

Protective Role of Vitamin C on Sperm Characteristics and Testicular Damage in Rats Exposed to Radiation

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Summary

The aim of this study was to investigate the protective effects of vitamin C on sperm characteristics and testes in male rats exposed gamma radiation (2 Gy). A total of 21 adult male wistar albino rats (8 weeks of age, weighing 180-220 g) were divided into three groups. Control, radiotherapy (received scrotal γ -radiation of 2 Gy as a single dose) and radiotherapy + vitamin C treated rats (during the 55 days after irradiation, 500 mg vitamin C/500 ml water daily orally). Testes samples from all groups were taken at day 55 post-irradiation and epididymal sperm characteristics, all-genital organs weights and testes histology were evaluated. Radiotherapy decreased significantly the sperm motility, concentration, left testes and epididymis weights, Johnsen's biopsy score and seminiferous tubular diameter but it increased the sperm head defects as compared to the Control group ($P<0.05$). The administration of vitamin C only reduced the harmful effects of radiotherapy on the seminiferous tubular diameter ($P<0.05$). It has been concluded that the radiotherapy may cause alteration in the genital organ weights, spermatologic and hystologic parameters in rats and administration of vitamin C may be slightly beneficial for seminiferous tubular diameter following testicular irradiation during the radiotherapy.

Keywords: Radiation, Vitamin C, Sperm, Testes, Rat

Radyasyona Maruz Bırakılmış Sıçanlarda Spermatozoa Özellikleri ve Testislerde Yarattığı Hasar Üzerine Vitamin C'nin Koruyucu Rolü

Özet

Bu çalışmada, vitamin C'nin gamma radyasyon (2 Gy) uygulanmış erkek sıçanlarda spermatolojik özellikler ve testis üzerine olan koruyucu etkilerini saptamak amaçlandı. Çalışmada, 8 haftalık yaşta ve ortalama 180-220 g canlı ağırlığa sahip toplam 21 adet Wistar Albino ırkı erkek sıçanlar kullanıldı. Sıçanlar rastgele 3 eşit gruba ayrıldı. Gruplar; Kontrol, radyasyon tedavi (scrotal bölgeye tek doz 2 Gy radyasyon ışınması yapıldı) ve radyasyon tedavi + vitamin C grubu (radyasyon ışınmasından sonraki 55 gün boyunca, günlük oral olarak, 500 mg/500 ml, vitamin C/su) şeklinde oluşturuldu. Tüm gruplardaki sıçanlardan radyasyon tedavisi sonrası 55. gününde genital organlar alınarak spermatolojik özellikler, genital organ ağırlıkları ile testis dokusuna ait histolojik özellikler değerlendirildi. Radyasyon tedavisi ve kontrol grubu, spermatolojik, morfometrik ve histolojik özellikleri bakımından karşılaştırıldığında, Radyasyon tedavisi uygulanan hayvanların motilite, konsantrasyon, sol testis ağırlığı, epididimis ağırlığı, seminifer tubul çapı ve Jhonsen biyopsi skorlarında istatistiksel olarak önemli düzeyde azalma ve anormal spermatozoon baş sayısında ise artış saptandı ($P<0.05$). Çalışmada vitamin C uygulamasının sadece seminifer tubul çaplarında radyasyonun zararlı etkisini önemli düzeyde azalttığı tespit edildi ($P<0.05$). Sonuç olarak, radyasyon tedavisinin sıçanlarda genital organ ağırlıkları, spermatolojik özellikler ve histolojik parametrelerde birtakım değişikliklere neden olduğu ve radyasyon uygulamasını takiben tedavi amaçlı vitamin C uygulamasının ise testislerdeki seminifer tubul çaplarındaki iyileşme gibi hafif düzeyde koruyucu etki gösterdiği tespit edildi.

Anahtar sözcükler: Radyasyon, Vitamin C, Spermatozoa, Testis, Sıçan



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INTRODUCTION

Exposure to a wide variety of external factors including certain drugs, environmental pollutants, heavy metals and ionizing radiation has been linked to a variety of adverse health outcomes that may have significant animal and public health consequences. Various tissues and organ systems of an individual differ in their response to radiation and as a rule; systems with proliferating cells are most sensitive. The testes which generates male germ cells is known to be a radiosensitive organ in the body. Testicular damage after local or whole-body irradiation by external sources (gamma radiation) has been well documented in both animals and man [1-3]. Ionizing radiation damages the biological systems in a major way by generating reactive oxygen species (ROS). These ROS interact with biological molecules producing toxic free radicals leading to lipid peroxidation and DNA damage [4,5]. ROS can also alter the balance of endogenous protective systems, such as glutathione and enzymatic antioxidant defence systems [6,7]. The endogenous antioxidant defences are inadequate to reduce the radiation-induced free radicals. Appropriate antioxidant intervention may inhibit or reduce free radical toxicity and thus offer protection against radiation. Because exogenous compounds contribute to the antioxidant capacity of the system, dietary antioxidants supplementation may have the ability to decrease an individual's susceptibility to oxidative damage. As such, various antioxidant supplements such as vitamins, carotenoids, carnitine, alpha lipoic acid and polyphenols [8-10], as well as the dietary consumption of high amounts of antioxidant-rich foods [11,12] have been shown to decrease an individual's susceptibility to oxidative damage. Vitamin C, also known as ascorbic acid, is a very important water-soluble vitamin. It is essential for preserving optimal health and it is used by the body for many purposes. Vitamin C is a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (e.g., smoking). The radioprotective effect of ascorbic acid seems to be due to its interactions with radiation induced free radicals [13]. Ascorbic acid pre-treatment inhibited the radiation-induced elevation in lipid peroxidation [14]. It protected the mice against radiation induced sickness, reduced the mortality and improved the healing of wounds after exposure to whole body gamma-radiation [15]. But some reports indicate that the capacity of vitamin C to reduce harmful effects of radiation is inconclusive [16-18], therefore further studies are needed. The aim of the present study was to evaluate the radioprotective effect of vitamin C on gamma-radiation-induced damage to testes and epididymal spermatozoa.

MATERIAL and METHODS

The experiment was performed in accordance with guidelines for animal research from the National Institutes of Health and were approved by the Dicle University Ethics Committee on Animal Research (approval no: 2012/36).

Chemicals

Vitamin C (Redoxon Ampul 500 mg/5 ml) was obtained from Roche, İstanbul, Turkey. It was dissolved in tap water at concentrations of 500 mg/500 ml and *ad libitum*, were given orally before and after irradiation.

Animals

Wistar albino male rats were obtained from the Experimental Animal Center of Dicle University. The animals were housed at 21°C under a 12 h light-dark cycle and were allowed tap water and standard pellet diet for rats (Elazığ Yem Inc. Elazığ, Turkey).

Experimental Design

Twenty-one rats (8 weeks old, weighing 180-220 g) were randomly divided into three groups of seven rats each. Control group did not receive any treatment. The radiotherapy group received scrotal γ -radiation of 2 Gy and radiotherapy + vitamin C group received scrotal γ -radiation plus vitamin C. The dose of vitamin C used in the present study was based on previous reports [19]. Because of vitamin C has been reported to be well tolerated without any toxic effects over the dose range 100-200 mg/d [19], its middle dose was chosen in our study protocol.

Scrotal-Irradiation

Prior to radiotherapy, the rats were anesthetized with xylazine/ketamine (10/90 mg/kg, i.p.) and immobilized from their 4 extremities on a tray. Irradiation was delivered by an ALCYON-II model cobalt-60 teletherapy unit (General Electric/GE Healthcare) at a source-surface distance of 80 cm. A single dose of 2 Gy radiations was given at a depth of 1 cm (thickness) with a dose rate of 0.4 Gy/min to an area of 30 x 30 cm of the scrotum in a supine position.

Sample Collection

All rats were kept under identical conditions for fifty days with free access to food and water. At the end of the fifty days, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine HCl (Rompun, Bayer) and 75 mg/kg ketamine HCl (Ketalar, Pfizer) for anesthesia. Afterward, the testes of each rat were located and the testes, epididymis, seminal vesicles, and ventral prostate were removed, cleared of adhering connective tissue. The left testes, epididymis, seminal vesicles, and ventral prostate weight were evaluated along with epididymal sperm concentration, sperm motility, and sperm

morphology. On the other hand, right testes was fixed in 10% Bouin fixative for histopathologic examinations.

Epididymal Sperm Count

The epididymis was finely minced with anatomical scissors in 10 ml of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:10 with a solution containing 5 g sodium bicarbonate, 1 ml formalin (35%), and 25 mg eosin per 100 ml of water. Total sperm number was determined using counting chambers. The cells were counted with the help of a light microscope (magnification, 200x).

Epididymal Sperm Motility Evaluation

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 ml with Tris buffer solution. A slide was placed on phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from 3 different fields in each sample. The mean of the 3 estimations was used as the final motility score. Samples for motility evaluation were kept at 37°C.

Epididymal Sperm Morphology Evaluation

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin-nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400x magnification. Two-hundred spermatozoa were examined on each slide, and the head and tail and total abnormality rates of spermatozoa were expressed as percent.

Hystological Analysis

The right testes from the rats were placed in 10% Bouin solution for 24 h for fixation and further pathologic examination. After fixation, the sections were subjected to routine histologic tissue preparation and dehydrated and embedded in paraffin. Paraffin blocks were sliced to 5- μ m thickness with a microtome and the slices were subjected to Periodic Acid Schiff-Hematoxylin (PAS-H) staining and were then examined under a light microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan). For each sample, 100 randomly selected seminiferous tubule diameters were measured. In addition, for each section, 100 randomly selected seminiferous tubules were evaluated using the Johnsen classification^[20].

Statistical Analyses

Statistical analyses were performed with SPSS for Windows 7 version 9.0 (SPSS, 1993) Results of the parameters were analyzed by One-way ANOVA procedure). Differences between means were tested by Tukey's least significant

difference when a difference between groups was significant. Data are presented as means and SEM. NC.

RESULTS

Epididymal Sperm Characteristics

When we evaluated the spermatologic results between the groups, it was determined that sperm motility and concentration of the control group were significantly higher than in the radiation group ($P < 0.05$; [Table 1](#)). But, vitamin C treatment did not prevent these decreases in motility and concentrations depend on irradiation ([Table 1](#)). In addition, the total morphologic defects and tail defects were similar in the control group and all the others groups, but it was determined that radiation and vitamin C-treated group had a higher ratio of spermatozoon head defect than control group ($P < 0.05$; [Table 1](#)).

Genital Organs Weight

Ionizing radiation no caused significant alterations in prostatic and seminal vesicles weight of the rats. In contrast, testicular irradiation resulted in significant decreases in testes and epididymis weights at 55 days post-irradiation ([Table 2](#)). Vitamin C treatment did not prevent these decreases in testes and epididymis weights depend on irradiation ([Table 2](#)).

Morphometric Parameters

The tubular diameter and the Johnsen's biopsy score were measured and a summary of these results are presented in [Table 2](#). As compared with control testes, all values of morphometric parameters were statistically significantly reduced in the irradiated testes at post-irradiation time periods. Vitamin C treatment significantly increased the diameter of seminiferous tubules at 55 days post-irradiation compared to radiation group. But, there was not difference between vitamin C plus radiation group and control group for the Johnsen's biopsy score.

Table 1. Effects of Vitamin C treatment on sperm characteristics in radiation treated rats

Tablo 1. Radyasyon uygulanmış ratlar da Vitamin C'nin spermatolojik özellikler üzerine etkileri

Measurements	Treatments		
	Control	Radiation	Vitamin C
Motility (%)	73.6 \pm 4.32 ^a	55.7 \pm 5.50 ^b	38.6 \pm 4.46 ^b
Concentration (x10 ⁶ /ml)	46.9 \pm 4.45 ^a	22.7 \pm 4.32 ^b	18.9 \pm 1.77 ^b
Tail Defects (%)	24.1 \pm 1.54	24.4 \pm 1.54	22.1 \pm 1.45
Head Defects (%)	3.4 \pm 0.84 ^b	8.0 \pm 0.61 ^a	7.1 \pm 0.85 ^a
Total Morphologic Defects (%)	27.6 \pm 1.41	32.4 \pm 1.87	29.3 \pm 0.77

The values given for continuous variables are Mean \pm Standard error
^{a,b,c} Means within line with different superscripts differ significantly ($P < 0.05$)

Table 2. Effects of Vitamin C treatment on genital organ weight and morphometric parameters in radiation treated rats

Tablo 2. Radyasyon uygulanmış ratlar da Vitamin C'nin genital organ ağırlıklarını ve morfometrik parametreler üzerine etkileri

Measurements	Treatments		
	Control	Radiation	Vitamin C
Left Testes (g)	1.36±0.02 ^a	0.91±0.04 ^b	0.95±0.03 ^b
Left Epididymis (g)	0.56±0.0 ^a	0.43±0.01 ^b	0.47±0.04 ^{ab}
Prostate (g)	0.73±0.04	0.77±0.08	0.84±0.03
Seminal vesicles (g)	1.30±0.07	1.31±0.16	1.50±0.08
Johnsen's Biopsy score (/10)	9.8±0.03 ^a	8.4±0.12 ^b	8.5±0.11 ^b
Seminiferous Tubules Dia. (µm)	355.7±5.39 ^a	298.5±4.47 ^c	326.9±2.74 ^b

The values given for continuous variables are Mean±Standard error
^{a,b,c} Means within line with different superscripts differ significantly (P<0.05)

Light Microscopic Findings

On histopathological examination, control rat testes showed normal morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen (Fig 1a, d). In contrast to control, the arrangement of the cells was disturbed in the seminiferous tubules of gamma irradiated rats. Germinal epithelial cells were separated from each other and the tubular basement membrane. There was desquamation of germinal cells

and consequent appearance of irregular spaces in the epithelium and spermatogenic cells were also decreased. The numbers of spermatozoa in the lumen were significantly low (Fig 1b, e). Vitamin C treatment improved the radiation-induced histopathological changes in rat testes. The tubular diameter in the vitamin C-treated rats was higher compared to the radiotherapy group and the disturbance in the arrangement of the cells was slight in this group (Fig 1c, f).

DISCUSSION

This study was undertaken to investigate the radio-protective effect of vitamin C on gamma-radiation-induced damage to testes and epididymal spermatozoa. We have determined the extent of changes in rat testes structure and epididymal sperms parameters following 60Co γ-Radiation. Radiation treatment no caused significant alterations in prostat and seminal vesicles weight of the rats. In contrast, testicular irradiation resulted in significant decreases in testes and epididymis weights at 55 days post-irradiation (Table 2). Various tissues and organ systems of an individual differ in their response to radiation and as a rule; systems with proliferating cells are the most sensitive. The testes which generates male germ cells is known to be a radiosensitive organ in the body. The loss of testes weight following irradiation has been reported by several workers [21,22]. The loss in testes weight might be in part

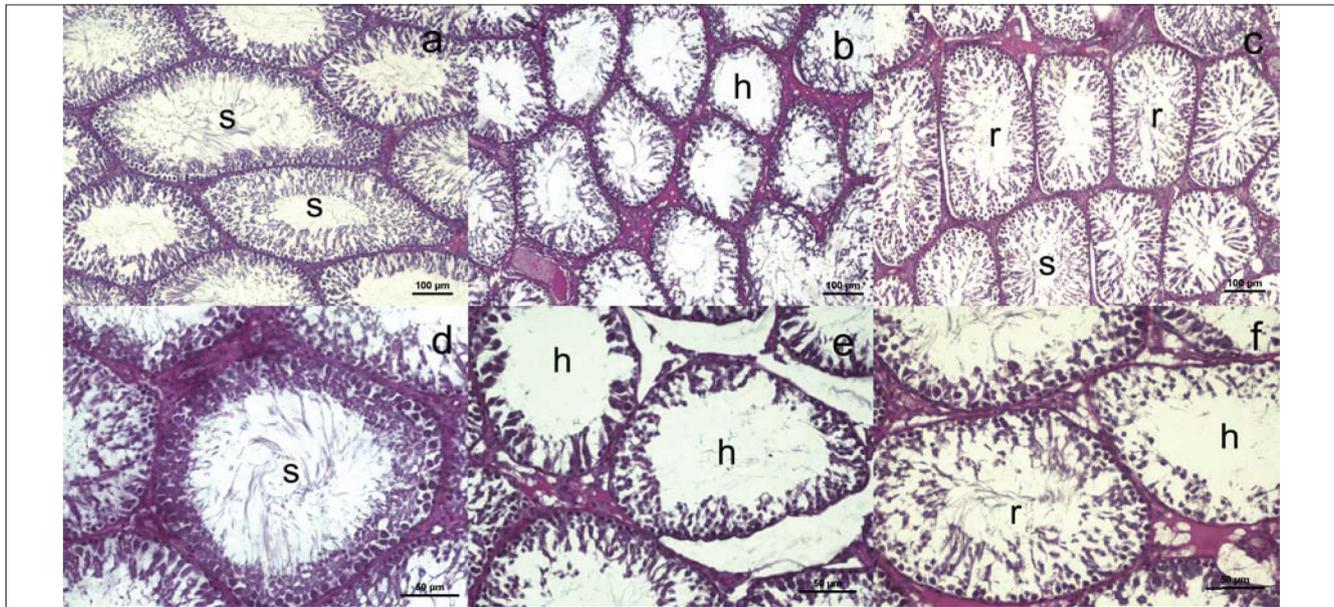


Fig 1. Testes histological sections: Normal spermatogenesis and seminiferous tubular organisation (s) in control group (a and d). In addition to tubular atrophy, hypospermatogenesis (h) and tubular degeneration is observed in 2 Gy Radiotherapy induced group (b and e). Although degenerated tubules and hypospermatogenesis, normal spermatogenesis (s) and regenerative tubules (r) are observed in radiotherapy and vitamin C induced group (c and f). In comparison with Radiotherapy induced group, tubular atrophy is decreased in Radiotherapy and Vitamin C dietary group

Şekil 1. Testes histolojik kesitleri: Kontrol grubunda spermatogenezis ve semimifer tübül organizasyonu normal gözlemlendi (şekil a ve d). 2 Gy Radyasyon uygulaması yapılan grupta hipospermatogenezis ve tübüler dejenerasyonun (h) yanı sıra tübüler atrofiye göze çarptı (şekil b ve e). Radyasyon ve vitamini c uygulanan grupta dejenere olmuş ve hipospermatogenezisli (h) tübüllerin yanı sıra rejeneratif profil gösteren (r) ve normal spermatogenezis gözlenen semimifer tübüller (s) gösterilmiştir (c and f). Vitamin C diyeti uygulanan grupta Radyasyon grubuna oranla tübüler atrofiyenin azalmış olduğu göze çarpmaktadır

due to loss of the body weight caused by radiation. The testes weight loss is also associated with cellular damage in the testes. Radiation inflicts lethal damage to spermatogonia, which adversely influences spermatogenesis and subsequently the sperm counts. In our study, we determined that sperm concentration and motility of the control group were significantly higher than in the radiation group ($P < 0.05$; [Table 1](#)). The sperm morphology is a characteristic of the genotype of the spermatogenic cells. Radiation is known to induce detrimental genotypic changes in the spermatogenic cells that affect the phenotype of the sperm ^[23]. Similarly, we found that radiation group had a higher ratio of spermatozoon head defect than control group ($P < 0.05$; [Table 1](#)). In addition to sperm parameters, both seminiferous tubule diameter and Johnsen's biopsy score are good predictors of fertility status ^[20]. In this study, as compared with control group testes, the tubular diameter and the Johnsen's biopsy score were significantly reduced in the irradiated testes. In histological examination under a light microscope, control rat testes showed normal morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen ([Fig 1a, d](#)). In contrast to control, the arrangement of the cells was disturbed in the seminiferous tubules of irradiated rats. There was desquamation of germinal cells and consequent appearance of irregular spaces in the epithelium and spermatogenic cells were also decreased. The numbers of spermatozoa in the lumen were significantly low ([Fig 1b, e](#)). This finding is in agreement with the previous reports documenting the degenerative effects of irradiation on spermatogenesis in bovine, mouse and rat ^[24,25].

The bone marrow and other tissues were often used to test the radioprotective action of different compounds, while there is no sufficient information concerning the modulating effect of vitamin C in testes and sperm of irradiated rat. The results of our present study indicate that orally administered vitamin C did not show protective effect against genetic damage (sperm motility, concentration and morphological defect) induced *in vitro* by exposure to gamma radiation. But, vitamin C treatment significantly increased only the diameter of seminiferous tubules at 55 days post-irradiation compared to radiation group. All these results suggested that vitamin C may be limited protective effects under different experimental condition. Previous study indicated that vitamin C at low concentration could protect DNA from radiation-induced damage in mouse bone marrow cells, but at high concentration enhanced the radiation-induced effect ^[26]. Alike, it was reported that vitamin C alone (0.01 μmol , 1 μmol) did not reduce radiation induced apoptosis in human lymphoblastic cell line, when given before 3 Gy gamma irradiation, but it showed radioprotective effect only at 0.01 μmol concentration after irradiation ^[27]. The reasons for the lack of radioprotection by dietary vitamin C are unclear. But, all of these findings suggest that presence or absence of radioprotective effect of vitamin C in examinations

depend on concentration of vitamin C in biological environment, time of administration, radiation dose rate and type of radiation (low or high). In our experiment, the vitamin C dissolved in water, were administered orally as, one day before irradiation, so the vitamin C was present in the tissue with nontoxic and appropriate concentrations before production of free radicals by irradiation. Lipid peroxidation takes place after irradiation or free radical attack ^[28]. Vitamin C is an antioxidant molecule and prevents lipid peroxidation in plasma and inside the cell ^[29,30]. Thus, the histological findings in rat treated with vitamin C in comparison with the radiation group suggest that vitamin C exert its radioprotective effect on the diameter of seminiferous tubules ([Table 2](#)). Therefore, also it was shown that vitamin C could have a slight radioprotective effect on rat testes.

In conclusion, present results confirm that the sperm parameter values and genital organ weights significantly decreased in the irradiated rat testes compared with the control testes. Treatment with vitamin C to irradiation decreases the germ cell apoptosis, suggesting that vitamin C can protect testes from radiation injury. The present results suggest that vitamin C may be beneficial to spermatogenesis and infertility following testicular irradiation by decreasing germ cell apoptosis. However, further investigations and clinical studies are required to elucidate the exact mechanism of antiapoptotic effects of vitamin C in testes.

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