Iran Red Crescent Med J. 2017 February; 19(2):e19624. doi: 10.5812/ircmj.19624.

Published online 2016 August 3. **Research Article**

In vivo Time-Dependent Radio-Protective Effect of Lycopene Against Whole-Body Gamma Radiation in Mice

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Received 2014 May 18; **Revised** 2015 May 23; **Accepted** 2015 September 07.

Abstract

Background: Ionizing radiation has become an essential part of modern medicine. However, applying the effective dose of ionizing radiation is compromised by its inevitable radiation-induced damage to normal tissues. Natural products with free radical scavenging, antioxidant activities, and immune stimulatory effects offer suitable radio-protective effects.
Objectives: This study was designed to evaluate the in vivo radio-protective activity of lycopene, a naturally dietary carotenoid, against whole-body gamma-irradiation-induced mortality and sickness in mice.
Methods: In this animal experimental study, lycopene was extracted from tomato paste, characterized, and then administered to mice. Consecutively, 80 healthy adult male mice were recruited and irradiated individually, and then monitored for irradiation sickness and survival parameters.
Results: Pretreatment with 7 mg/kg dose of lycopene 7 days before irradiation was found to reduce the severity of symptoms of radiation sickness in a 30-day post-radiation monitoring. Lycopene treatment increased the survival rate. At the radiation dosage of 8 and 9 Gy, the difference between the survival rates of control and sample groups became significant after day 15 and 25. Lycopene was also shown to have a protective effect on white blood cell count against 8 and 9 Gy gamma radiations.
Conclusions: Natural and exhibiting whole-body radio-protective activity, lycopene can be proposed as an effective radio-protector in healthy people who are exposed to radiation, such as radiographers, nuclear pharmacists, and radiologists.

Keywords: Lycopene, Radiation Protection, Gamma Rays, White Blood Cell

1. Background

Ionizing radiation has become an essential part of modern medicine. However, applying the effective dose of ionizing radiation is compromised by its inevitable radiation-induced damage to normal tissues. This damage is obvious in radiosensitive tissues, such as lymphoid organs, bone marrow, testes, and ovaries (1). Accidental exposure to irradiation is also a concern among healthcare providers, such as radiographers, nuclear pharmacists, and radiologists. The effects of low linear energy transfer radiations begin by the generation of reactive oxygen species (ROS). These ROS interact with biological molecules to produce toxic free radicals that lead to lipid peroxidation and DNA damage (2). Apart from lipid peroxidation, ROS can also alter the balance of endogenous protective systems, such as glutathione and enzymatic antioxidant (SOD, CAT, and GPx) defense systems (3).

To date, synthetic radio-protectors, such as amino thiol or S-2-(3-aminopropyl-amino) ethyl phosphorothioic acid, have not shown to be promising compounds for clinical use because of their high toxicity at effective concentrations (4, 5). Less- or non-toxic natural products with free radical scavenging, antioxidant activities, and immune stimulatory effects have advantages over their synthetic counterparts (4). A number of dietary antioxidants have been reported to decrease the harmful effect of free radicals on biomolecules (6). Carotenoids are a class of hydrophobic pigments generally found in plants, and they are strong antioxidants associated with scavenging of free radicals (7, 8). They protect the photosynthetic machine in plants by dissipating excess energy; several studies supported the idea that carotenoids also protect human skin against ultraviolet (UV)-induced lesions (9). Among carotenoids, lycopene has been demonstrated as a highly efficient antioxidant in cell protection against free radicals (10). It is an acyclic isomer of ß-carotene with 11 conjugated double bonds, normally in the all-trans configuration (11). The antioxidant activity of lycopene has been extensively

evaluated on the basis of its scavenging activity in cell culture and animal models (7). The anti-cancer activity of this carotenoid has also been demonstrated in many studies (12-14). Lycopene has attracted attention for several therapeutic potentials because of its abundance in vegetables and fruits, low cost, and safety. Although lycopene is extensively studied for its photoprotection activity against UV, only a limited number of studies have been conducted on its potential use against gamma radiation, especially in vivo.

2. Objectives

We investigated the in vivo radio-protective activity of lycopene, which was extracted from tomato, against accumulated doses of gamma radiation in mice.

3. Methods

Eighty healthy adult male mice were recruited into four controlled groups, irradiated individually, and monitored for irradiation sickness and survival parameters. The sample size was selected on the basis of similar studies and statisticians' expert recommendation. This study was conducted in Mashhad University of Medical Sciences, Mashhad, Iran.

3.1. Extraction and Purification of Lycopene from Tomato Paste

The tomato fruits used to prepare the tomato paste were purchased from the local market of Mashhad. To extract lycopene from the tomato paste, a modified procedure was performed according to the literature (15). Briefly, tomato paste was spread on an aluminum sheet and powdered after completely dried at 37°C. The powder was then transferred to a flask and mixed with n-hexane. After 24 hours of soaking in n-hexane, the mixture was sonicated and then filtered. n-Hexane was then added to the precipitate at 50°C in a water bath and filtered. The filtrate was then collected. The extraction procedure was repeated for 6 times, and the obtained collected extract was concentrated under reduced pressure at 45°C. First, 1 mL and then 2 mL of 1-2 propandiol were added to the concentrated extract and stirred at 50°C to obtain a homogenous dark red mixture. Afterwards, saponification was performed in 50 mL 45% (w/v) KOH at 100°C for 30 minutes. After adding 20 mL distilled water, red lycopene crystals appeared in the suspension. The lycopene crystals were then collected after filtration, washed with cold distilled water, and dried.

3.2. Characterization of Lycopene by Spectrophotometry Analysis

Lycopene spectrum was obtained by a UV-visible (UV-vis) spectrophotometer and then compared with that of standard lycopene. The UV-visible spectra were collected at room temperature on a double-beam V-630 spectrophotometer (Shimadzu, Japan) in 1 cm quartz cells.

3.3. Animals

Healthy adult male mice in the weight range of 37 ± 2 g were used for the experiments. The mice were housed in ventilated rooms at a controlled temperature (23 ± 2°C) and suitable humidity with 12 hours light/dark cycle. All animals were provided with enough food and water. They were cared for according to the guiding principle in the care and use of animals.

3.4. Ethical Consideration

The protocols used in this study conformed to the guidelines of the conduct of animal experiments issued by the School of Pharmacy and were approved by the committee on the ethics of animal experiments in Mashhad University of Medical Sciences.

3.5. Irradiation, Exposure Time, and Required Doses

The mice were placed in perforated plastic holders and irradiated individually. To avoid the generation of hypoxic conditions in the irradiation chamber, fresh air was circulated continuously through a rubber tube connected to an air pump. A Cobalt-60 Gamma Chamber (AECL, Canada Ltd.) was used to deliver the desired whole-body radiation. The mice were irradiated with different gamma rays at a dose rate of 119.27 cGy/min with irradiation conditions of 80 cm source skin distance and 87.55 of cGy percentage depth dose. The irradiation facility was provided by Omid hospital (Mashhad University of Medical Sciences, Mashhad, Iran).

3.6. Treatment of Mice With Lycopene and Monitoring Gamma-Irradiated Mice

To determine whether or not lycopene conferred any advantage over the lethal whole-body irradiation, the effect of lycopene on the survival of mice was investigated. Four radiation doses of 8, 9, 10 and 11 Gy were used to study the effect of lycopene on survival rate. All of the mice in the experiments were randomly (simple randomization method) divided into two groups of sample (lycopene-treated) and control (lycopene-untreated), with each group including 20 mice.

Lycopene at a dose of 7 mg/kg and a volume of 0.26 mL/mouse was given intra-peritoneally to the sample

groups for 7 consecutive days before irradiation. Mice in the control group received the same volume of control solution (DMSO + tween80 + distilled water) without lycopene for the same number of days before irradiation. One hour after the last administration, the mice were exposed to the above-mentioned doses of gamma irradiation.

The prevalence and severity of complications and the number of surviving mice reported as the percentage of survival were recorded daily up to 30 days post-irradiation.

3.7. Determination of the Dose-Reduction Factor (DRF)

A parameter indicating the effect of radiation protection in different situations is defined by the Equation 1 (16): in which D_{sample} is the dose of radiation expected to cause death to 50 percent of an exposed sample groups within 30 days and $D_{control}$ is the dose of radiation expected to cause death to 50 percent of an exposed control groups within 30 days:

$$A = \frac{LD50/30}{LD50/30} = \frac{D_{sample}}{D_{control}} \quad (1)$$

3.8. Hematological Study

To examine their survival, mice were pretreated with either lycopene (7 mg/kg) or control solution for 7 consecutive days before irradiation as mentioned above. Blood samples taken from the retro-orbital sinus of mice just before whole-body irradiation and 30 days after irradiation were transferred to vials containing 0.5 ethylenediamine tetraacetic acid. The total number of white blood cells (WBC) was determined using light microscopy standard procedures.

3.9. Statistical Analysis

Statistical comparisons were made with Kruskal-Wallis H and Mann-Whitney tests were used according to the non-normal distribution of the variables assessed by the Kolmogrov-Smirnov test. Differences were considered significant when P values were less than 0.05. The cumulative survival rates were determined by the Kaplan-Meier method. In the case of WBC data in the peripheral blood smear, the variance test of one-way ANOVA was used. For intra-group analysis, Tukey's test was performed for the WBC count experiment.

4. Results

4.1. Lycopene Extraction and Characterization

The concentration of lycopene in tomato was reported to be 30 - 200 mg/kg in fresh fruit and from 430 - 2950 mg/kg on a dry basis and accounted for more than 85% of the total carotenoid content (17). In the current study, the yield of tomato paste from fruits was 10% (w/w). From each kilogram of tomato paste, 314 mg crystalized lycopene, which corresponded to an extraction efficiency of 0.0314%, was extracted.

4.2. Survival Analysis

The control group exhibited several radiation-induced symptoms, such as loss of appetite, weakness, weight and hair loss, ocular injury, diarrhea, and bloody stools in the 30-day period post-irradiation. The severity of the symptoms was correlated with the applied radiation dose. A 7-day repetitive lycopene administration at a dose of 7 mg/kg could significantly protect the mice in the sample group from radiation effects. However, the radiation complications at dosages of 10 and 11 Gy were too severe to gain recovery from lycopene pretreatment.

Figure 1A-1D represents the cumulative survival analysis of either the sample (lycopene-treated) or the control (untreated) group at different radiation doses of 8, 9, 10 and 11 Gy in 30 days plotted by the Kaplan-Meier test. The data are presented as survival fractions considering the survival fraction of 1 on the first day when no death occurred. As shown in Figure 1, the survival rate generally decreased when the radiation dose was increased. For example, at day 30, the percentages of survival in the control group for exposure radiation doses of 8 and 10 Gy were 70% (Figure 1A) and 0% (Figure 1B), respectively. At a dose of 11Gy radiation (Figure 1D), the cumulative survival analysis revealed that the mice could not survive up to the end of the 30-day monitoring period. A comparison of the survival rates of the lycopene-treated group with the control group at days 10, 15, 20, 25 and 30 after gamma radiation analyzed by the Mann-Whitney test is presented in Table 1.

At the radiation dosages of 8 and 9 Gy, the difference in the survival rates between the control and the sample group became significant after days 15 and 25, respectively. At the radiation dosage of 10 Gy, a significant difference was found in the survival rates between the control and the sample group only on day 30. No substantial difference was observed between the treated and non-treated group at the radiation dosage of 11Gy ($P > 0.05$).

Table 1. Survival Rates Obtained at 10, 15, 20, 25, and 30 Days After Exposure to Radiation Doses of 8, 9, 10, and 11 Gy Compared Between the Control and the Sample Group by the Mann-Whitney Test

	8 Gy		9 Gy		10 Gy		11 Gy	
Days	**Mean Rank**	**P Value**	**Mean Rank**	**P Value**	**Mean Rank**	**P Value**	**Mean Rank**	**P Value**
10								
Control	9.50	0.147	9.25	0.285	10.25	0.816	9.75	0.552
Sample	11.50		11.75		10.75		11.25	
15								
Control	12.30	0.010	13.57	0.213	14.83	0.662	14.63	0.584
Sample	18.70		17.43		16.17		16.37	
20								
Control	15.95	0.007	17.50	0.099	18.75	0.331	19.73	0.670
Sample	25.05		23.50		22.25		21.28	
25								
Control	20.20	0.007	21.02	0.028	22.10	0.091	24.78	0.716
Sample	30.80		29.98		28.90		26.22	
30								
Control	24.00	0.003	24.35	0.006	25.17	0.015	29.82	0.744
Sample	37.00		36.65		35.83		31.18	

4.3. Time Dependency of the Protective Effect of Lycopene

Time dependency of the lycopene effect on the survival rate is shown in Figure 2. As observed, the protective effects of lycopene against irradiation were shown to be time dependent at doses of 8 and 9 Gy (Figure 2A - 2E). That is, the difference between the control and the sample group increased during the periods after irradiation exposure. Although only significant on day 30 post-radiation, this trend also applied to the radiation dose of 10 Gy (Figure 2E). However, lycopene could not protect the animals against the fatal rate of irradiation because of the severity of the complications at 11 Gy radiation.

4.4. Determination of the DRF

As described above, the rate of radiation protective effect by a protective agent can be determined by deducting the DRF. The LD50/30 values of the sample and control groups (i.e., the required dose to reduce survival percentage to 50% within 30 days calculated using the equation stated above) resulted in a DRF of 1.03 for lycopene.

$$DRF = \frac{8.85}{8.57} = 1.03 \quad (2)$$

4.5. Effect of Lycopene Administration on Peripheral WBC Count in Irradiated Mice

The effects of lycopene administration on peripheral WBC count in mice after 30 days of exposure are shown in Figure 3. The results represent the mean data obtained from seven mice in each group. At dose of 10 Gy, lack of information in the control group was caused by the death of all the mice before day 30. A significant difference was observed at doses of 8 and 9 Gy ($P < 0.05$).

5. Discussion

Total body irradiation can lead to complications that mostly cause damage to gastrointestinal epithelial cells and subsequently bone marrow cells, thus resulting in hematologic disorders. Symptoms of these complications include weight loss, anorexia, lethargy, hematemesis, anemia, hair loss, irritability, and convulsion. The main biological effect of radiation is the induction of oxidative stress that leads to both DNA damage and cell death (18). As ionizing radiation is an important part of modern medicine, the administration of radio-protective agents can simultaneously protect normal tissues against radiation while permitting the application of higher radiation doses for more efficient therapeutic purposes (19).

To our knowledge, the current study is one of the first studies attempting to use herbal supplements to decline

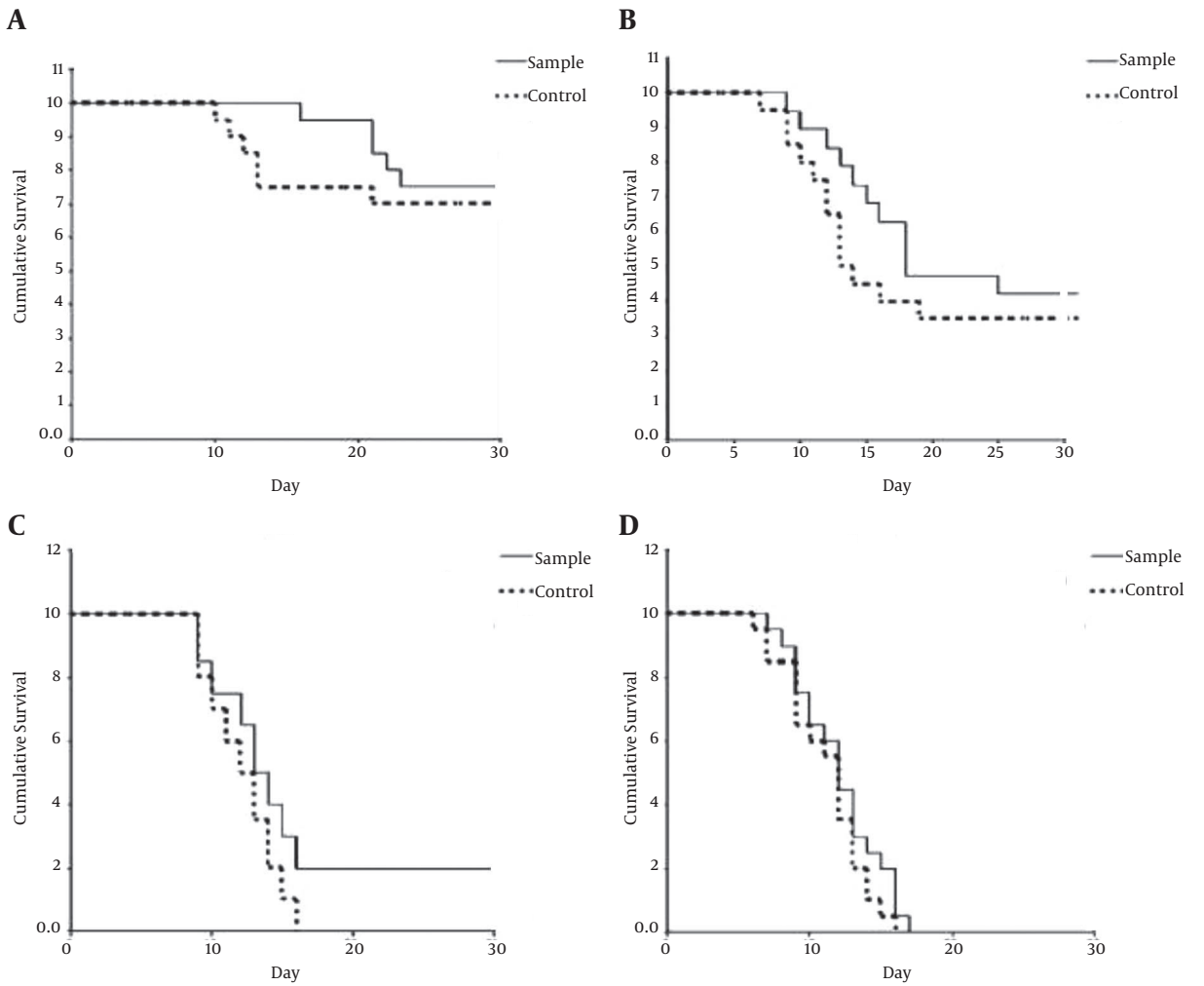

Figure 1. Kaplan-Meier Analysis of Survival of the Sample (Lycopene Treated) and Control (Untreated) Groups at Radiation Doses of A, 8 Gy; B, 9 Gy; C, 10 Gy; D, 11 Gy in 30 days

post-radiation complications by using lycopene, an inexpensive and accessible food product.

In the present study, the carotenoid lycopene was evaluated for its protective effect against gamma irradiation. Lycopene has been shown to be effective in cell protection against free radicals as it has the highest antioxidant activity among the carotenoids (20, 21). Total body irradiated mice have been monitored for 30 days after exposure to gamma irradiation. The above-mentioned irradiation-induced complications were initiated in the first week and became evident in the second week. To evaluate the rate of damage to bone marrow cells, the peripheral WBC were counted. In the 30-day monitoring of the irradiated mice, the administration of lycopene reduced the prevalence and severity of these symptoms and also delayed the appearance of the symptoms. Lycopene was then shown to cause a significant difference in leukocyte count between the sample and the control group at radiation doses of 8 and 9 Gy. The counting of peripheral WBC confirmed that lycopene could protect bone marrow from radiation.

In this study, the Kaplan-Meier curves were used to compare the mortality rate or change in survival length between the control and the sample group at specific radiation doses. In the survival curves, mean percentage was expressed to show the cumulative survival rate of different radiation doses in specific days for both control and sample groups. As time passed from the radiation exposure, the difference between the control and the sample group increased. This trend was similarly observed for the appearance of complications of irradiation. Moreover, more time is required to observe the difference in survival rates between the control and the sample group at higher

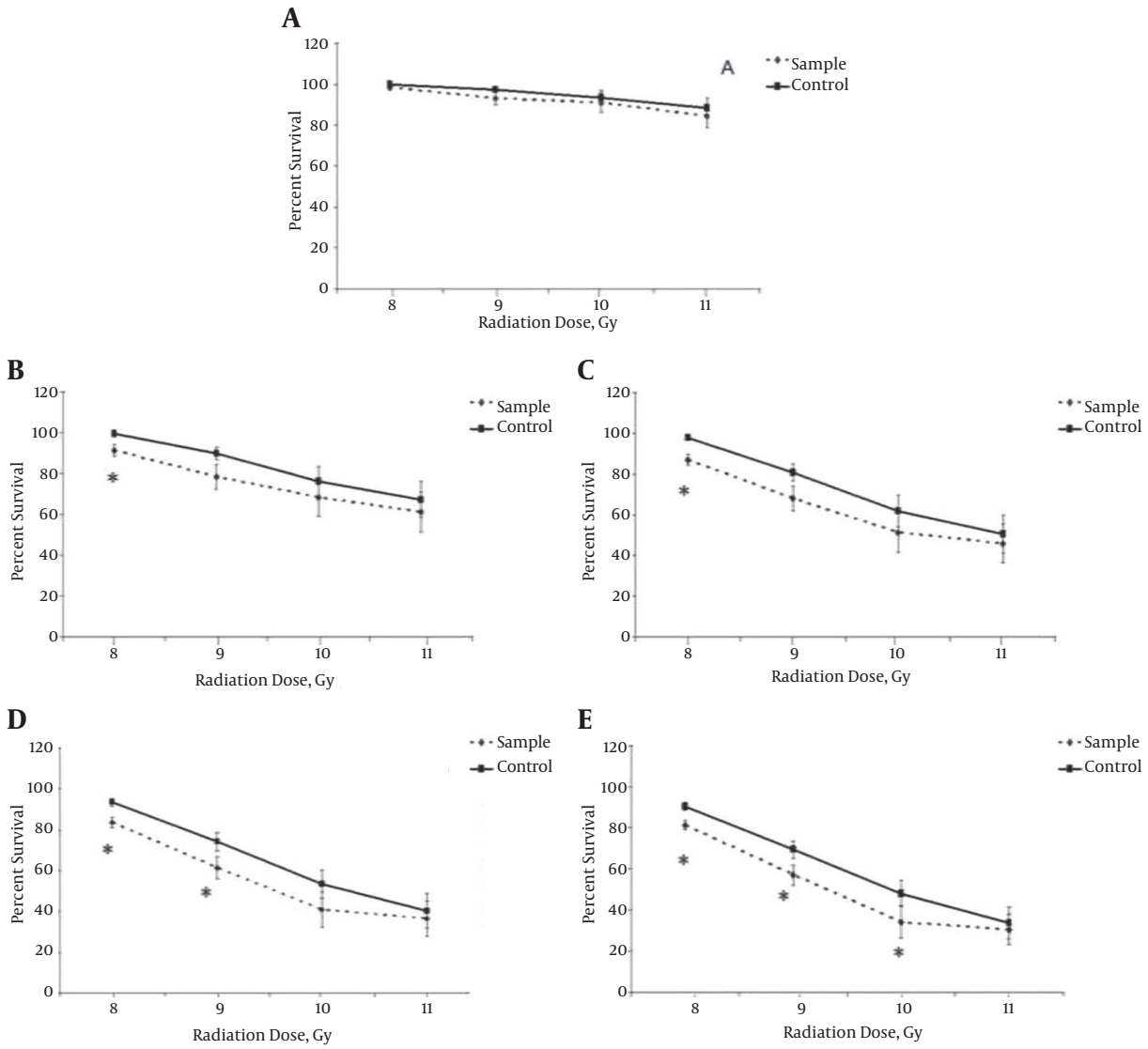

Figure 2. Comparison of the Percent Survival Means of Mice in the Lycopene-Treated and Untreated Groups at Different Doses (8, 9, 10, and 11 Gy) of Radiation After A, 10 days; B, 15 Days; C, 20 Days; D, 25 Days; E, 30 Days From Irradiation

doses of irradiation. Therefore, at doses of 8, 9, and 10 Gy, a significant difference between the control and the sample group ($P < 0.05$) was observed on days 13, 25, and 30 post-radiation, respectively. Therefore, by increasing the radiation dose, more time may be required to observe the protective effect of lycopene. Moreover, the protective effect of lycopene against irradiation was improved by time. Preliminary reinforcement of the mice bodies against irradiation and increased ability of the animals' recovery by time are the probable reasons for the observed time dependency trend.

Peripheral WBCs were counted to investigate the protective effect of lycopene on bone marrow after the 30-day monitoring. Significant differences in the WBC count between the control and the sample group at 8 and 9 Gy radiation doses were observed ($P < 0.05$). Thus, lycopene is assumed to be preventive against severe reduction in blood leukocytes by protecting the bone marrow. Other studies also reported similar in vitro protection effects of lycopene. In Srinivasan et al. (22), pre-treatment with lycopene (1.86, 9.31, and 18.62 μM) showed a significant decrease in DNA damage in cultured rat hepatocytes examined by comet assay. The maximum protection of hepatocytes was observed in a pre-treatment trial at 9.31 μM of

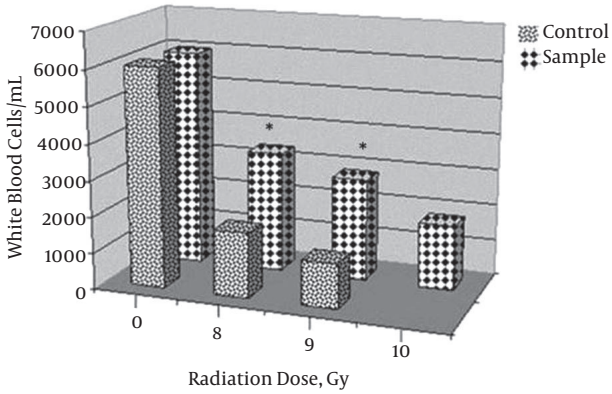

Figure 3. Comparison of the Effects of Lycopene Administration on WBC Count Between the Sample Group and the Control Group at Different Applied Radiation Doses (*P < 0.05)

lycopene. Moreover, lycopene pre-treatment (1, 5, and 10 μg/mL) significantly decreased the frequency of cellular changes and increased the previously reduced glutathione levels compared with the gamma-radiated control group with no lycopene pre-treatment (23). The dose of 5 μg/mL of lycopene was found to be more effective than the other two doses (23). Consistent with the mentioned studies, we also demonstrated that pre-treatment with lycopene could offer protection of normal lymphocytes against gamma-radiation-induced cellular damage. We used the DRF to compare quantitatively the properties of the radiation protective effect of lycopene. A higher DRF indicates a higher ability of a substance to protect. DRF values for the radiation protection effect of extracts of Adhatoda vasica, Spirulina fusiformis, and Mentha piperita were reported as 1.6, 1.3, and 1.78, respectively (24). The DRF value for lycopene in our study was measured as 1.03.

5.1. Limitations

We were constrained in obtaining a larger sample size and accurately matching the samples in case of strains. Moreover, we had limited time for follow-up, and we used simple randomization method for allocating to groups without controlling the sample matching after allocation. In addition, we did not have calibrated observers for multiple measurements.

5.2. Conclusions

As a potent antioxidant, lycopene can increase the resistance of cells against gamma radiation. In the acute whole-body irradiation of mice, we observed an inhibitory effect of lycopene against the complications of gamma rays, such as gastrointestinal and hematological side effects and reduction in WBC. In the sample group that received lycopene, the mortality rate significantly decreased compared with the control group. A remarkable difference in the average of cumulative survival data at 8, 9, and 10 Gy doses of radiation was also observed after lycopene administration.

The current study proposes lycopene as a potential radio-protective agent against gamma radiation. Lycopene administration may be useful in people who are exposed to radiation, such as radiographers, nuclear pharmacists, and radiologists. Nevertheless, lycopene administration still requires to be optimized in terms of the appropriate dosage and period of treatment for human use.

Acknowledgments

The authors would like to thank Sara Amel Farzad for her assistance in the lycopene extraction procedures.

Footnote

Authors' Contribution: Study concept and design: Mohammad Reza Oladi; analysis and interpretation of data: Ameneh Sazgarnia and Hamideh Parhiz ; drafting of the manuscript: Mostafa Amrollahi and Mohammad Ramezani; statistical analysis: Mohammad Reza Ghavam Nasiri.

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