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Combined action of X-rays and nonylphenol on mouse sperm

Research Article

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Abstract: The aim of this study was to assess the effects of 2-weeks' X-ray and/or nonylphenol (NP) exposure on male mice's sperm count and quality. Pzh:SFIS mice were exposed to X-rays (0.05 Gy, 0.10 Gy, 0.20 Gy) or to nonylphenol (25 mg/kg bw, 50 mg/kg bw, 100 mg/kg bw) or to both agents (0.05 Gy + 25 mg/kg bw NP, 0.10 Gy + 50 mg/kg bw NP). At 24 h and 5 weeks after the end of exposure the sperm count, morphology and frequency of DNA damage in the male germ cells were estimated. Each agent alone diminished sperm count and morphology. The dose of 0.05 Gy of X-rays decreased the frequency of DNA damage. Combined exposure to lower doses of both agents significantly improved sperm morphology and decreased the level of DNA damage compared to one agent alone. Combined exposure to higher doses reduced the frequency of DNA damage compared to the effect of the appropriate dose of NP. Results of combined exposure to low doses of both agents suggest that 0.05 Gy of X-rays stimulate the DNA damage-control system and in consequence repair of DNA caused by X-rays and NP. It may be correlated with increased antioxidant capacity.

Keywords: *Sperm count and morphology • Dna damage • X-Rays • Nonylphenol • Combined exposure*

1. Introduction

Humans are equally exposed to numerous physical and chemical agents, which may adversely affect spermatogenesis at different stages of differentiation, giving rise to changes in the number, morphology, motility, viability, fertilization capacity, chromatin structure and possibly DNA integrity of germ cells [1]. Over the past 50-60 years, several groups of scientists reported deterioration of quantity and quality of sperm among human males. The incidence of congenital malformations of the male reproductive tract among newborns and testicular cancer among young men has also been increased [2-4]. It was suggested that such reproductive abnormalities may be attributed to endocrine-disrupting chemicals which are widespread in the environment [3,5].

Endocrine-disruptors are exogenous compounds that, due to their configuration similar to natural hormones, mimic or inhibit the action of oestrogens or other hormones. Endocrine-disruptors enter the body of mammals through food, drinking water, air and skin [3]. Nonylphenol (NP) is the final degradation product of alkylphenol polyethoxylates, which are widely used in the preparation of lubricating oil additives, resins, plasticizers, surface-active agents, detergents, paints, cosmetics and other formulated products [4,6]. It is also widely used in the manufacture of nonionic surfactants, polymer stabilizers, and antioxidants [7]. NP has been found in polyvinyl chloride, which is used in the food processing and packaging industry and contaminates the water flowing through polyvinyl chloride (PVC) pipes [6]. During anaerobic sewage digestion, alkylphenol polyethoxylates are biodegraded by hydrolytic removal of ethoxylate groups forming short-chain ethoxylates, carboxylic acid derivatives and nonylphenols [8]. NP possesses oestrogenic properties and has been shown to disrupt the male reproductive system of fish, reptiles and mammals [9-11]. The residual nature of nonylphenol and its lipophilic character leads to its accumulation

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in the lipids of living organisms [7,12,13]. Neonatal exposure of rats to NP had an effect on the weight of the reproductive organs and delayed testes descent, whereas exposure of adult rats affects spermatogenesis [14,15].

Radiation comes from natural and man-made sources and is a known mutagen and carcinogen. The greatest source of human exposure is radiodiagnosis and radiotherapy, where radiation is used for both diagnosis and treatment of disease [16], so medical personnel and patients may receive low doses of radiation. Irradiation induces micronuclei in spermatids and an enhanced frequency of abnormal sperm heads [17,18]. Ionizing radiation may cause damage at a variety of points during spermatogenesis. Spermatogonial stem cells and differentiating spermatogonia are highly sensitive to ionizing radiation. Spermatocytes are less sensitive, whereas spermatids are rather radioresistant [19-20].

Radiation and endocrine disruptors such as nonylphenol belong to those agents most often present in the environment and people are often exposed to both of them, especially at low doses. There are no published reports describing the effects of the combined exposure of germ cells to X-rays and nonylphenol. Therefore, investigations related to the effects of such exposure seem to be important to reproductive health.

This study was undertaken to evaluate the effects of 2-weeks' combined X-rays-nonylphenol exposure on the induction of changes in sperm count, morphology and DNA integrity in comparison to the effects caused by each agent acting alone.

2. Experimental Procedures

2.1 Animals

Outbred male Pzh:SFIS mice were obtained from the Laboratory of Animal Breeeding "Górzkowska" (Warsaw, Poland). Mice were housed in plastic cages in a room designed for control of temperature, humidity and light cycle (12 h dark, 12 h light). Tap water and rodent diet were available *ad libitum*.

The author obtained permission no. 03/2004 from the Fourth Local Ethical Commission (part of the National Ethical Commission) for conducting studies on laboratory animals.

2.2 Groups of mice

After one week's acclimatisation, 8-week old males were assigned randomly to either control or exposed groups. Male mice were irradiated with X-rays (0.05 Gy, 0.10 Gy and 0.20 Gy), intraperitoneally injected with nonylphenol diluted in oil (25 mg/kg bw, 50 mg/kg bw and 100 mg kg bw) or exposed to a combination of both agents (0.05 Gy + 25 mg/kg bw NP and 0.10 Gy + 50 mg/kg bw NP) daily. Total accumulated doses were 0.5 Gy, 1 Gy, 2 Gy and 250 mg/kg bw, 500 mg/kg, 1000 mg/kg bw NP and 0.5 Gy + 250 mg/kg bw NP, or 1 Gy + 500 mg/kg bw NP for combined exposure. Males were exposed to X-rays or NP or to both agents for 2 weeks, 5 days/week. Five or six males of each experimental and control group were weighed and sacrificed at 24 h or 5 weeks after the last treatment. Two-weeks' exposure was chosen as typical for a short-term toxicity study. Spermatozoa observed at 24 h after 2-week treatments were exposed as late spermatids and young spermatozoa. Waiting 5 weeks after the end of exposure allowed the observation of mature spermatozoa, which were exposed at the youngest stage of spermatogenesis, as spermatogonia.

2.3 X-ray exposure

A therapeutic Roentgen unit type THX-250 (Medicor, Budapest, Hungary) was used as the X-ray source. It was operated with the following parameters: 175 kV, 20 mA, added filtration 0.5 mm Cu and HVL 0.8 mm Cu. Mice were whole-body irradiated at the dose rate of 0.20 Gy/min. This meant that, for example, to obtain 0.05 Gy, mice were exposed for 15 seconds daily. Simultaneously, the dosimeter VA-J-18 (VEB RFT Masselelektronik, Dresden, Germany) was used to measure the correct dose. The dose of 0.05 Gy has been chosen for a combined exposure study because this dose was earlier the lowest which induced changes in the quality of mouse spermatozoa [21].

2.4 Nonylphenol application

Nonylphenol (CAS no 84-852-15-3, Sigma-Aldrich, Seelze, Germany) was mixed exactly with sunflower oil "Bartek" (Factory of Fatty Industry, Warsaw, Poland) in a laboratory mortar to obtain a homogenous mixture containing NP in an appropriate dose. Males received one injection per day, with a volume of 0.1 ml per 10 g body weight. Sunflower oil was chosen due to its frequent use in diet in Poland. The route of administration (i.p.) was chosen to enhance the sensitivity of the assay and to be sure that NP reached the reproductive system.

2.5 Combined exposure of mice to X-rays and nonylphenol

In the combined exposure study animals were injected with NP within 15-20 min after irradiation.

2.6 Control mice

Control mice for irradiated animals were sham-irradiated. Control mice for NP and combined exposures were injected with sunflower oil only.

2.7 Sperm count

Both testes and epididymes were removed from each male. Testes were weighed. For sperm count estimation the method described by Searle and Beechey [22] was used with some modifications. Briefly, one epididymis was removed and placed in 2 ml of 0.9% NaCl solution, minced with scissors to release the spermatozoa, and dispersed in the saline. A drop of the suspension was spread on the haemocytometer and counted.

2.8 DNA damage analysis

A Comet assay was used for estimation of DNA damage. For Comet assay analysis, one testis from each animal was decapsulated, placed in RPMI (Roswell Park Memorial Institute) 1640 medium and minced. Before using the cells, tubes were swirled so that single cells remained in the suspension. The basic technique of Singh *et al*. [23] and further described by Anderson *et al*. [24] was followed. 5 µl of cell suspension was mixed in an Eppendorf tube with 75 µl low melting point agarose (LMPA) for embedding on the slides previously covered with normal melting point agarose (NMPA). After solidifying the agarose at 4°C, another layer of LMPA was added and allowed to solidify at 4°C again. The slides were immersed in lysing solution overnight at 4°C. Slides were removed from the lysing solution, drained and placed in a gel electrophoresis tank, and incubated in the electrophoresis solution for 20 min to allow the unwinding of DNA. Electrophoresis was conducted for 20 min at 4°C using 24 V and 300 mA. After neutralisation, slides were stained with EtBr and examined using a fluorescence microscope. Images of 200 randomly chosen cells from each animal (100 cells from each of two slides) were analysed from each mouse. According to the method described by Kumaravel and Jha [25], cells were graded by eye into five categories based on the distance of migration and perceived proportion of DNA in the tail, and given a value from 0 (undamaged) to 4 (maximally damaged). The percentage of cells with each level of damage was calculated. In this way the total score ranging from 0 (all undamaged) to 400 (all totally damaged) was determined.

2.9 Sperm morphology

The second epididymis was used to study the frequency of morphologically abnormal spermatozoa. The epididymis was placed in 2 ml of 0.9% NaCl solution, minced and dispersed in the saline. Smears were prepared on microscope slides, air dried overnight and stained with eosin Y. Then 1000 spermatozoa per mouse were analysed using a light microscope and on the basis of classification proposed by Wyrobek and Bruce [26] the abnormalities of sperm heads such as lacking hook, amorphous, banana-shaped, folded on themselves, and multiple headed were recorded. Examples of abnormal sperm morphology compared to normal spermatozoa are shown in Figure 1.

2.10 Statistical evaluation

Statistical differences were analyzed between each experimental group and corresponding control group by using the Student's t-test at $P<0.05$. Instead of percentage values, the numbers of abnormal spermatozoa from each experimental and control group were compared. Results of each combined group were

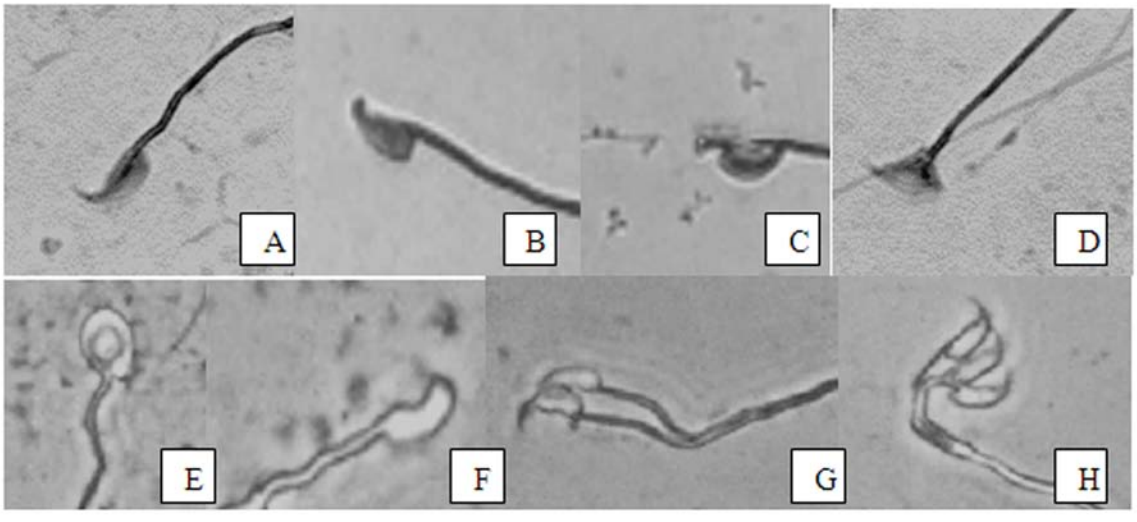

Figure 1. Sperm abnormalities in mice, A - normal shape of sperm head, B – lack of the hook, C – head folded on itself, D, E – amorphous, F – banana like head, G, H – multiple heads.

compared to results of X-rays and NP alone at the appropriate dose.

3. Results

X-rays as well as nonylphenol acting alone diminished sperm count and quality of male gametes. Combined exposure of spermatozoa and spermatids as well as spermatogonia to both agents at low doses (0.05 Gy + 25 mg/kg bw NP) significantly decreased the percentage of abnormal spermatozoa and the level of DNA damage in germ cells compared to results of at least one agent alone. Combined exposure to both agents at high doses (0.10 Gy + 50 mg/kg bw NP) significantly reduced the level of DNA damage after exposure of spermatozoa and late spermatids compared to the effect of NP, and not significantly diminished sperm count and morphology following the exposure of the above cells and spermatogonia.

Results are shown in Table 1. Mean and relative testes weights of males sacrificed 24 h after the end of irradiation or/and exposure to nonylphenol did not reveal any change. Five weeks following the end of 2-weeks' irradiation with 0.10 Gy and 0.20 Gy testes weights were significantly reduced compared to the control.

At 24 h after the end of exposure as well as 5 weeks later the sperm counts of irradiated mice were significantly decreased. Just after the end of 2-weeks' treatment with NP alone sperm count was also significantly reduced compared to the control group. Five weeks later, a significant decline sperm count was observed only in the group administered 25 mg/kg bw NP. Just after the end of combined exposure to 0.05 Gy + 25 mg/kg bw NP, the sperm count was not significantly higher than after exposure to either agent alone; however, it was significantly lower compared to the control. Five weeks following the end of the combined exposure to lower doses of both agents, results of the sperm count were not significantly changed compared to the control as well as to each agent alone. Just after the end of combined exposures to higher doses the sperm count was not significantly decreased in relation to results obtained after the exposure to each agent alone and significantly reduced compared to the control. Five weeks later, the sperm count was similar to that after irradiation alone and not significantly lower than following treatment with NP alone, and significantly decreased compared to the control.

The frequency of morphologically abnormal spermatozoa of irradiated males or males treated with NP alone was significantly higher. At 24 h after the end of combined exposure (0.05 Gy + 25 mg/kg bw NP) the frequency of abnormal spermatozoa was higher than after exposure to 0.05 Gy of X-rays alone and to the control group, but lower than when compared to 25 mg/kg bw of NP alone. Just after the end of 2 weeks' exposure the frequency of abnormal spermatozoa of males receiving 0.10 Gy + 50 mg/kg bw NP was not significantly different from results of each agent alone, but significantly higher than the control. Five weeks after the end of exposure to doses of 0.05 Gy + 25 mg/kg bw NP the level of abnormal spermatozoa was significantly reduced compared to results of 0.05 Gy alone and to control animals. Five weeks following the end of combined exposure to higher doses of both agents, the frequency of abnormal spermatozoa non-significantly exceeded the results of each agent alone, but was significantly different compared to the control group. There was no correlation between NP and/or X-ray treatment and elevated percentage of specific sperm abnormality type. The small differences were rather between individual animals than between groups. Among animals from each groups folded forms (33-54% of all malformed spermatozoa) and amorphous (21-40%) were the most frequent. Spermatozoa lacking a hook and banana-like forms were less frequent, 4-16% and 2-5%, respectively. Multiple heads were very rare in the males from all groups.

Irradiation at the lowest dose of X-rays (0.05 Gy) decreased the frequency of DNA damage in male mice's haploid germ cells at 24 h following the end of 2-weeks' exposure as well as 5 weeks later, however only results at 24 h were significantly different from the control group. Two-weeks' irradiation with 0.20 Gy daily induced statistically significantly enhanced levels of DNA damage in germ cells. Exposure to each dose of NP induced an increased frequency of DNA damage at 24 h after the end of exposure. Both at 24 h and at 5 weeks later, there were statistically significantly decreased frequencies of DNA damage in germ cells of males exposed to the doses of 0.05 Gy + 25 mg/kg bw NP compared to control and NP alone values. At 24 h after the end of 2-weeks' exposure to 0.10 Gy + 50 mg/kg bw NP and 5 weeks later, the level of DNA damage was not significantly changed compared to the control group, however result at 24 h was significantly decreased compared to the males from group receiving 50 mg/kg bw NP alone.

4. Discussion

Exposure to two agents can lead to exacerbating effects or one agent can play a protective role in the induction of harmful effects by the other. Ionizing radiation affects

tissues by two mechanisms: directly, which is the result of energy deposition in critical molecular structures within the cell, and indirectly due to interactions between the critical molecules and the variety of free radicals ($OH^{\cdot}$ and $H^{\cdot}$) produced in the radiolysis of water [27]. Endocrine disruptors, including NP, may affect spermatogenesis by the following possible routes: an alteration of the hypothalamic pituitary functions, and/or the alteration of other functions leading to adverse effect on spermatogenesis and direct disruption of cells in the testis [28].

Environmental contaminants are able to induce oxidative stress by generating reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$). On the other hand, cells possess antioxidant enzymes such as superoxide dismutase and glutathione reductase, which are helpful in the neutralisation of ROS. Oxidative stress appears when there is an imbalance between the production and removal of ROS. Acute or chronic exposure to ionising radiation results in immediate formation of free radicals in living organisms leading to oxidative damage to DNA, proteins and lipids [29-31]. Also, oestrogenic compounds appear to produce ROS, especially hydrogen peroxide, at a level that disrupts DNA structure significantly in spermatozoa and in peripheral blood lymphocytes [32,33].

Postmeiotic stages such as elongating spermatids and spermatozoa are vulnerable to DNA damage because DNA repair in those stages is impossible. Following their release from germinal epithelium, the gametes can no longer take advantage of protection afforded to them by the Sertoli cells. The cytoplasm of spermatozoa is very small, thus polyunsaturated fatty acids bound the sperm plasma membrane are very susceptible to ROS attack. To protect them from the effect of ROS, spermatozoa, similar to other cells, are equipped with an antioxidant defence system [6,34]. The mechanism of action of NP on the production of ROS remains unclear. Probably nonylphenol disrupts the pro-oxidant antioxidant balance and increases the formation of ROS causing oxidative stress in epididymal sperm of rats [6]. As previously shown, nonylphenol may have short-term effects, as it causes elevated ROS production and decreased SOD and GSH activity, and also long-term effects as it causes apoptosis of Sertoli and germ cells [6,35,36]. Gong and Han [35] observed that NP induces an adverse oxidative stress in rat Sertoli cells. Because Sertoli cells are primarily supporting cells for spermatogenesis, damage to these cells may lead to the impairment of male reproduction.

In the present study, 2-weeks' exposure to NP decreased sperm counts and diminished sperm quality, especially after exposure of late spermatids and spermatozoa. It is known that vegetable oils are used for injection when prolonged diffusion in organisms is needed. In this paper, the level of NP in the animal body was not estimated, however the effects observed at 5 weeks might be partly dependent on this phenomenon. Several authors have observed that NP causes testicular abnormalities such as a lack of differentiation of seminiferous tubules and decreased sperm count and motility [37-39]. In the present study, the diminished sperm count after treatment with NP did not correlate with decreased testes weights. In contrast, Chitra *et al.* [6] after 45-days exposure of rats to doses of 1, 10 and 1000 μg/kg daily of NP observed decreased testes and epididymes weights as well as diminished sperm production. Nagao *et al.* [40,41] and Tyl *et al.* [42] did not observe the influence of NP on the quantity and quality of male gametes. As reported by Chitra *et al.* [6] in rats, an adverse effect of NP on the male reproductive system could be due to the induction of oxidative stress in the epididymal sperm.

Testes are one of the most radiosensitive organs. The damage caused by radiation may result in cell death or modifications that can affect the normal functioning of organs and tissues. The loss of male germ cells after exposure to ionising radiation is mainly attributed to apoptosis [43]. Sensitivity to radiation-induced apoptosis is characteristic for different types of cells present in testes [44,45]. Liu *et al.* [43] report that low-level radiation in the dose-range of 25-200 mGy induces a significant increase in apoptosis in both spermatogonia and spermatocytes. This finding may be one of the reasons for diminished sperm count after exposure of spermatogonia. The increased apoptosis is most likely associated with increased levels of Trp53 protein [43]. As Yin *et al*. [46] note, predominant expression of *Trp53* is possible in spermatocytes of mice and in spermatogonia and spermatocytes of rats and humans. It suggests Trp53 protein's potential role in initiating apoptosis to eliminate germ cells with genomic abnormalities. Irradiation at a dose range of 0.5-3.0 Gy causes a significant decrease in the number of germ cells at meiotic prophase (4C) [47,48]. Results of Beumer *et al.* [49,50] show that the dose of 4 Gy of X-rays activates the *Trp53* gene, which results in spermatogonia apoptosis 9-18 h after the treatment.

In the case of DNA damage induced in spermatogonia, if there is no repair or misrepair, this may lead to cell death or transmission of damage to the next cell stages up to mature spermatozoa. In our work, exposure to the lowest dose of X-rays (0.05 Gy) markedly decrease the level of DNA damage compared to the control group as measured by Comet assay just after the end of irradiation as well as 5 weeks later.

Although it was expected that there would be little or no response in DNA damage at 5 weeks after the end of exposure, DNA damage was assessed to remain the same at 24 h and 5 weeks later. Haines *et al.* [51,52] report that DNA damage is still present in spermatozoa of mice irradiated with 0.25-4 Gy of X-rays 24 or 120 days earlier.

Combined exposure to low doses of both agents (0.05 Gy + 25 mg/kg bw NP) markedly decrease the level of DNA damage compared to the effects of NP alone. At the dose of 0.05 Gy of X-rays, the response observed as the reduction of sperm count and the enhanced percentage of abnormal spermatozoa show opposite results compared to results of the Comet assay. Such differences are possible because Comet assay measures DNA damage in the population of haploid germ cells, *i.e.* late spermatocytes, spermatids and spermatozoa, whereas sperm count and morphology were observed in mature spermatozoa. The DNA damage-control biosystem is suppressed by high doses and stimulated by low-dose radiation. The hormetic effect of low-dose radiation may be explained by its increase of biosystem efficiency [53]. Pollycove and Feinendegen [54] show that low dose radiation induces DNA damage and stimulates physiological mechanisms which repair and remove damage. The above may explain the reduced DNA damage observed after exposure to a lower dose of X-rays (0.05 Gy) alone and in combination with NP (0.05 Gy + 25 mg/kg bw NP).

Ultraviolet B (UVB) induces the degradation of nonylphenol polyethoxylates due to the production of ROS. Higher doses of UVB destroy the benzene ring, which decreases the toxicity [55,56]. Combined exposure of late spermatids and spermatozoa to low doses of X-rays and nonylphenol (0.05 Gy + 25 mg/kg bw NP) reduces the effect induced by one or both agents acting alone, *i.e.* decreases percentage of abnormal spermatozoa as well as DNA damage. The above results suggest that low doses of X-rays stimulate the DNA damage-control system and in consequence repair of DNA damage caused not only by X-rays but also by NP. This response may be correlated with an increased anitioxidant capacity.

Results of combined exposure to high doses of both agents (0.10 Gy + 50 mg/kg bw NP) are different. In the present study, the combined exposure of late spermatids and spermatozoa as well as spermatogonia did not change sperm count or the frequency of abnormal spermatozoa compared to results obtained after exposure to each agent alone. On the other hand, after combined exposure of late spermatids and spermatozoa to higher doses of both agents, the level of DNA damage was markedly decreased compared to the results of NP alone. The mechanisms involved in this process might be explained in further investigations.

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Time	Doses	Number of animals	Mean body weight (g) ±SD	Mean testes weight (mg) ±SD	Relative testes weight	Sperm count x 10^6 ± SD	% of abnormal spermatozoa	Score of DNA content in the tail ± SD
24 h after the end of exposure	Control (without oil)	6	36.0±1.6	198.3±25.8	0.6±0.1	8.9±2.4	6.6± 3.6	129.6±28.5
	0.05 Gy	6	35.9±2.4	210.3±35.7	0.6±0.1	5.0±0.6*	13.5± 2.9*	85.9±19.6*
	0.10 Gy	6	37.5±3.7	176.8±43.7	0.5±0.1	4.3±0.5*	17.5±11.1*	158.8±16.0
	0.20 Gy	6	38.0±2.4	195.0±34.9	0.5±0.1	3.2±1.6*	14.5± 5.1*	193.0±20.6*
	Control (oil)	6	40.7±3.1	234.0±25.0	0.6±0.1	8.8±2.9	8.1± 1.6	142.2±28.0
	25 mg/kg NP	6	35.9±3.0	230.3±28.9	0.6±0.1	5.8±0.8*	28.4± 5.8*	210.0±19.7*
	50 mg/kg NP	6	38.0±4.3	223.7±42.4	0.6±0.1	5.2±1.1*	28.1± 6.5*	207.1±15.6*
	100 mg/kg NP	5	39.2±3.1	220.5±21.5	0.6±0.1	4.3±1.9*	25.3± 8.5*	215.9±33.8*
	0.05 Gy+25 mg/kg NP	6	35.6±2.9	219.8±50.4	0.6±0.1	6.4±1.8*	17.7±3.1*[a,b]	88.2± 7.4*[,b]
	0.10 Gy+50 mg/kg NP	6	40.2±3.5	220.5±21.5	0.6±0.1	3.8±2.2*	24.3±10.0*	129.7±44.0[b]
5 weeks after the end of exposure	Control (without oil)	6	39.2±2.4	242.3±16.8	0.6±0.1	8.3±1.3	8.7± 5.9	128.7±52.9
	0.05 Gy	5	36.8±1.9	228.0±37.7	0.6±0.1	5.8±2.1*	15.4± 2.5*	71.0±12.7
	0.10 Gy	6	39.5±3.2	186.8±39.2*	0.5±0.1	4.0±1.0*	21.8± 8.2*	170.4±29.2
	0.20 Gy	5	36.9±3.0	156.8±47.6*	0.4±0.1	2.2±1.0*	23.8±10.1*	146.2±63.6
	Control (oil)	6	40.4±2.9	217.7±23.0	0.5±0.1	7.9±2.4	8.8± 0.9	126.3±22.4
	25 mg/kg NP	6	38.3±3.1	199.7±36.0	0.5±0.1	4.9±1.6*	27.8±15.2*	145.6±28.4
	50 mg/kg NP	6	45.3±4.3	226.3±26.7	0.5±0.1	6.0±1.8	28.5± 7.8*	106.8±33.1
	100 mg/kg NP	6	39.6±2.4	212.3±23.0	0.5±0.1	6.0±1.7	23.6± 7.1*	138.5±14.1
	0.05 Gy+25 mg/kg NP	6	39.7±2.9	223.7±19.6	0.6±0.1	5.3±3.2	24.0± 5.0*[,a]	80.8±13.6*[,b]
	0.10 Gy+50 mg/kg NP	6	41.5±3.6	210.5±52.7	0.5±0.1	4.0±1.5*	30.7±11.9*	152.7±35.7

Table 1. The effects on the testis and germ cells following 2-weeks' exposure to X-rays, nonylphenol or a combination of both agents.

**P<0.05; compared to control by Student t-test*
[a]P<0.05; compared to appropriate dose of X-rays by Student t-test
[b]P<0.05; compared to appropriate dose of NP by Student t-test

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