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Evaluation of the Effects of Curcumin and Lycopene Treatment on Human Lymphocytes before 2 and 6 Gy of 6 MV X-Ray Irradiation

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ABSTRACT

Introduction: Today, the use of ionizing radiation in medicine has grown as an important tool for diagnosis and treatment of diseases. However, the harmful effects of radiation should be also considered. Some substances such as lycopene and curcumin can reduce or increase the harmful effects of radiation on humans. So the aim of this study was to evaluate the radioprotective effects of lycopene and curcumin based on the MN assay.

Material and Methods: In this study, the effects of lycopene and curcumin on reducing or increasing the harmful effects of radiation were studied using the micronucleus assay. The effects of lycopene (5 µg/mL) and curcumin (5 µg/mL) were evaluated at radiation doses of 2 and 6 Gy.

Results: The results indicated that the simultaneous use of curcumin and lycopene can be radioprotective at low radiation doses (2 Gy; $P<0.001$) and radiosensitizing at high doses (6 Gy; $P>0.05$).

Conclusion: Based on the present results, further research using other methods may contribute to our understanding of the effect of simultaneous use of curcumin and lycopene at low and high doses of X-ray radiation.

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Introduction

Recent developments in radiation technology have led to its extensive use in various medical fields, such as medical imaging, nuclear medicine, and cancer therapy [1, 2]. Ionizing radiation (IR) is an indispensable part of modern medicine; however, its hazardous biological effects on the human body are not inevitable [3]. One of the main hazards of IR exposure can be induced by reactive oxygen species (ROS), causing oxidative reformation of cellular macromolecules. On the other hand, oxidative stress is known as a leading cause of cancer [4, 5]. Today, researchers are seeking substances and materials that can protect human cells against IR. These substances must lack toxic components and protect the cells against genotoxic stress. According to the literature, natural plant extracts and phytochemicals not only inhibit oxidative stress, but also induce less toxicity [6, 7].

Curcumin is a phenolic compound, found in the roots of *Curcuma longa* Linn. Scientific investigations have shown that curcumin is a radiosensitizer for tumors and a radioprotector for normal tissues [8]. This compound interacts with various molecular targets, enabling it to affect various biological reactions, such as gene regulation or apoptosis. Therefore, it is difficult to clearly explain the protection mechanism of curcumin against radiation [9]. Many yellow or red fruits and vegetables contain large amounts of lycopene. Lycopene is a red insoluble non-cyclic carotenoid. Many studies have shown that lycopene has strong free radical scavenging and anti-inflammatory characteristics [10]. Pretreatment with lycopene before radiation protects irradiated lymphocytes against the genotoxic effects of ionizing radiation and significantly reduces the number of micronuclei (MN) and mutations [11-15].

Although many studies have been conducted on curcumin and lycopene in low-dose or chronic experiments, there is little evidence on how curcumin or lycopene-treated cells respond to high-dose radiation. Also, there are some controversies about the effects of these substances. For instance, curcumin

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at low doses can decrease radiation-induced damage, but at higher doses, it may increase radiation-induced damages [16]. Only few studies have investigated the effects of concurrent use of lycopene and curcumin. In this regard, a histopathological study by Lopez-Jornet et al. [6] showed that the use of lycopene and curcumin at 24 hours before irradiation reduced the structural damage of salivary glands. However, further research, using genotoxicity tests, can shed light on the radioprotective effects of combined use of lycopene and curcumin to substantiate the histopathological evidence. Therefore, in this study, we aimed to evaluate the radioprotective effects of lycopene and curcumin, used concurrently at high doses of ionizing radiation, on normal human lymphocyte cells, based on the MN assay.

Materials and Methods

Preparation of drugs

In this study, lycopene and curcumin stocks were prepared. For this purpose, 5 µg/mL of lycopene (Lot. #11365700, 21st Century Healthcare, Inc., USA) was prepared in RPMI-1640 medium (1X) (Lot. #RPL09, CBSA, Iran) and dimethyl sulfoxide (DMSO; Lot. #STBG3187V, Sigma-Aldrich). Also, 5 µg/mL of curcumin (Sigma) was prepared in the RPMI medium and DMSO. The final concentration of DMSO in the lymphocyte culture was 0.01% (w/v), and 0.01% DMSO was used as the sham control.

Blood sampling and experimental design

Blood samples (7 mL) from three healthy donors (2 males and 1 female, age: 20-25 years) were collected in sterile heparinized tubes. The donors were non-vegetarian, non-smoker, and non-pregnant. Written informed consent was obtained from each of the donors. A total of 13 groups were evaluated in the present study. Table 1 presents different groups, which were categorized based on radiation exposure and drug treatment.

Sample preparation, X-ray irradiation, and MN assay

The whole blood samples (400 µL) were cultured in the RPMI-1640 medium (1X), supplemented with 10% fetal bovine serum (FBS; Gibco, USA), 1% penicillin/streptomycin (Gibco, USA), and 100 µL of phytohaemagglutinin (PHA; Gibco, USA) in a 15 mL falcon tube. The cells were incubated at 37˚C, and after one hour, the drugs were added to the samples. Next, the samples were irradiated with 2 and 6 Gy of X-ray radiation (1 Gy/min, 6 MV X-ray), using the Elekta Compact linear accelerator (Stockholm, Sweden) at Nemazee Hospital in Shiraz, Iran. After radiation, the samples were returned to the incubators.

Forty-four hours after irradiation, 100 µL of cytochalasin-B (Lot. #42F650K, Sigma) was added to the samples. Then, the cells were incubated for another 22 hours. After incubation, the cells were harvested, placed on slides, and finally stained with Giemsa (Merck, USA). According to the Countryman and Heddle's criteria [17], the binucleated cells (BNCs), which were considered as MNs, contained two distinct nuclei of approximately the same size, either separated or connected by a narrow nucleoplasmic bridge. MNs were considered within the cell cytoplasmic range, with identical morphological characteristics to BNCs and a smaller size. Also, MNs did not contain a nucleoplasmic bridge.

Table 1. The groups under study (5 µg/mL of lycopene and 5 µg/mL of curcumin were prepared in the RPMI medium and DMSO. $DMSO^-$ refers to samples without DMSO as the solvent, and $DMSO^+$ refers to samples with DMSO as the solvent).

Groups #	Treatments and irradiation
1	Control ($DMSO^-$)
2	Sham-Control ($DMSO^+$)
3	2 Gy ($DMSO^+$)
4	6 Gy ($DMSO^+$)
5	Lycopene (5 µg/mL)
6	Curcumin (5 µg/mL)
7	Lycopene (5 µg/mL) + 2 Gy
8	Lycopene (5 µg/mL) + 6 Gy
9	Curcumin (5 µg/mL) + 2 Gy
10	Curcumin (5 µg/mL) + 6 Gy
11	Lycopene (5 µg/mL) + Curcumin (5 µg/mL)
12	Lycopene (5 µg/mL) + Curcumin (5 µg/mL) + 2 Gy
13	Lycopene (5 µg/mL) + Curcumin (5 µg/mL) + 6 Gy

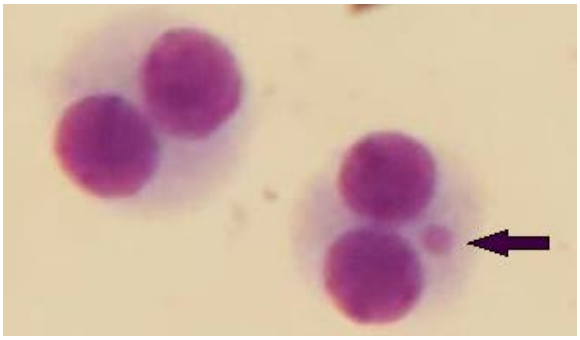

Figure 1. Cells regarded as MNs based on the Countryman and Heddle's criteria [17]. The pale purple color indicates the cell cytoplasmic range. The cells with two distinct nuclei of approximately the same size are considered as BNCs. MNs are considered within the cell cytoplasmic range, with identical morphological characteristics to BNCs and a smaller size. The tip of the arrow represents MNs.

The slides were counted randomly at 10X magnification, using a bright-field microscope (BX61; Olympus, Tokyo, Japan). At least 1000 BNCs were counted in each slice, and then, the number of MN per 1000 BNC MNs was reported. The cells that were considered as BNCs and MNs are shown in Figure 1. In this study, the MN assay for each sample was repeated three times to increase the reliability of data.

Statistical analysis

In this experimental-interventional study, each group was assayed in triplicate. Data are expressed as

mean±SD. Different study groups were compared, using one-way analysis of variance (ANOVA) in Microsoft Excel 2016. P-value less than 0.05 was considered statistically significant.

Results

In this study, the effects of curcumin and lycopene as radioprotectors were evaluated, using the MN assay. As expected, IR significantly increased the number of MNs in lymphocytes (Figure 2). As can be seen in Figure 2, the number of MNs produced by exposure to both 2 and 6 Gy of radiation showed a significant increase, compared to the control and sham-control groups.

The average number of MNs in the control and sham-control groups was 1.33 and 2, respectively, which is not significantly different (P>0.05). Therefore, the used concentration of DMSO, as a solvent for drugs, caused low toxicity. Figure 3 shows the effect of lycopene on the MN production. As can be seen, the lycopene-treated cells slightly increased the average number of MNs, compared to the sham-control group; however, the difference was not statistically significant (P>0.05). In exposure to 2 Gy of radiation, pretreatment with lycopene decreased the average number of MNs from 9 to 7.67 (P>0.05), while in exposure to 6 Gy of radiation, lycopene increased the average number of MNs from 20.33 to 27, relative to the group without lycopene (P>0.05).

Besides, the effects of curcumin-treated lymphocytes are shown in Figure 4. Curcumin slightly increased the average number of MNs, compared to the sham-control group; however, the difference was not statistically significant (P>0.05). In exposure to 2 Gy of radiation, curcumin increased the average number of MNs from 9.00 to 21.67 (P>0.05). The same trend was observed in exposure to 6 Gy of radiation, as curcumin increased the average number of MNs from 20.33 to 21.33 (P>0.05).

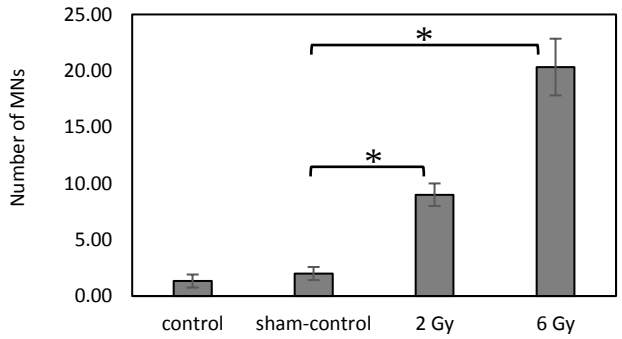

Figure 2.The effect of an increase in the MN number by increasing the dose of irradiation in lymphocyte cells (*P<0.05). At least 1000 BNCs are scored per slide, and the results are presented as the number of MNs per 1000 BNCs. Data are presented as mean ± SD. P<0.05 is considered statistically significant.

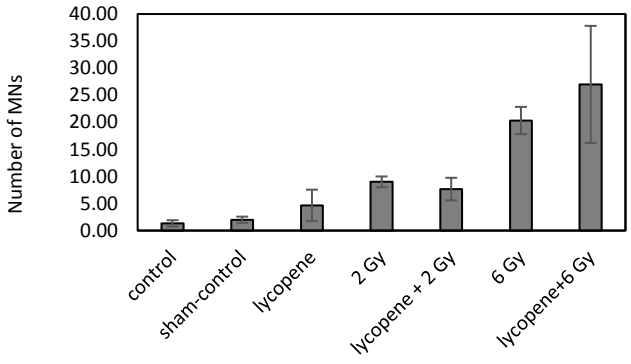

Figure 3. Effects of lycopene pretreatment on the number of MNs in lymphocyte cells before irradiation. At least 1000 BNCs are scored per slide, and the results are presented as the number of MNs per 1000 BNCs. Data are presented as mean±SD. P<0.05 is considered statistically significant.

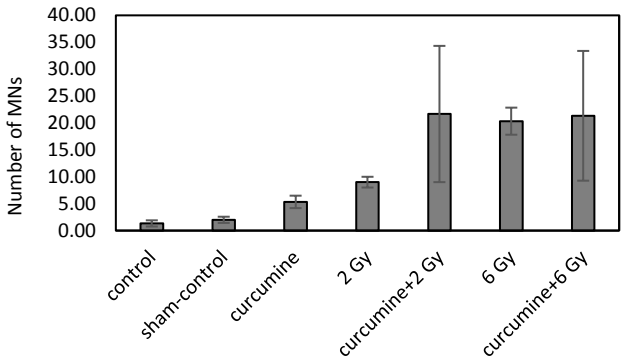

Figure 4. Effects of curcumin pretreatment on the number of MNs in lymphocyte cells before irradiation. At least 1000 BNCs are scored per slide, and the results are presented as the number of MNs per 1000 BNCs. Data are presented as mean±SD. P<0.05 is considered statistically significant.

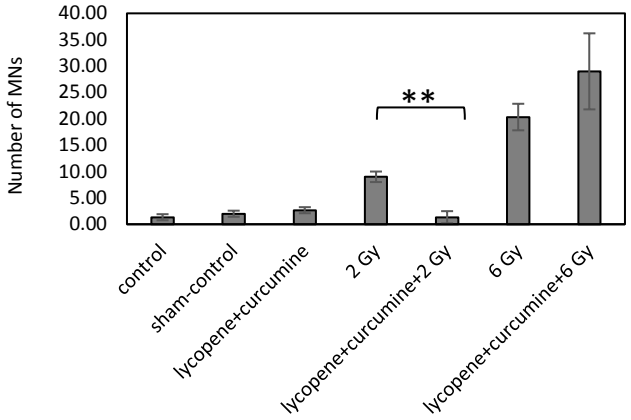

Figure 5. Effects of co-treatment with curcumin and lycopene on the number of MNs in lymphocyte cells before irradiation (**P<0.001). At least 1000 BNCs are scored per slide, and the results are presented as the number of MNs per 1000 BNCs. Data are presented as mean±SD. P<0.05 is considered statistically significant.

Discussion

The main purpose of this study was to evaluate the protective effects of lycopene, curcumin, and their combination at high doses of IR on normal lymphocyte cells. IR induces DNA damage both directly and indirectly. DNA damage in lymphocytes is significantly increased by IR [11, 15]. The MN analysis in this study indicated that IR increases DNA damage in a dose-dependent manner (Figure 2).

The consumption of tomatoes and turmeric is generally beneficial due to their antioxidant effects [18, 19]. Since these two substances are widely included in the diet of most people, it is important to evaluate whether they can be used for radiation protection or sensitization. So far, some studies have been conducted in this area. It has been shown that the use of curcumin and lycopene in radiotherapy may protect the cells against harmful radiation [20]. The strong antioxidant properties of lycopene reduce the adverse effects of oxidative stress. Lycopene is a non-toxic substance with the ability to detoxify. We evaluated the effect of lycopene and curcumin before irradiation, because all doses of lycopene used before irradiation significantly reduced the lymphocyte DNA damage, while adding lycopene after irradiation did not affect the cellular response [11-13].

In the present study, it was found that pretreatment of whole blood cells with lycopene at 2 Gy of radiation insignificantly reduced the average number of MNs (P>0.05) (Figure 3). In contrast, Srinivasan et al. [13] reported that lycopene significantly reduced the average number of MNs at 2 Gy of radiation. Despite the same lycopene dose (5 µg/mL), Srinivasan et al. [13] used 1.6 Gy/min of cobalt-60, while we used 1 Gy/min of 6 MV X-ray. This contradiction may be attributed to the use of different dose rates. Moreover, Nakamura et al. reported that oral administration of tomato juice, containing 16.9±0.2 mg of lycopene, insignificantly increased the average number of MNs at 2 Gy of radiation (150 kVp and 20 mA X-ray with a 0.5 mm Al and 0.3 mm Cu filter at 1 Gy/min). Although both studies were conducted at the same dose rate, different routes of drug administration might have led to different results.

As shown in Figure 3, 6 Gy of IR insignificantly increased the average number of MNs in lycopene-treated cells (P>0.05). It has been suggested that lycopene might be a radiosensitizer. To the best of our knowledge, there is no study on human lymphocytes in exposure to 6 Gy of IR. The present results showed that curcumin insignificantly sensitized the cultured

lymphocytes at 2 and 6 Gy of radiation (P>0.05) (Figure 4) by increasing the MN production. Further studies on curcumin may help us develop a distinct view about the effect of curcumin on the lymphocyte response.

Based on the present results, pretreatment of cultured lymphocytes with lycopene and curcumin before 2 Gy of radiation significantly protected the cells against IR (P<0.001) by decreasing the number of MNs (Figure 5). Our results indicated that combining lycopene with curcumin (5 µg/mL) had a significant protective potential against radiation. In contrast to 2 Gy of irradiation, simultaneous use of lycopene and curcumin increased the average number of MNs at 6 Gy of radiation. Consequently, the combination of these two substances at 6 Gy of radiation can act as a radiosensitizer.

At 6 Gy of IR, the average number of MNs increased insignificantly in all drug-treated groups. Further studies with different doses of these substances may clarify the possible underlying radiosensitizing effects. As mentioned earlier, the effect of lycopene at 2 Gy of IR is somehow controversial. While Srinivasan et al. [21] claimed that at 2 Gy of irradiation, the number of MNs reduced in curcumin-treated cells, compared to the control group, our results did not support this claim.

In the present study, the combination of lycopene with curcumin had different sensitizing and protective effects at high and low doses of radiation, respectively. Therefore, further studies using other methods may contribute to our understanding of the exact effects of lycopene, curcumin, and their combination at low and high doses of X-ray radiation.

Conclusion

The results of this study showed that combination of curcumin with lycopene could act as a radioprotector at low radiation doses (2 Gy; P<0.001). However, at 6 Gy of radiation, it increased the number of MNs (P>0.05). Considering the adverse effects of using these two substances either separately or in combination, the claim that either of these substances or their combination can act as radiosensitizers or radioprotectors needs to be further investigated.

Acknowledgment

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