Original Article

The Protective Effect of Curcumin on the Proliferation and Colonization of Spermatogonial Stem Cells in Gamma-Irradiated Rats

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Abstract

Background & Objective: One of the side effects of radiotherapy can be damage to spermatogonial stem cells that may lead to spermatogenesis disorders and sterility. Protective effects of curcumin on normal cells against radiotherapy side effects have already been shown. In the current study, the protective effects of curcumin on the spermatogonial stem cells against gamma radiation were evaluated.

Materials & Methods: This study was done on 50 adult rats in 10 experimental groups. Four groups were injected 0, 25, 50, or 100mg/kg of curcumin in 1ml olive oil for 15 days intraperitoneally, then exposed to radiation at 2 Gy on the next day. Also, four groups were treated like above but without radiation; and two groups as control with and without radiation. The day after radiation, all of the rats were euthanatized, their testes were removed, and they underwent enzymatic digestion to co-culture spermatogonial stem cells. After 12 days, the colonization of spermatogonial stem cells was assessed.

Results: There was a significant decrease in the colonization of spermatogonial stem cell proliferation in groups that had taken radiation but not curcumin. There was a significant increase in the colonization of spermatogonial stem cells in the group which had taken radiation whit maximum curcumin compared with the other irradiated groups and was similar to non-irradiated control animals. Colonization of spermatogonial stem cells in non-irradiated animals treated with curcumin had increased compared with control groups.

Conclusion: Injection of curcumin can protect spermatogonial stem cells against radiation. Thus, curcumin may prevent sterility in men who undergo radiotherapy.

Keywords: Curcumin, Radiation therapy, Radiation protective agents, Spermatogonial stem cells, Sterility

Introduction

One of the therapeutic methods in cancer therapy is radiotherapy. Radiotherapy uses high-energy rays, usually x-rays and similar rays (such as gamma), to treat disease. It destroys cancer cells in the area that is treated. Normal cells can also be damaged by radiotherapy (1).

In nature, we are also exposed to natural electromagnetic radiation (2). Gamma rays induce DNA damage and oxidative stress. Oxidative stress is the inappropriate exposure to reactive oxygen species (ROS) and results from the imbalance between prooxidants and antioxidants leading to cell damage (damage of lipids, proteins, carbohydrates, and nucleic acids) and tissue injury, and eventu-ally cell death (3, 1).

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Much research has been conducted into the subject of compounds that can protect normal cells from the harmful effects of radiation without any effects on cancerous cells. Some natural or indus-trial compounds seem to be used to protect patients exposed to radiotherapy or individuals who work in a radiotherapy center against radiation side effects. One of these compounds is curcumin. Curcumin is a diarylheptanoid (also known as Diphenylheptanoids). It is the principal curcuminoid of turmeric, which is a member of the ginger family (Zingiberaceae) (1). Its chemical name is (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione. Curcumin incorporates several functional groups. The aromatic ring systems, which are phenols, are connected by two α,β-unsaturated car-bonyl groups. The diketones form stable enols and are readily deprotonated to form enolates; whereas the α,β-unsaturated carbonyl group is a good Michael acceptor and undergoes nucleophilic addition. Its hydroxyl groups need antioxidant activity and methoxy groups for anti-inflammatory activity. Curcumin is insoluble in water and ether and soluble in ethanol, dimethyl sulfoxide, and acetone.

It is more than one thousand years that curcumin has been used in traditional medicine. It has been traditionally used in Asian countries as a medicinal herb due to its antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer properties (4).

Curcumin has antioxidant and anti-inflammatory properties. Two primary mechanisms explain the majority of their effects on the various conditions. Curcumin has been shown to improve systemic markers of oxidative stress. There is evidence that it can increase serum activities of antioxidants such as superoxide dismutase (SOD), a significant effect of curcuminoids supplementation on all investi-gated parameters of oxidative stress including plasma activities of SOD and catalase, as well as serum concentrations of glutathione peroxidase (GSH) and lipid peroxides (5). Curcumin's effect on free radicals is fulfilled by several different mechanisms. It can clean up different forms of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS, respectively) (6). It can regulate the activity of GSH, catalase, and SOD enzymes active in the neutralization of free radicals (7, 8). Also, it can inhibit ROS-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine hydro-genase/oxidase (7).

Curcumin applies its protective effect against free radicals produced by radiation by its antioxidant effect. However, it seems that it has a dose-dependent dual effect exposure to radiation. A radiosensitizer for various malignancies and a radioprotector for normal tissues. Examples of radiosensitization have been seen for various cancers including Pediatric, lymphoma, sarcoma, prostate, gynecologic, pancreas, liver, colorectal, breast, lung, head/neck, and glioma. But almost all evidence of radiosensitization comes from laboratory data, and clinicallyapparent benefits of curcumin as a ra-diosensitizer are yet to be determined (9). The evidence for curcumin's radioprotection is less varied as compared to radiosensitization.

One of the side effects of radiotherapy is the negative effect on the survival of spermatogonial stem cells and subsequent spermatogenesis that eventuate in sterility. Since many people who are exposed to radiotherapy are in the age of fertility, the protection of their germ cells against the harmful effect of radiation can save their fertility. In this study, we survey the radioprotective effect of curcumin on the colonization and proliferation of spermatogonial stem cells of rats exposed to 2 greys of gamma-ray.

Materials & Methods

Ethical considerations

This study was approved by the Ethics Committee of the veterinary medi-cine faculty, Tehran University, Tehran, Iran (ethics committee reference number: 750016/6/26).

The study included 50 healthy experimental male rats (aged 12-14 weeks and weighed 170-200g). The rats were placed in close cells as 10 experimental groups.

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Plant materials

Curcumin was prepared as 95% sterile powder (Barij Essence™) and added to autoclaved sterile olive oil in the below-mentioned amounts and incubated in a shaker for 24 hours at 37© to dissolve completely.

Evaluation of LD50 of curcumin

Before doing the main experiment the LD50 of curcumin was assayed by intraperitoneal injection of olive oil dissolved curcumin in 15 rats that LDL50 was found to be 200mg/kg body weight.

Experimental groups

In the main experiment, the rats were divided into 10 groups as follows: group 1- Control (without any treatment). group 2- Radiation Control (expose to radiation without other treatments). Groups 3, 4, 5, and 6 - as curcumin controls which were treated with 0 (as solvent control), 25 (low or minimum) 50 (medium), and 100mg/kg (high or maximum) curcumin for 15 days. Groups 7, 8, 9, and 10- radiation+ curcumin as main treatment groups, were treated with 0 (as solvent control), 25 (low or minimum) 50 (medium), and 100mg/kg (high or maximum) for 15 days and then exposed to radiation. Prescription of olive oil (with or without curcumin) was as an intraperitoneal injection.

Irradiation of Rats

Radiation exposure was done at 2 Gy on the next day of the completion of injections at the radiotherapy section of Imam Khomeini hospital (Tehran, Iran). All irradiated rats received Ketamine 75-95 mg/kg and Xylazine 5 mg/kg before irradiation. In this study, cesium137 was used for gamma irradiation. On the day after irradiation, the rats were sacrificed by intravenous administration of ketamine-xylazine. Then, their testicles were removed, weighed, and considered for anomalies.

Isolation and Culture of Spermatogonial Stem Cells

Spermatogonial stem cells were extracted from one testis of every rat as follows according to Izadyar (10). The testes were rinsed with 70% ethanol and then with sterile PBS. Tunica albuginea was removed and the testis tissue was minced and then was washed with PBS several times. For the first step of enzymatic digestion, the minced tissue was incubated in DMEM containing 1% pen-strep, 0.5mg/ml Collagenase Type IV, and 0.5mg/ml Trypsin in a shaker incubator (37 ©) for about 30 min. After digestion of interstitial tissue, the tubes were centrifuged at 400g for 2min. The supernatant was removed and the sediment underwent the second step of digestion by incubating in DMEM contain-ing DMEM containing 1% pen-strep, 0.5mg/ml Collagenase Type IV, Hyaluronidase type II 0.5 mg/ml, and 0.5mg/ml Trypsin in a shaker incubator (37 ©) for about 30 min. After this to stop enzymatic digestion FBS 20% was added. To extract cells from seminiferous, tubes pipetting was done for two min. Then, tubes were centrifuged at 50g for three minutes to precipitate seminiferous tubes debris and then the supernatant was moved to another tube and was centrifuged at 400g for 4 minutes. The pellet was resuspended in one ml of DMEM and after cell counting and viability assay were cultured in three wells of the 24-well cell culture plate in DMEM containing 20% FBS and 10% pen-strep and incubated in 37© and 5%CO_2 for 8 days. The culture media was changed every 3 days. After 12 days, stem cell colonies in each well were counted and each colony size was calculated under an inverted microscope by an ocular micrometer.

Data analysis

Data were analyzed using statistical software SPSS® (ver 18) by ANOVA, expressed as the mean±SEM and when ANOVA revealed a significant effect, values were compared by the least significant difference pairwise multiple comparison post hoc tests (Duncan). Differences were considered statistically significant at $P < 0.05$.

Results

In this experience radioprotective effect of curcumin on proliferation and colonization of spermatogonial stem cells in rat was surveyed. No significant difference in body weight gain in testes' weight was seen between the treated and control groups (Chart 1 and Table 1).

Table 1. Comparison of mean values ± standard deviation of testes weight in the experimental groups. No signifi-cant difference in testes' weight was seen between the treated and control groups

Groups	Testes Weight (gr)
Control	2.6±0.5
Radiation control	2.2±0.4
Solvent control	2.4±0.5
Radiation+solvent	2.4±0.5
curcumin-min	2.6±0.8
curcumin-mid	2.2±0.4
curcumin-max	2.4±0.5
curcumin-min-rad	2.8±0.8
curcumin-mid-rad	2.8±0.8
curcumin-max-rad	2.2±0.4

Min=minimum min=medium max=maximum rad=Radiation exposure

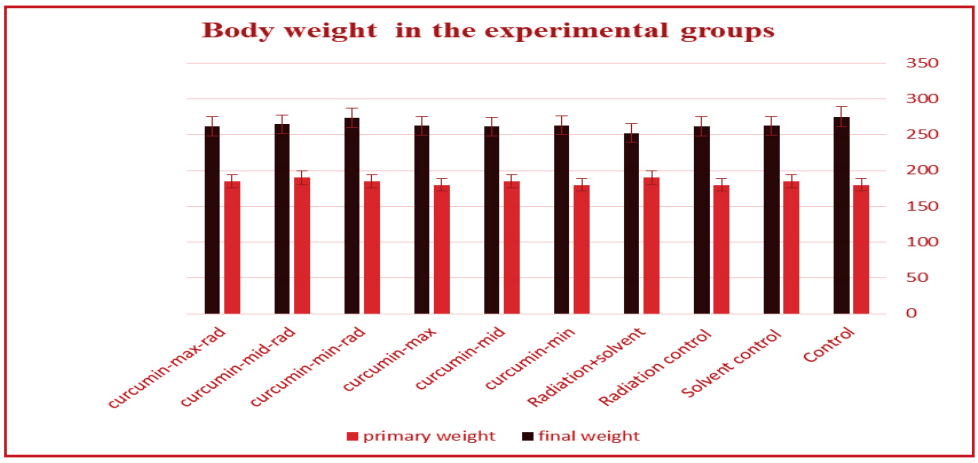

Chart 1. Comparison of body weight between treated and control groups before and after treatment. No significant difference in body weight gain was seen between the treated and control groups. min= minimum mid=medium max=maximum rad=Radiation exposure

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However, enlargement and congestion of epididymis were observed in curcumin-treated groups. In irradiated rats there was petechiae in the surfaces of the testes.

The mean number of spermatogonial stem cell colonies and mean total colony size (Colony di-ameter) of different treatments are shown in Chart 2 and Chart 3. There was no significant difference between control, solvent control, and low curcumintreated in the numberof colonies. Also, there was no significant difference between irradiated control, irradiated solvent, and irradiated low curcumintreated groups. But the number of colonies in irradiated medium and high curcumintreated groups was significantly more than the irradiated control, irradiated solvent, and irradiated low curcumintreated groups. Also, it was more in the irradiated high curcumintreated group than the medium curcumintreated one. As expected, the number of colonies in irradiated groups was significantly lower than their nonirradiated counterparts. As it is shown in Chart 2, the number of colonies in the high curcumintreated group was statistically identical to the nonirradiated control group. Also, the number of colonies was more in nonirradiated medium and high curcumintreated groups than in the nonirradiated control group.

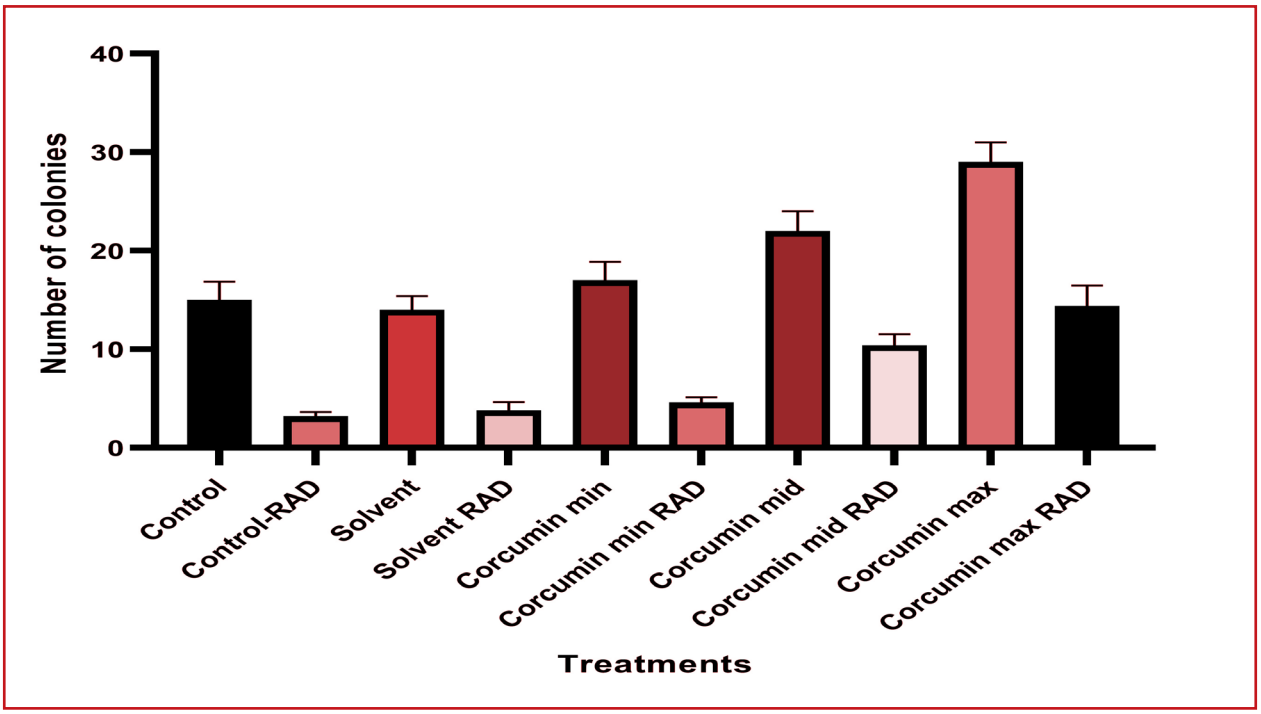

Chart 2. The number of spermatogonial stem cell colonies in controls and treatment groups. The number of colo-nies in irradiated groups was significantly lower than their non-irradiated counterparts. The number of colonies in the high curcumin-treated group was identical to the non-irradiated control group. Different letters indicate statistically sig-nificant differences at $p < 0.05$. Bars with no common letters are significantly different ($p < 0.05$). min=minimum mid=medium max=maximum rad=Radiation exposure

As it is shown in Chart 3, the statistical findings which were seen in the number of spermatogonial stem cell colonies in controls and treatment groups almost were seen in the size of spermatogo-nial stem cell colonies. There was no significant difference between control and solvent control, in the size of spermatogonial stem cell colonies. Also, there was no significant difference between irradiated control, irradiated solvent, and irradiated low curcumintreated groups. The size of colonies in irradiated medium and high curcumintreated groups was significantly more than the irradiated control,

irradiated solvent, and irradiated low curcumintreated groups. However, the size of colonies was larger in the irradiated high curcumintreated group than the medium curcumintreated one (P= 0.0401). Also, the size of colonies in irradiated groups was significantly lower than their nonirradiated counterparts. There was no significant difference between the high curcumintreated group and the non-irradiated control group. Also, the size of colonies was more in nonirradiated medium and high curcumintreated groups than in the nonirradiated control group.

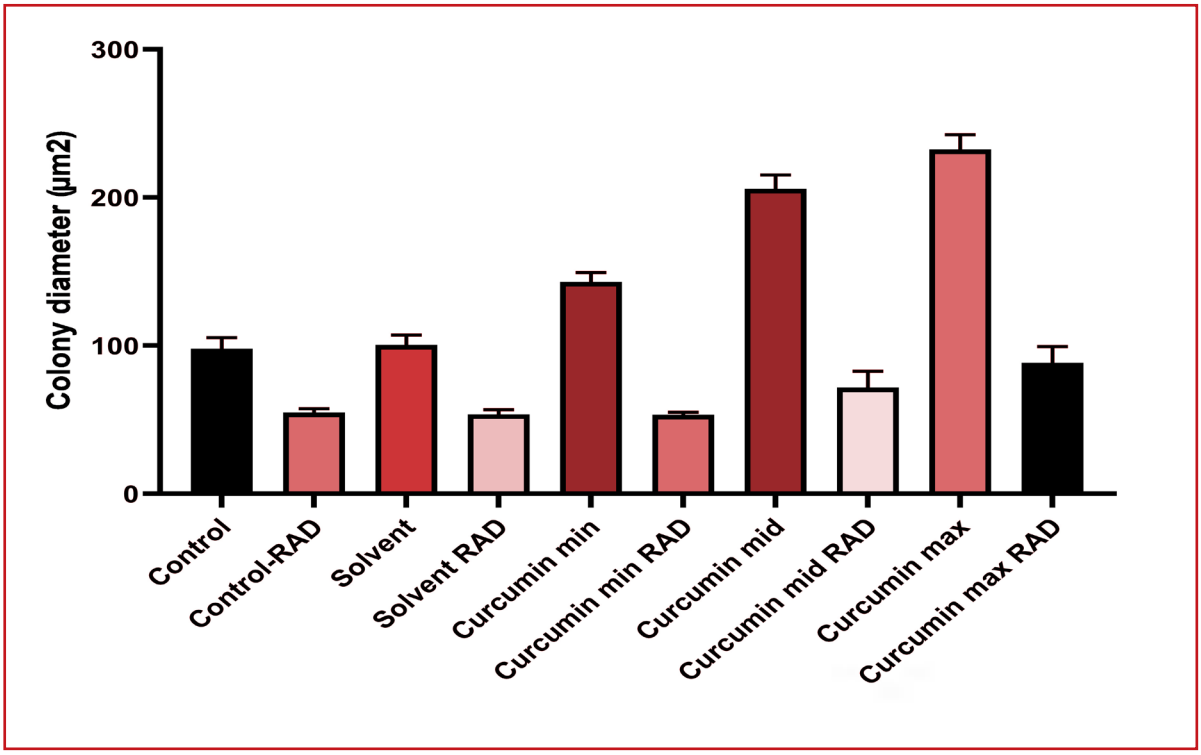

Chart 3. The size of spermatogonial stem cell colonies in controls and treated groups. There was no significant dif-ference between the high curcumin-treated group and the non-irradiated control group. Also, the size of colonies was more in the non-irradiated medium and high curcumin-treated groups than in the non-irradiated control group. Differ-ent letters indicate statistically significant differences at $p < 0.05$. Bars with no common letters are significantly differ-ent ($p < 0.05$). min=minimum mid=medium max=maximum rad=Radiation exposure

Discussion

In recent years, curcumin has been shown to have a wide range of pharmacological activities, in-cluding antioxidant, antiinflammatory, antimutagen, and anticarcinogenic effects (11, 12) and is a prominent biomolecule in the field of drug development. One of these pharmacological effects is to prevent or repair chemical or physical harms to testis and spermatogonial. For instance, curcumin can repair the harmful effects of testicular ischemia and improve sperm chromatin quality in mice (13). Also, it has been shown that curcumin is one of the antioxidants that could be used potentially to inhibit the harmful effects of Benzo(a)pyrene on sperm parameters, though it is ineffective in preventing all abnormalities (14).

Irradiation in multiple fractionated doses is frequently used for the treatment of various neo-plastic disorders. It produces both acute and late effects on exposed tissue. Ionizing Radiationinduced free radicalmediated events are primarily responsible for IR-induced injury (15). Curcumin has remarkable medicinal - including

antineoplastic properties. It has been shown to have an effect as a radiosensitizer for various malignancies and an effect as a radioprotector for normal tissues. The sensitivity of human and rat glioma cells to radiation increased following curcumin treatment, and both AP-1 and NF-κB expression were inhibited (16).

There is well-approved clinical evidence for the radioprotective effect of curcumin, and small clinical trials implicating the efficacy of curcumin for RT toxicities (vs placebo/current therapies) are also detailed. Albeit, it must be recognized that risks of adverse effects are exceedingly low, and clinicians may need to judge the yet-unproven rewards with low toxicity risks (9).

Many of toxicities associated with radiotherapy are inflammatory in nature, so curcumin's potent antiinflammatory effects (17-20) could decrease these inflammatory toxicities, likely through de-creased inflammatory molecule production (21), as well as increasing the balance of antioxidants to oxidants (22). It is shown that curcumin pretreatment has a helpful effect on the irradiated wound and could be a beneficial therapeutic strategy for improving radiationinduced skin injuries (23). Curcumin showed potential for protection against radiotherapyinduced oxidative injury to the skin (24) Also, curcumin treatment reduces the liver damage caused by radiation through the inhibition of the "nuclear factor kappa-light-chain-enhancer of activated B cells" (NF-κB) pathway. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens (25).

One of the side effects of radiotherapy used in cancer treatment is damage to the male reproductive system and a negative effect on the survival of spermatogonial stem cells and subsequent spermatogenesis that leads to sterility (26, 27).

It is shown that curcumin has a high potent effect on the prevention of damage to gonads when it is prescribed along with cisplatin, a chemotherapy drug (28), or aflatoxin (2), and in lead toxicity (29, 30). It can be used as a potent antioxidant against oxidative stresses and their harmful effects (31). Also, curcumin is shown to have a protective effect on sperm production in rats treated with sodium arsenite, an air pollutant, and cadmium chloride (32).

Gamma-radiation with a dose of 2 Gy caused severe degeneration changes in testicular tissue in-cluding a significant decrease in epithelium thickness and the number of spermatogonia, spermatid, and spermatozoa. Treatment with curcumin prevented from adverse effects of Gamma-radiation on testis structure and spermatogenesis (33). Also, curcumin could ameliorate the adverse effects of cadmium chloride on sperm plasma membrane integrity (34).

So far, there has been no report on the effect of ionizing radiation on the colonization of spermatogonial stem cells and the protective effect of curcumin on the reverse effect of gamma irradiation on spermatogonial stem cells proliferation.

Our findings show that there was a significant difference in the size and number of spermatogonial stem cell colonies between irradiated and non-irradiated control groups confirming that irradiation can damage spermatogonial stem cells. Although low dose curcumin (25mg/Kg) did not protect the side effect of irradiation on spermatogonial stem cells, medium and high doses (50 and 100mg/Kg) of curcumin increased the number and size of spermatogonial stem cell colonies so that in the high curcumin-treated radiated group the size and number of spermatogonial stem cell were the same as the noneradiated control group. These findings corroborate the Brinster findings (35, 36).

The size of colonies in non-irradiated curcumin-treated groups was significantly larger than in non-irradiated non-curcumin-treated groups in a dosedependent manner. This can be due to the antioxidant effect of curcumin on even healthy cells.

Conclusion

It seems that curcumin can be prescribed as a radio-protective agent to reduce infertility

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risk in males who must undergo radiotherapy and those who are exposed to radiation in their workplaces. Although the oral delivery of curcumin is limited mostly due to its low water solubility, low bioaccessibility, and fast degradation throughout the gastrointestinal tract, curcumin oral bioavailability can be boosted via new generations of delivery systems.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

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