group at 13 % more than other groups.

*Corresponding Author: Tel: +964 060850110; E-mail: prof.lai2014@gmail.com

According to the literature, the reproductive system has been represented to be one of the systems in humans sensitive to electromagnetic radiation especially ionizing radiation [14] Not only can it detect the proportion of the human body, but it can also influence population movements and the balance of environmental units. Those with increased female arthropods are more radiosensitive than male radiosensitive cells in general as well as Al-Dulamey et al ,2020 irradiated mice with same radioactive source emitted gamma ray by two dose rate (110 and 310 mGy/h) with period 3-41 days [15-17]. In addition there are multiple exclusions, in this regard. In the hemipteran Pyrrrhocoidae groups for example, males may be more radiosensitive than females. [18]. The amount of radiation doses is also very important .Accordingly, high doses of gamma ray lead to sperm inactivation and small doses have important effects on sperm production if the treatment time changes developing sperm cells [19].Abnormal sperm count gradually increases by raising the rate of gamma radiation dose [20]. A blood suction insect and Chagas disease vector was identified during spermatogenesis apoptosis in Triatoma infestans after exposure to gamma radiation. [21] . A another study used soft gamma radiation and investigated the effect of a low dose or soft dose of gamma ray in (m Gy) on the reproductive system of the Earthworm Eisenia fetida (Oligochaeta) using chronic gamma radiation. In the aforementioned study, it was shown that the ability of the reproductive system reduced with increasing the dose rate [22]. The present study aimed to determine the effects of the soft activity of gamma radiation emitted from americium -241 as a low dose rate in (m Gy) as oxidation stress on the physiological properties of sperm namely concentration,normal and abnormal counts, and live and dead counts. Black seed oil is an edible product containing beta-carotene,iron, calcium, potassium and sodium. Out of nine essential amino acids, eight essential amino acids cannot be produced in the human body. In addition to chief active constituents of black seed oil, it also has thymoquinone, myristic acid ,beta sitosterol, palmitic acid , palmitoleic acid , oleic acid, stearic acid, linolenic acid, linolenic acid, arachidonic acid, protein, folic acid ,copper, zinc, phosphorous, as well as vitamin B1 ,B2 and B3 [23] . With this background in mind, the current study was conducted to investigate the effects of gamma ray as oxidation stress on the physiological properties of sperm including concentration, normal and abnormal counts and live and dead counts.

Materials and Methods

Nigella sativa (i.e., black seed) was purchased from the local market in Mosul, Iraq, and filtered to remove any impurities. Using a breaking system inside the snowy bath, black seed oil was obtained by equipment breaking 50 g of seed with 250 cm3 of ethanol with 95% concentration.. The mixture was shaken with an electric motor generator (Ahlstrom Germany GmbH Company, Germany) for 1h at room temperature. The filtration was performed using multiple layers of filter paper. Then the filtered mixture was centrifuged at a speed of 1000 rpm for (15) min. Subsequently, the seed oil was collected from the surface layers. The albino Blub/c male rats in this study were within the age and weight ranges of 2-3 months and 170-200 g respectively. The sterile rats were obtained from the College of Veterinary Medicine at the University of Mosul and placed in cages of plastic materials with dimensions of (20 x 30 x20 cm). A set of steps were taken to ensure good hygiene and care. Throughout the experiment, the temperature and humidity were optimally maintained for both control (without irradiation) and irradiation groups at 25.5 ±2 C^o and 34.5 ± 5 respectively .In addition the sawdust was changed every week. The animals food consisted of wheat (35%), corn (34%), soybeans (20%),

Protein (10%) and dried milk (1%), and they were provided with water through the testing period [24].

Americium- 241 properties

Americium-241 has 59.5 keV energy and 50 μCi radiation activity with exposure constant or gamma constant Γ= 0.013 mR. /hr . μCi . The half-life of this source is equivalent to 432 years and adds that with a few centimeters, the radiation source emits alpha particles passing through the grid[25].

Program of work

Americium- 241 as a radioactive source emitted gamma radiation doses of 9.1 , 16.2 and 36.4 (mGy/h) to the rats . The gamma radiation was a chivied with daily 7 hours exposure for 90 days .A portable Geiger counter (INVOVA GmbH company , www.alphaaix.com ,Germany) was used to determine the radiation dose used in this study with R/h and covert to mGy/h by the following formula: (Dose rate R/h = Γ x A / d^2 with unit R. m /Ci .h : where Γ is gamma constant , A is the specific activity of the source in (Ci), d, is the distance between the source and detector in (m). The detector has other functions such as the radiation dose of X-ray. Alpha, and beta were provided from natural sources as other types of radiation in addition to gamma ray.

Semen collection

The animals were weighed and deeply anesthetized with ether at (100mg/kg) leading to the effect on the breath and expiration. The samples of semen were collected from the cauda epididymidis. The percentage of sperm cells in a unidirectional progressive movement over a field was observed, on a slide using a light microscope according to the literature [26] . Briefly, a small drop of semen was placed on a warm slide, mixed with one drop of warm sodium citrate and covered with a glass slip. The sperm cells moving in a straightforward unidirectional motion were counted however, the sperm cells moving in circles, in a backward direction or showing pendulating movement were excluded.

Live/Dead ratio or Percentage liveability

One drop of semen was mixed with one drop of eosin-nigrosin stain on a warm slide as described by Wells and Awa (1970) [27] .Then a thin smear was made from the mixture of semen and stain. Subsequently, the smear was air-dried and a total of 400 sperms were observed under the microscope. The live and the dead sperm cells were separately counted and the ratio of the live to dead sperm cells was calculated according to the Zemjanis method (1977).

Sperm morphological defects

Morphological defects in a total of 400 sperm cells were determined using the Wells-Awa technique (1970). Briefly, each drop of Wells - Awa stain and semen was placed on a warm slide and mixed, and a smear was made with another slide .The stained smear was then air-dried and viewed under the microscope. The defects were classified as described by Bloom (1973) [28] and Parkinson (2001) [29].

Experimental design

A total of 70 rats were used in two experimental designs as follows:

Experimental design 1

The present experiment was carried out on 20 rats divided into five groups and received the selected dose as follows:

1- The first (n=5) was the control group, without irradiation including five rats who received water and food. In addition the number of rats was similar in all groups of the experiment.
2- The second (n=5) group received a radiation dose of 9.1 m Gy/.h
3- The third (n=5) group received a radiation dose of 16.2 m Gy/h
4- The fourth (n=5) group received a radiation dose of 36.4 m Gy/h
5- 5- The number of rats used in the second to fourth category was up to 15 male rats and subjected to a radiation dose for duration of 90 days at an average of 7 h / day.

The experiments were performed using gamma ray for 7 h /day with different exposure doses reported as (9.1, 16.2 and 36.4 m Gy/h) from Americium-241 source to male rats for 90 days

Experimental design 2

The current experiment was carried out on 50 rats divided into five groups and received the selected dose as follows:

1- The first group (n=5) was the control group without irradiation, including five rats who received water and food. In addition, the number of rats was similar in all groups of the experiment.
2- The second group (n=5) received an oral 20 mg/kg dose of black seed oil.
3- The third group (n=5) received a radiation dose of 9.1 mGy/h.
4- The fourth group (n=5) received a radiation dose of 9.1 mGy/h and oral 20 mg/kg dose of black seed oil.
5- 5- The number of rats used in the second to fourth category was up to 15 male rats and subjected to a radiation dose for duration of 30 days at an average reaction time of 7 hours/day with other doses of 16.2 and 36.4 m Gy/h.

Statistical analysis

In this study one way analysis of variance was used for Statistical analysis (SAS software, GitHub company, USA) Variables were identified with the measurement of the Duncan Involve significant status. The statistical variance was analyzed for the test groups (irradiation groups) and control group (i.e non-irradiation group).A p-value ≤ 0.05 was considered statistically significant. .The percentage and number of sperm were calculated for the irradiation groups and control group.

Results

Table 1 tabulates the sperm counts and percentage of normal, abnormal, live, and dead sperm cells.

As shown in Table 2, the irradiation by gamma ray at doses of 9.1, 16.2, and 36.4 mGy/h for 7 h/day and treatment with black seed oil at a dose of 20 mg/kg/day for 30 days resulted in a significant decrease in some parameters of sperm count, namely total sperm count, normal sperm count, and live sperm count, in groups 3 and 4, compared to those reported for group 1. In addition, group 2 showed a significant increase in these parameters in comparison to group 1 based on the obtained results presented in Table 2.

Table 1. Mean values of sperm counts and percentage of normal, abnormal, live, and dead sperm cells

Measurement / Groups	Number of sperm cells in epididymis (mean±standard deviation)	Percentage of live sperm cells (mean±standard deviation)	Percentage of dead sperm cells (mean±standard deviation)	Percentage of normal sperm cells (mean±standard deviation)	Percentage of abnormal sperm cells (mean±standard deviation)
Control	31.46± 0.99	88.12±0.82	11.88±0.81	85.31±0.83	14.69±0.73
9.1 mGy/h	24.20±0.49	48.02±0.44	51.98±0.43	46.71±0.55	53.83±0.55
16.2 mGy.h	16.36±0.23	34.45±0.69	65.55±0.52	31.44±0.72	68.56b±0.46
36 .4 mGy/h	10.18±0.26	28.77±0.21	71.23±0.21	26.32±0.45	73.68±0.45

Experimental design 1. *Mean ± S.D. ** p= 0.05 ***n= 5 ****T= 20 , *Means and standard deviations are reported in Tables 1. **Level of significance (p<0.05).as a,b,c,d between groups. *** Number of animals per group.****Total number of animals used
Experimental design2. *Mean ± S.D. ** p= 0.05 ***n= 5 ****T=50, *Means and standard deviations are reported in Tables 1. **Level of significance (p<0.05).as a,b,c,d between groups. *** Number of animals per group.****Total number of animals used

Table 2. Effect of low-dose gamma irradiation and black seed oil for 30 days on parameters of sperm count in male rats

Parameters of sperm	Group 1 Control (mean±standard deviation)	Group 2 Black seed oil at 20 mg/kg (mean±standard deviation)	Group 3 Irradiation with gamma ray at mGy/h for 7 h/day (mean±standard deviation)	Group 4 Black seed oil with 20 mg/kg + irradiation with gamma ray at mGy/h for 7 h/day (mean±standard deviation)	Group2 due to group 1 (i.e oral treatment with black seed only group per control group) Ratio %	Group3 due to group 1 (i.e irradiation with gamma ray at mGy/h for 7 h/day group per control group) Ratio %	Group4 due to group 1 (i.e oral treatment with black seed + irradiation with gamma ray at mGy/h for 7 h/ day group per control group) Ratio %
First dose 9.1 m Gy/h							
Sperm count	32.05 ± 0.40	36.62 ±0.41	24.22±0.33	27.36 ±0.45	114	75	65
Normal sperm count	27.29± 1.81	32.38± 0.74	19.32±1.84	22.56 ±1.92	118	70	82
Abnormal sperm count	4.76± 1.47	4.24 ±1.21	4.90±0.41	4.80 ±0.45	89	102	100
Dead sperm count	3.46±0.12	3.11±0.31	4.10± 0.23	3.60 ±0.27	89	118	104
Live sperm count	28.59± 89.58	33.51± 4.55	20.12± 0.91	23.7± 41.53	117	70	83
Second dose16.2 m Gy/h							
Sperm count	32.05 ± 0.40	34.72 ±0.41	23.91±0.33	26.33 ±0.45	108	74	82
Normal sperm count	27.29± 1.81	30.61± 0.74	17.34±1.84	21.32 ±1.92	112	63	78
Abnormal sperm count	4.76± 1.47	4.11 ±1.21	5.85±0.41	5.01 ±0.45	86	122	105
Dead sperm count	3.46±0.12	3.12±0.31	4.20± 0.23	3.80 ±0.27	90	121	109
Live sperm count	28.59± 89.58	31.6± 5.55	19.71± 0.91	22.53± 41.53	110	68	78
Third dose36.4 m Gy/h							
Sperm count	32.05 ± 0.40	33.02 ±0.41	22.01±0.33	25.643 ±0.45	103	68	80
Normal sperm count	27.29± 1.81	29.41± 0.74	16.09±1.84	20.44 ±1.92	107	58	74
Abnormal sperm count	4.76± 1.47	3.61 ±1.21	5.92±0.41	5.20 ±0.45	75	124	109
Dead sperm count	3.46 ±0.12	2.92±0.31	4.80± 0.23	4.0 ±0.27	84	138	115
Live sperm count	28.59± 89.58	30.10± 5.55	17.21± 0.91	21.64± 41.53	105	60	75

Table 3. Comparison of irradiation groups and control group

Parameters / Groups	Number of sperms in epididymis (mean±standard deviation)	Ratio of sperm irradiation count due to control group (%)	Exposure duration (h)	P-value
No. irradiation (control)	31.46± 0.99	100	0	NS*
Irradiation at 9.1 mGy/h	24.20±0.49	77	7h/day	NS
Irradiation at 16.2 mGy/h	16.36±0.23	52	7h/day	< 0.05
Irradiation at 36.4 mGy/h	10.18±0.26	32	7h/day	< 0.05

*Not significant

Table 4. Comparison of results of present study and previous studies

Authors (year)	Radiation type	Sperm count million/ml (mean±standard deviation)	Dose rate	Exposure duration	Type of sample
(George et al,2009) [8]	Gamma ray	13.0±1.2 10.0±1.1 6.0±0.89	4Gy/h 5Gy/h 6Gy/h	30 min /day	Mice Rats
(Kumer et al ,2013) [10]	Ionizing radiation	64.16±3.2	5.4 m Gy/h	6 h/day	Humans
(Dobrzynska,2011) [11]	X-ray	3.2±1.6	0.02 m Gy/h	2 weeks	Mice
(Reid,1981) [12]	Heat	0.45±0.1	0.002 m G/h	30min/ day for 56 days	Mice
(Eissa,2007) [9]]	Gamma-ray	32.94±68.25	3-6 Gy/h	1 week	Rats
Present study	Gamma –ray	24.20 ±0.49 16.36 ±0.23 10.18 ±0.26	9.1 m Gy/h 16.2 m Gy/h 36.4 mGy/h	7h/day for 90 days	Rats

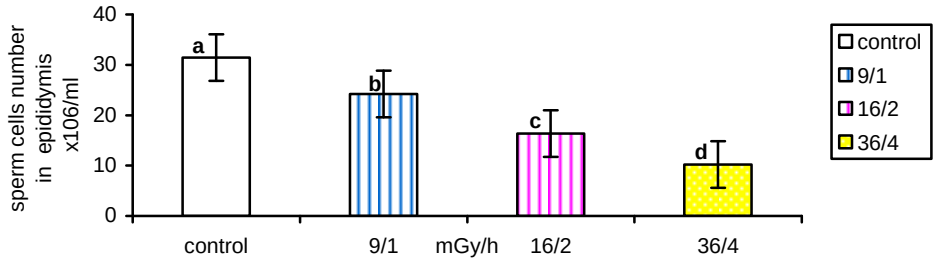

Figure 1. Effect of daily exposure to gamma radiation at doses of 9.1, 16.2, and 36.4 m Gy/h for 7 h in 90 days on number of sperm cells in epididymis in standard error mean

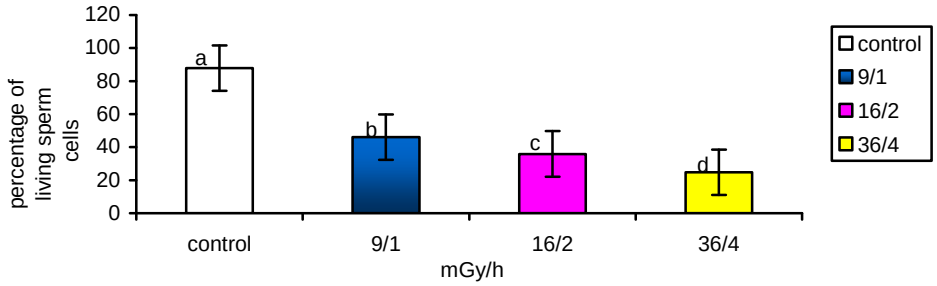

Figure 2. Effect of daily exposure to gamma radiation at doses of 9.1, 16.2, and 36.4 m Gy/h with interval for 7 h in 90 days on percentage of live sperm cells

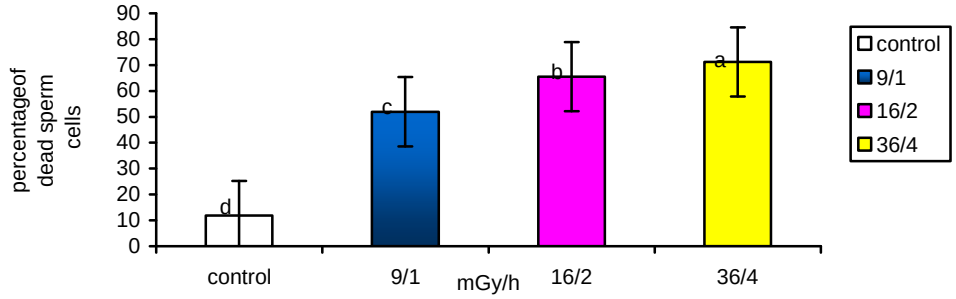

Figure 3. Effect of daily exposure to gamma radiation at doses of 9.1, 16.2, and 36.4 m Gy/h with interval for 7 h in 90 days on percentage of dead sperm cells

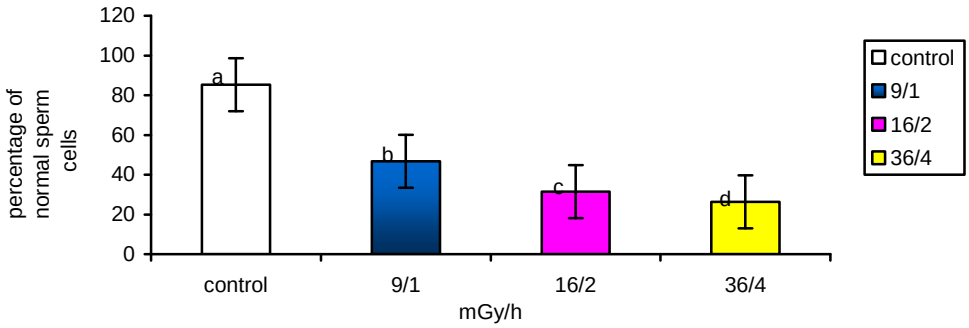

Figure 4. Effect of daily exposure to gamma radiation at doses of 9.1, 16.2, and 36.4 mGy/h with interval for 7 h in 90 days on percentage of normal sperm cells

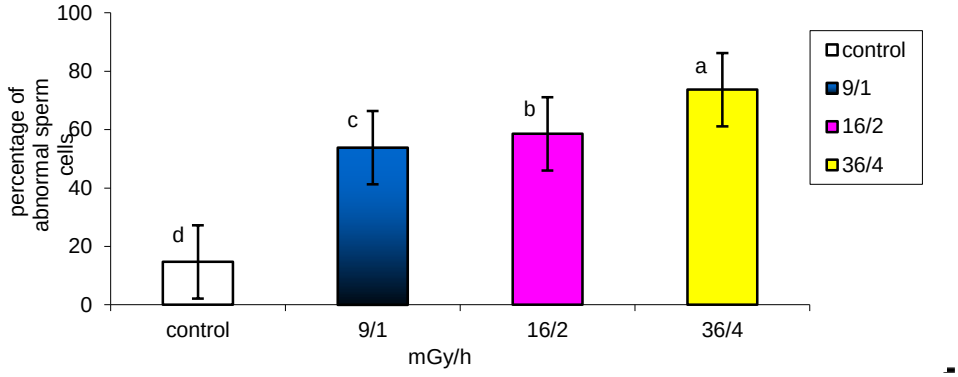

Figure 5. Effect of daily exposure to gamma radiation at doses of 9.1, 16.2, and 36.4 mGy/h with interval for 7 h in 90 days on percentage of abnormal sperm cells

Discussion

Figure 1 illustrates a significant reduction ($P<0.05$) in the number of sperm cells in the epididymis of the rats irradiated with gamma ray doses of at 9.1, 16.2, and 36.4 mGy with the interval for 7 h daily in 90 days in comparison to that reported for the control group. The findings of the present study are in line with the results of another study [30]. The aforementioned study reported that the sperm concentration also decreased (close to 0) at 42 and 49 days of the treatment. Although all live creatures are at risk of damage in response to ionizing radiation, the mammalian testes are much more sensitive to ionizing radiation. In humans and majority of animals, the testes lie outside the body and are susceptible to radiation damage (Abuelhija et al. 2013).

Figures2 and 3 depict a significant reduction ($P<0.05$) in the percentages of live and normal sperm cells during the irradiation with gamma ray doses of 9.1, 16.2, and 36.4 mGy for 90 days with a daily interval of 7 h. The percentages of dead and abnormal sperm cells were illustrated in figures 4 and 5with an observed elevation ($P<0.05$) when irradiated to a similar previous case.

Damage to the testes is directly proportional to the dose and time of exposure to artificial radiation or treatment. Based on the evidence, there was a reduction in sperm count after the testis treatment with low-dose irradiation [31]. The tests of monkeys were exposed to a single dose of 2 Gy with X-ray radiation. In this regard, the mean number of sperm concentration per any state of shoot scientifically reduced to 9.2±3.5×106 in 35 days following irradiation, compared to the first treatment value (60.3±15.5×106) [32].

Furthermore, another study demonstrated that gamma radiation leads to a significant decrease in the measurement of sperm, especially the sperm count of irradiated rats, compared to that reported for the control group. This finding is in line with the results of another study by Attla and Nasr (2009) [33].

It was concluded in Table 3 that the sperm count values for the irradiation groups differed compared to those recorded for the control group. With the radiation dose increasing, the change in the number of sperm dramatically increased at a value of less than 0.05, especially 36.4 mGy/h was a 32 percent ratio of sperm irradiation count due to the control group. The irregular sperm count also indicated this improvement.

Based on Table 4, a comparison was made between the results of the present study and other studies regarding radiation type and samples. Due to similar conditions, Eissa [9] investigated the impact of gamma ray on rats in a week. According to the results of the aforementioned study, the outcomes showed a significant decrease obtained at doses of 3 and 6 Gy with 32.9 percentage .In another study conducted by George et al. in (2009) [8], there was a significant decrease at 13, 10, and 6 in sperm counts with the effect of gamma ray at doses of 4, 5, and 6 Gy for 30 min/h, respectively

They reported the effect of the gamma ray emitted from cobalt-60 radiation source on the rat with a daily dose of 2 Gy with a source-to-surface distance of 80 cm. In the aforementioned study, there was a significant decrease in the group exposed to radiation in the numbers of spermatogonia, primary spermatocyte around spermatid, and spermatozoa [33].

In another study, Diagnox-60 (Meditonic) at 30 min daily was irradiated with X-ray in mice over two periods (i.e., 24 h and 35 days) at a dose of 0.5 Gy. Moreover, a reduction was observed in the concentration, number, and percentage of sperm [8]. Furthermore, the male mice were continuously irradiated within the dose range of 4-6 Gy leading to a significant increase in the percentage of abnormal sperm.

The results of the present study are similar to the findings of another study [34] carried out on the role of gamma radiation in adult male mice. The irradiation dose of 4-6 Gy achieved a small interval of sterility connected with oligospermia, and the sperm count significantly decreased with a gradual increase in radiation dose. The number of sperm count significantly decreased with increasing the dose (i.e., 150-250 Gy) of gamma radiation within the period of irradiation, which was emitted from cobalt-60 radioactive source [34, 35].

Abnormal sperm count in all groups as indicated in Table 2 was noticed in comparison to control magnitude

in almost all doses when the rats were exposed to doses of 0.675, 1.350, 2.700, and 4.050 Gy. The abnormalities became more clear at doses of 2.700 and 4.045 Gy [36]. This finding is consistent with the results of other studies regarding the sperm count of morphology while different testes of mice strain were exposed to X-ray radiation doses of 0, 0.3, 1.0, and 3 Gy. The last dose (3 Gy) led to a decrease in sperm counts observed in the Cauda epididymis with both effects of 42 and 49 days with final irradiation. Subsequently, the rise in the percentage of sperm abnormalities was noticed after 21 days in both groups [37].

The results of the current study are in line with the findings of other studies [38, 39]. In the aforementioned studies, there was a significant reduction in epidermal sperm count in the irradiated group, compared to that of the control group. The mice were exposed to the whole-body irradiation dose of 2 Gy for 28 days after treatment similar to the method of the present study using antioxidant materials. In addition, in 1999, Foppiani et al. irradiated monkeys from 14 weeks a and reached when one monkey was castrated after irradiation and in the 27th week when the remaining five monkeys were bilaterally biopsied. A decrease in body weight, with total value (30% of the pre-treatment size), and sperm count was observed after irradiation [39].

A decrease in the number of sperm was observed in 60 days of radiation and several monkeys showed azoospermia return in that period [40]. In another study [41], different results were obtained indicating no change in abnormal sperm count when exposed to gamma ray emitted from many radioactive sources as environmental radiation on tests and spermatogenesis.

These physiological changes in the measurement of sperm to decrease lipid peroxidation activity lead to the elevation of free radicals in sperm metabolism raising the spermatozoa activity to react with zona pellucida. However, increasing patients' lipid peroxidation of sperm membranes may unbalance emotion. For a long time, it has been known that oxygen metabolism led to the observation of the cells and tissues and the effects of reactive oxygen species on male infertility [37, 38].

Free electrons allow unbelievers of the external orbit to refer to the impact of ionizing waves on deoxyribonucleic acid, proteins and lipids between the cell effect .The precision of sperm completion, spermatogenesis, enabling energies, acrosome reaction, and increasable membrane liquidity were the lipid structures placed in the sperm membranes. [42]. They also correspond to a similar system [43] for the calculation of weak gamma wave impact on several parameters of blood.

A comparison of the results of the present study with the results of previous studies revealed a median radiation dose of 36.4 mGy/h as a chronic dose (10.18 ±0.26) with an exposure period of 7 h/day for 90 days. This finding (30.%) is consistent with the findings of a study conducted under a similar scenario by Eissa[9].George et al. in 2009 [8] also studied the effect of gamma irradiation on rats but with different conditions

According to the results obtained from the current research, the effect of gamma ray on soft wave bands was close to that of rats when the acute dose was used for a short irradiation period.

Based on the evidence [10, 11, 12], different radiation doses of 5.4, 0.02, and 0.002 m Gy were used as irradiation doses for different samples, including humans, mice, and mice, respectively. The comparison of the results of the present study and findings of previous studies for different samples and radiation impacts at doses of 9.1, 16.3, and 36.4 m Gy/h showed a change in sperm count. The sperm count increase was expressed by (3.92, 0.19, and 0.02) percent relative to the first dose defined by (2.67, 0.13, and 0.01) percent and also due to the second dose relative to the sperm count of our work respectively. The sperm count changed with regard to the third dose with 6.30, 0.31, and 0.04 percent due to the sperm count of our job, respectively.

The relation between the radiation doses of 99.1, 16.3, and 36.4 mGy/h in the current study and the radiation doses in previous studies showed a difference of [0.59, 0.002, and 0.0002] percent with respect to the first dose and also a change of [0.33,0.001,0.00006] percent with respect to the second dose. Finally, due to the third dose in the current analysis, the change in the radiation dose rate from the previous dose rate to (0.14, 0.0005, and 0.00003) percent.

The results of the present study showed a significant decrease in the mean values of sperm count parameters, namely total sperm count, normal sperm count, and live sperm count. In addition, there was a significant increase in the abnormal sperm and dead sperm counts in groups 3 and 4. However, a significant increase was observed in these parameters in group 2 after gamma irradiation at doses of 9.1, 16.2, and 36.4 m Gy/h for 7 h/day in 30 days and treatment with a 20 mg/kg/day dose of black seed oil for a similar time.

On the other hand, after irradiation with gamma ray for 7 h /day at doses of 9.1, 16.2, and 36.4 mGy/h and treatment with a 20 mg/kg/day dose of black seed oil for 30 days, there was a significant decrease in the sperm parameters in group 4, compared to that reported for group 1. In addition, in three cases of radiation exposure, the values in groups 3 and 4 were less than the values in group 2 regarding the total sperm count, normal sperm count, and live sperm count; however, there was an increase in the abnormal and dead sperm counts in the 3 and 4 groups.

The results of the present study revealed that the treatment of normal rats with blacked seed oil (*Nigella sativa*) at a dose of 20 mg/kg resulted in significant variations in the levels of total sperm count, normal sperm count, live sperm count, abnormal sperm count, and dead sperm count after 30 days. Nevertheless, the treatment of the group irradiated with gamma ray showed a significant decrease ($P \leq 0.05$) in the similar parameters of sperm count after 30 days, compared to

that reported for the control group. These results led to the protective effect of phenolic antioxidant during the investigation of blacked seed oil on rats body from irradiation of gamma ray . This antioxidant is capable of reducing abnormal and dead sperm counts and changing the consequences of their ability to release free radicals. However the development of prostaglandins, which enhance flow and shift as immune modulators, is unimpaired [44].

Finally, the results of the present study showed that group 2 treated with black seed oil was reported with a significant increase in the parameters of sperm count in comparison to that of the control group. The control group in the second process received a dose of 20 mg/kg for 7 h/day in 30 days. These results are in line with the findings of another study [45] investigating the effect of oral administration of the black seed oil (at a dose of 20 mg/kg daily) at several gamma doses on rats at 6 h/day for 30 days. The selected dose of black seed oil in the present study was similar to that reported for the aforementioned study. In the aforementioned study, there was a significant increase in sperm parameters in the group of mice receiving black seed oil, compared to that reported for the control group.

Conclusion

To date, a limited number of studies were carried out regarding the effect of a low dose of gamma ray as a chronic dose and treatment with radioprotective materials (e.g., black seed oil). The current study investigated the effect of black seed oil as an antioxidant substance leading to the reduction of free radicals. Based on the obtained results, this substance may be able to reduce the oxidative stress caused by low-dose gamma radiation. Therefore, this substance can be used as a therapeutic option for several types of cancers, especially those under the treatment of low-dose gamma radiation, through the enhancement of protection for a long time. According to these findings, black seed oil (*Nigella sativa*) is considered a good protective agent against oxidative stress in case of oral administration.

Acknowledgment

The authors would like to extend their gratitude to Physics Department for help with performing the present study and staff of Physics, Biologics, and Biophysics departments in the College of Science at the University of Mosul, Iraq, for their support.

References

1. Adams NR. Permanent infertility in ewes exposed to plant oestrogens. Australian Veterinary journal. 1990; 67(6) :197-201.
2. Matalka KZ, Barth RF, Bailey MQ, Wilkie DA, Koestner A, Hopewell JW. Radiation effects of boron neutron capture therapy on brain, skin, and eye of rats. International Journal of Radiation Oncology* Biology* Physics. 1994; 28(5):1089-97.
3. Yang W, Barth RF, Rotaru JH, Boesel CP, Wilkie DA, Bresnahan JC, et al. Boron neutron capture therapy of brain tumors: functional and neuropathologic effects of blood‐brain barrier disruption and intracarotid injection of sodium borocaptate and boronophenylalanine. Journal of neuro-oncology. 2000; 48(3):179-90.
4. Geraci JP, Mariano MS, Jackson KL. Radiation hepatology of the rat: microvascular fibrosis and enhancement of liver dysfunction by diet and drugs. Radiation research. 1992;129(3):322-32.
5. Kangasniemi M, Veromaa T, Kulmala J, Kaipia A, Parvinen M, Toppari J. DNA-flow cytometry of defined stages of rat seminiferous epithelium: effects of 3 Gy of high-energy X-irradiation. Journal of andrology. 1990;11(3):312-7.
6. Mian TA, Suzuki N, Glenn HJ, Haynie TP, Meistrich ML. Radiation damage to mouse testis cells from [99mTc] pertechnetate. Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine. 1977;18(11):1116-22.
7. Satoh H, Nishikawa K, Suzuki K, Asano R, Virgona N, Ichikawa T, et al. Genistein, a soy isoflavone, enhances necrotic-like cell death in a breast cancer cell treated with a chemotherapeutic agent. Research communications in molecular pathology and pharmacology. 2003;113:149-58.
8. George S, Chuttani K, Basu SK. Radiation protection of male fertility in mouse and rat by a combination of 5-hydroxy-L-tryptophan and a thiol compound (AET). Acta Oncologica. 1992; 31(6):669-72.
9. Eissa OS, Moustafa NA. The protective role of Septilin against Gamma radiation-induced testicular toxicity in rats. The Egyptian Journal of Hospital Medicine. 2007;27(1):176-87.
10. Kumar D, Salian SR, Kalthur G, Uppangala S, Kumari S, Challapalli S, et al. Semen abnormalities, sperm DNA damage and global hypermethylation in health workers occupationally exposed to ionizing radiation. PLoS One. 2013;8(7): e69927.
11. Dobrzynska MM. Combined action of x-rays and nonylphenal on mouse sperm. cent Eur.J.Biol. 2011;6(3): 320-9.
12. Reid BO, Mason KA, Withers HR, West J. Effects of hyperthermia and radiation on mouse testis stem cells. 1981; 141: 4453-7.
13. Penfold JS. Assessment of the radiological impact of coal-fired power stations in the United Kingdom. InProceedings of NORM II, Second International Symposium on the Treatment of Naturally Occurring Radioactive Materials, Krefeld, Germany. 1998: 67-71.
14. Cogburn RR, Tilton EW, Brower JH. Almond moth: gamma radiation effects on the life stages. Journal of Economic Entomology. 1973 Jun 1;66(3):745-51.
15. Hooper G.H.S. The effect of ionizing radiation on reproduction , In: World crop pests ,Volume 3A, Fruit flies , their biology, natural enemies and control (Robison , A. S. and Hooper , G. eds .), Elsevier ,Amsterdam , The Netherland. 1989: 1553-164.
16. Hallman GJ. Ionizing radiation quarantine treatments. Anais da Sociedade Entomológica do Brasil. 1998;27(3):313-23.
17. Al-Dulamey Qusay Kh., Aldabag ,Shaimaa T.., Shafeek Raghad R. Gamma Ray Effects on White Blood Cells of Male Mice .NeuroQuantology ,

August 2020.| Volume 18 . Issue 8 pp 19-23.| doi: 10.14704/nq.2020.18.8.NQ20200
18. Radiation Mutagenesis. World of Microbiology and Immunology. 2013. Available from: http://www. Encyclopedia.com.
19. Woon JH, Shin SC, Kang YM, Kim HS. Sperm abnormalities in high-and low-dose-rate Γ-irradiated Korean dark-striped field mice, Apodemus agrarius coreae. Radiation protection dosimetry. 2011;146(1-3):280-2.
20. Alvares-Garcia RD. Efeitos da radiação gama sobre os fenotipos nucleares de alguns tipos celulares de Triatoma infestants klug (Hemiptera, reduviidae). Masters thesis , UNICAMP, Campinas, Sp. 1988.
21. Hasan M, Khalequzzaman M, Khan AR. Development of Tribolium anaphe irradiated as larvae of various ages with gamma rays. Entomologia experimentalis et applicata. 1989;53(1):92-4.
22. Aljanabi KA. The effect of aqueous extract of grassed on oxidative stress induced by hydrogen peroxide in male rat. Msc. Thes, Tekrit University; IRAQ. 2008: 70-5.
23. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity-the secret of pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer therapy. 2008;6(b):495.
24. IRSN Institute De Radioprotection Et De SORETE NUCLEAR. Principle emissions de I americium-241. 2012:1-10. Available from: http//www.irsn.org.
25. Hassan AB, Ayoub MM, Doghaim RF, Yousef HL, Eid MA. Studies of the effect of some antihistamines on the reproductive organs of male rats. Vet. Med. J. 1988;31(2):277-86.
26. Lalinde-Acevedo PC, Mayorga-Torres BJ, Agarwal A, Du Plessis SS, Ahmad G, Cadavid AP, et al. Physically active men show better semen parameters than their sedentary counterparts. International Journal of Fertility & Sterility. 2017;11(3):156.
27. Wells ME, Awa OA. New technique for assessing acrosomal characteristics of spermatozoa. Journal of Dairy Science. 1970;53(2):227-32.
28. Bloom E. The ultrastructure of some characteristic sperm defects. Nord. Vet. Med. 1973; 25:283.
29. Parkinson TJ. Fertility and infertility in male animals. Veterinary Reproduction and Obstetrics. 7th edn. WB Saunders Co. Ltd., London, England. 1996:572-633.
30. Ahmad G, Agarwal A. Ionizing radiation and male fertility. InMale Infertility. 2017;185-96.
31. Abuelhija M, Weng CC, Shetty G, Meistrich ML. Rat models of post-irradiation recovery of spermatogenesis: interstrain differences. Andrology. 2013; 1:206‐15.
32. Fabiani R, Rosignoli P, Bartolomeo A, Fucceili R, Serrili M, Montedoro GF, et al. Oxidative DNA damage is prevented by extracts of olive oil ,hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells . J Nutr. 2008; 138(8): 441-16.
33. Attia AM, NASR HM. Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed (Nigella sativa L.) oil. Slovak Journal of Animal Science. 2009;42(2):87-94.
34. Koudelová J, Cook PA. Effect of gamma radiation and sex-linked recessive lethal mutations on sperm transfer in Ephestia kuehniella (Lepidoptera: Pyralidae). Florida Entomologist. 2001:172-82.
35. Chatterjee J, De K, Basu SK, Das AK. Alteration of spermatozol structure and trace metal profile of testis and epididymis of rat under chronic low-level x-ray irradiation. Biol Trace Elem Res. 1994; 41:305-19.
36. Bruce WR, Furrer R, Wyrobek AJ. Abnormalities in the shape of murine sperm after acute testicular x-irradiation . Muttat Res. 1974; 23:381-6.
37. Marzban M, Anjamshoa M, Jafari P, Masoumi H, Ahadi R, Fatehi D. Effects of gamma rays on rat testis tissue according to the morphological parameters and immunohistochemistry: radioprotective role of silymarin. Electronic physician. 2017;9(6):4524.
38. Searle AG, Bechey CV. Sperm count. egg-fertilization dominant lethality after X-irradiation of mice. Mutant Res. 1974: 22: 63-72.
39. Foppiani L, Schlatt S, Simoni M, Weinbauer GF, Hacker-Klom U, Nieschlag E. Inhibin B is a more sensitive marker of spermatogenetic damage than FSH in the irradiated non-human primate model. Journal of Endocrinology. 1999;162(3):393-400.
40. Okano T, Ishiniwa H, Onuma M, Shindo J, Yokohata Y, Tamaoki M. Effects of environmental radiation on testes and spermatogenesis in wild large Japanese field mice (Apodemus speciosus) from Fukushima. Scientific reports. 2016; 6:23601.
41. John Aitken R, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biology of reproduction. 1989; 41(1):183-97.
42. Sanocka D, Jędrzejczak P, Szumała-Kaękol A, Frączek M, Kurpisz M. Male genital tract inflammation: The role of selected interleukins in regulation of pro-oxidant and antioxidant enzymatic substances in seminal plasma. Journal of andrology. 2003;24(3):448-55.
43. Qusay Kh. Al-Dulamey, Yasir A, Al-Jawwady, Laith Ahmed Najam . Effect of low gamma ray dose on some blood factors in adult rats. Iranian journal of medical physics. 2020; 17(3):137-41.
44. Bashandy AES. Effect of fixed oil of Negilla Savita on male fertility in normal and hyperlipidemic rats. International journal of pharmacology. 2007;3(1): 27-33.
45. Gadelha IC, Fonseca NB, Oloris SC, Melo MM, Soto-Blanco B. Gossypol toxicity from cottonseed products. The Scientific World Journal. 2014 Oct;2014.