Scientific Research Publishing

Journal of Biosciences and Medicines, 2024, 12, 202-213
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

In Vitro Antioxidant and Radio Protective Activities of Lycopene from Tomato Extract against Radiation—Induced DNA Aberration

Safaiatul Islam*, Abu Hena Mostofa Kamal, Md. Ziaur Rahman, Protul Kumar Roy, A.Y.K. Md. Masud Rana

Molecular Radiobiology and Biodosimetry Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Savar, Bangladesh
Email: *safaiatul.bge28@gmail.com

How to cite this paper: Islam, S., Kamal, A.H.M., Rahman, Md.Z., Roy, P.K. and Masud Rana, A.Y.K.Md. (2024) *In Vitro* Antioxidant and Radio Protective Activities of Lycopene from Tomato Extract against Radiation—Induced DNA Aberration. *Journal of Biosciences and Medicines*, **12**, 202-213.
https://doi.org/10.4236/jbm.2024.122015

Received: January 4, 2024
Accepted: February 17, 2024
Published: February 20, 2024

Abstract

Background: The accumulation of free radicals is linked to a number of diseases. Free radicals can be scavenged by antioxidants and reduce their harmful effects. It is therefore essential to look for naturally occurring antioxidants that come from plants, as synthetic antioxidants are toxic, carcinogenic and problematic for the environment. Lycopene is one of the carotenoids, a pigment that dissolves in fat and has antioxidant properties. **Materials and Methods:** The antioxidant and free radical scavenging activity were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The impact of lycopene on bacteria (*E. coli*) susceptibility to *γ*-radiation was examined by radio sensitivity assay. The study also examined the induction of strand breaks in plasmid pUC19 DNA and how lycopene extract protected the DNA from *γ*-radiation *in vitro*. **Results:** At varying concentrations, lycopene demonstrated its ability to scavenge free radicals such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH). IC_{50} for lycopene was determined at 112 μg/mL which was almost partial to IC_{50} of standard antioxidant L-ascorbic acid. The D_{10} value 180 Gy of *E. coli* was found to be >2-fold higher in the extract-containing lycopene sample than in the extract-free controls. The lycopene extracts inhibited the radiation-induced deterioration of the plasmid pUC19 DNA. At an IC_{50} concentration, lycopene provided the highest level of protection. **Conclusion:** Lycopene functions as an efficient free radical scavenger and possible natural antioxidant source. For cancer patients and others who frequently expose themselves to radiation, lycopene may be a useful plant-based pharmaceutical product for treating a variety of diseases caused by free radicals.

Keywords

Radio Protective, Antioxidants, Free Radical, DNA Damage, pUC19 Plasmid, Gamma Irradiation, DPPH

1. Introduction

Radio-protective agents or radioprotectors are chemical or biological compounds that are employed to alleviate the damage that radiation causes to tissues and cells [1]. Ionizing radiation (IR) can damage bio-macromolecules directly, as well as produce reactive oxygen species (ROS), which can indirectly breakdown proteins and DNA [2]. Free radicals, such as reactive oxygen species (ROS), comprise unpaired electrons or singlet oxygen, which tend to be highly chemically reactive in cells [3]. These reactions result in oxidative stress and damage, namely causing single-strand breaks, double-strand breaks, and oxidized DNA bases, among other DNA diseases [4]. Radiation dosages too high can harm our cells' DNA and result in Acute Radiation Syndrome (ARS). Moreover, radiation caused DNA lethality, mutagenesis, and apoptosis. Therefore, DNA damage can result in aging, cancer, and disruptions of the cell's critical functions [5]. Antioxidants act as radioprotectors, able to neutralize rogue free radicals by giving them an electron. These free radicals scavenging process tends to postpone cellular damage [6]. Antioxidants can repair DNA damage by controlling the redox-sensitive transcription factor NRF2 and regulating apurinic/apyrimidinic endonuclease [7]. Both natural and synthetic antioxidants are frequently used in food, cosmetics, and pharmaceutical products. However, due to consumer preferences and the carcinogenic nature of some synthetic antioxidants, manufacturers are now focusing more on natural antioxidants [8]. Out of all the common carotenoids found in tomatoes and other red fruits, lycopene is the most powerful naturally occurring antioxidant. Lycopene is an isomer of beta-carotene with a molecular weight of 536.89 and molecular formula $C_{40}H_{56}$ [9]. Because of its eleven linear conjugated double bonds and two unconjugated double bonds, it is a lipophilic compound with hydrophobic properties that make it more soluble in organic solvents like petroleum ether, methylene chloride, acetone, hexane, and chloroform. It is suggested that lycopene's chemical structure increases its capacity to scavenge radicals and has a greater affinity for singlet oxygen [10]. Because of lycopene's biological and physiochemical characteristics, which have drawn attention to it as a natural antioxidant, there is a high demand for its extraction and purification [11]. Due to their high nutritional content, remarkable yield, affordability, and versatility as a model plant for various scientific studies, tomatoes are regarded as the most superior vegetable in the world [12]. Additionally, lycopene has been demonstrated to be effective in reducing the risk of cancer recurrence, diabetes, heart problems, oxidative stress-related dysfunctions, liver, neurological, and reproductive issues [13]. It has been shown that

lycopene, a fat-soluble pigment with antioxidant and antitumor properties, reduces oxidative stress by either reducing oxidative damage to lipids, proteins, and deoxyribonucleic acid or trapping reactive oxygen species to enhance antioxidant potential [14]. The pUC19 plasmid vector is a frequently used cloning vector that is high copy number, cultivable, and has multiple cutting sites. The 2686 base pair long molecule is a small double-stranded circle that is widely used as a model plasmid in research [15]. In this study, we will extract and purify lycopene from the available local variety of tomatoes (*Solanum lycopersicum*) in Bangladesh and evaluate the free radical scavenging activity (antioxidant) of lycopene and radioprotective activities of pretreated bacteria and plasmid DNA (pUC19) with lycopene at various radiation doses. It will be the first study of natural radioprotection against radiation-induced DNA aberration in Bangladesh.

2. Materials and Methods

2.1. Extraction of Lycopene

The fully mature, bright red tomatoes were thoroughly ground and homogenized to break down their cell structure and facilitate the effective extraction of lycopene. Tomato paste was filtered by strainer to remove seeds and other debris. First of all, 100 g was taken in the 250 mL of the conical flask. The samples were then extracted using a solvent mixture consisting of 200 mL of hexane and acetone at room temperature in a 3:1 ratio for an entire night in an orbital shaker. The extract from flask was filtered with Whatman No. 1 filter paper using vacuum filtration system. Following filtration, only crude extract remained after the solvent from the extract was separated at 50˚C in a rotary vacuum evaporator (Heidolp, Germany). This crude extract of sample was strongly attached with surface of beaker and could not isolate directly. Then, this crude extract of sample was separated from beaker of vacuum evaporator by resuspending in ethanol. This ethanol was removed by vacuum concentrator and then stored our earmarked lycopene at 4˚C until use [16].

2.2. Free Radical Scavenging Activity

The scavenging capacity of lycopene was measured against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in accordance with Brand-Williams *et al*. with a slight modification [17]. When the DPPH free radical interacts with hydrogen donors, it is reduced to the corresponding hydrazine. A 2.5 ml of the crude lycopene in methanol at different concentrations (200 - 300 µg/mL) was added to 500 µl of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution (1 mM in methanol). Methanol was conducted as control. The spectrophometer was set by zero with Methanol and the absorbance of the control reaction was taken at 517 nm [blank sample (only DPPH in Methanol) (t = 0 min)]; After giving the mixture a thorough shake and letting it sit at room temperature in the dark for 30 minutes, the absorbance at 517 nm was measured using a spectrophotometer.

For comparison, L-ascorbic acid was used as the standard.

$$\text{DPPH\% inhibition} = \left[\left(\text{A1} - \text{A2}\right)/\text{A1}\right] \times 100,$$

where A1 = the absorbance of the control reaction; [blank sample in Methanol (t = 0 min)]; A2 = the absorbance in the presence of the lycopene [Lycopene extract solution (t = 30 min)]. The concentration at which the scavenging activity was 50% is known as the IC_{50} value. The analysis was done thrice, and the results are shown as mean values.

2.3. Radio Sensitivity of DNA

The large and complex bacterial genomes make it challenging to measure DNA damage in agarose gel electrophoresis. So, the induction of strand breaks in plasmid pUC19 DNA *in vitro* by γ-radiation was studied using the agarose gel electrophoresis method. Exposure to γ-radiation at 50 and 100 Gy doses was performed on ultra-purified Plasmid pUC19 DNA (Promega, USA) at a concentration of 75 ng/µl, either with or without lycopene. The supercoiled and open circular forms of DNA were separated by agarose gel electrophoresis using 1% agarose gel in Tris-acetate-EDTA (TAE) buffer (pH 8.0). DNA bands after staining with ethidium bromide were photographed using a gel documentation system (Aplegen, USA) [18]. ImageJ (nih.gov. USA) software was used for gel band intensity analysis.

2.4. Radio Sensitivity Test of Bacteria

50 µl of the suspension of the vegetative cells of the *E. coli* ATCC 11303 (Absorbance$_{600nm}$ = 0.1 = 10^8 CFU/mL) were added to 50 µl of the aqueous extract of the lycopene at IC_{50} [19]. The suspension of the test organism without the lycopene extract was used as control. Gamma irradiations were carried out in a research irradiator (Cobalt-60) at Gamma source Division, Institute of Food and radiation Biology, Bangladesh Atomic Energy Commission. The cell suspensions were irradiated at doses of 0.1, 0.2, 0.4, 0.6, 0.8 and 1 kGy. The test organisms irradiated and non-irradiated suspensions were serially diluted to ascertain the viable bacterial count, which was then spread out on LB agar plates. Following an 18-hour incubation period at 37˚C, the plates' colony-forming units were counted [20]. A semi logarithmic survival graph was created by plotting the number of average colony of each type bacterial cells against the irradiation dose. Percent survivals of bacterial cells were plotted on Y-axis in logarithmic scale where γ-irradiation dosages applied were plotted on X-axis.

3. Results

3.1. Lycopene Content

Total content of lycopene from tomato paste of our local variety extract was 200 ppm. According to one study on tomatoes and tomato-related products, the lycopene content of fresh tomatoes was about 120 ppm, whereas that of tomato

paste was about 160 ppm. The lycopene content of tomato boiled sauce was about 40 ppm, tomato ketchup was 170 ppm, and spaghetti sauce was 160 ppm [21]. Variations can be ascribed to differences in the treatment's experimental design, raw material composition, and methodology. The UV/visible absorption spectrum was used as a first clue for the identification of lycopene. Figure 1 displayed the shape of the spectrum along with the λ max values of the lycopene in methanol purified from our local tomato cultivar.

3.2. DPPH Radical Scavenging Activity

Antioxidants postpone the oxidation of oxidizable materials by scavenging free radicals and reducing the oxidative stress resulting from the reactive oxygen and nitrogen species [22]. These species have the ability to initiate membrane lipid peroxidation and damage DNA. The DPPH assay quantifies the possibility of contributing an electron or α hydrogen atom to a particular ROS or RNS. The crude lycopene extract's inhibitory percentage of DPPH scavenging adequacy was displayed in (Figure 2). The DPPH scavenging capability of lycopene extracted was compared with standard ascorbic acid. It was found that the IC50 DPPH inhibition percentage of lycopene was 112 µg/ml compared to 74 µg/ml for ascorbic acid, meaning that lycopene has partially antioxidant power of ascorbic acid.

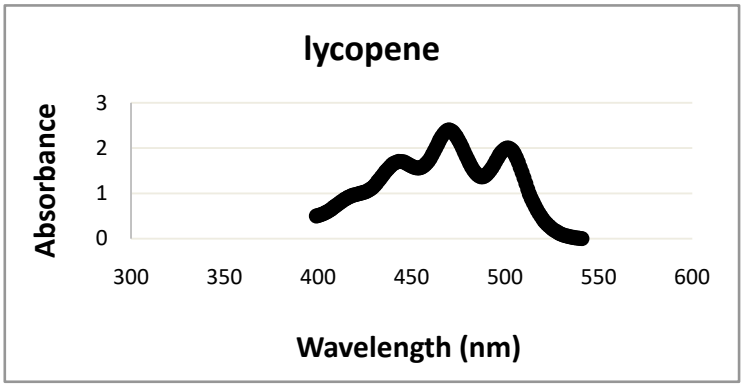

Figure 1. UV-Vis spectrum of lycopene extract from tomato sample.

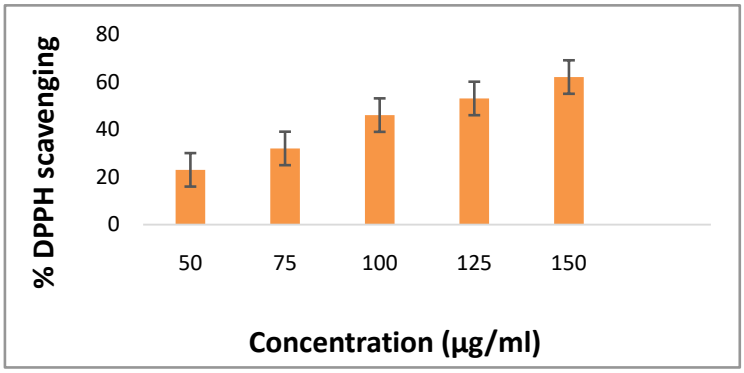

Figure 2. Determination of a DPPH radical scavenging activity of lycopene.

3.3. Protective Effect of Lycopene on *E. coli*

The survival of *E. coli* ATCC 11303 cells at the various doses of γ-radiation (100 Gy - 1 kGy) without and with the lycopene is shown in Figure 3. After overnight incubation of *E. coli* ATCC 11303 on LB broth, bacterial suspension was optimized (absorbance$_{600nm}$ ~ 0.1) in spectrophotometer. Bacterial colony was monitored after overnight incubation at 37˚C on LB agar media. The bacterial cells in PBS without the lycopene (control) decreased with an increase in the dose of γ-radiation. Thus, at 0.4 kGy in PBS without the lycopene (control), no colony forming units were detected, indicating complete elimination of the microflora (Figure 4). Conversely, 4 log cycle reductions in microflora were noted in the presence of lycopene. At a higher dose of 1 kGy no colony forming units were observed in the presence of lycopene, indicating lack of survivors. However, in the presence of lycopene extracts the population was reduced by ~4 log cycles, indicating a protective effect of these extracts. *E. Coli* D_{10} value in PBS was discovered to be 75 Gy. The D_{10} value 180 Gy was found to be >2-fold higher in the extract-containing lycopene sample than in the lycopene extract-free controls.

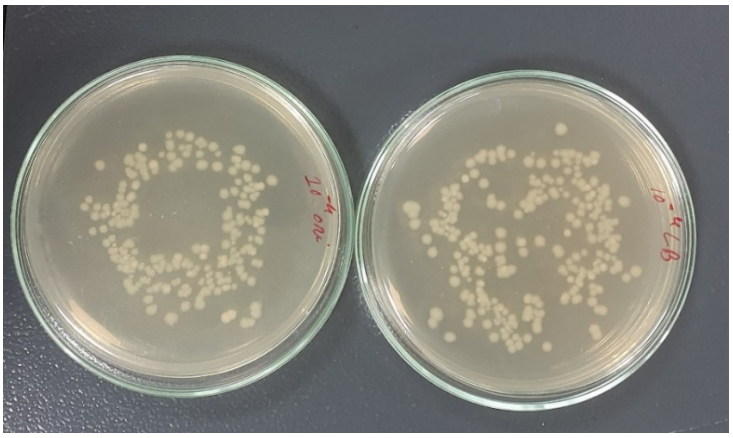

Figure 3. Bacterial colony at 100 Gy without (left) and with (right) lycopene extract.

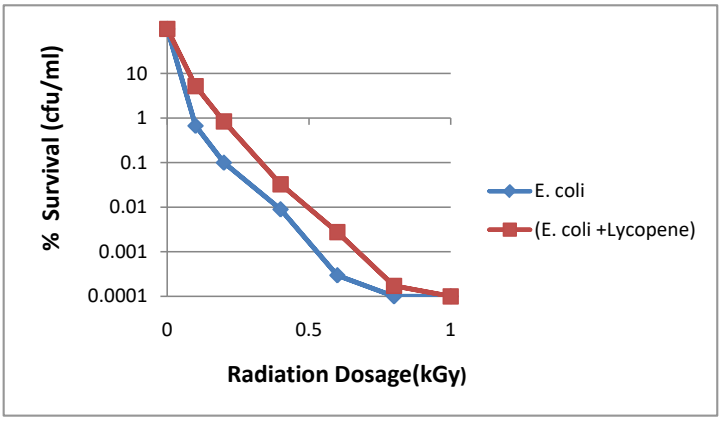

Figure 4. γ-radiation survival curve of *E. coli* ATCC 11303 in PBS with and without the lycopene.

3.4. Inhibition of Radiation Induced Strand Breaks in Plasmid DNA

The plasmid pUC19 DNA was irradiated with and without the lycopene at 50 and 100 Gy doses of γ-radiation. It was observed how the supercoiled form changed into an open circular form. The pUC19 is a 2.69 kb plasmid with an ampicillin resistance marker on it. It is clear from the Figure 5 that the plasmid DNA was protected during irradiation in the presence of the lycopene extracts, as indicated by its inferior degradation (lanes 5 - 7) compared to that in lanes 4, which was without the lycopene extracts. Lycopene has no effect on plasmid DNA. Supercoiled plasmid ladder used to measure the length of circular form DNA and 1 kbp DNA ladder was used to measure the length of linear DNA. The appearance of more than one band of plasmid in gel electrophoresis is due to the presence of multimeric (Supercoiled, open circular and linear) forms of the plasmid. In ImageJ analysis for Figure 5, intensity of linear band due to radiation was higher in lane 4 rather than lane 5, 6 and the intensity of intact circular form was higher than in lane 5, 6 rather than lane 4. In lane 7, data is not clear due to poor migration. Similar results were obtained with lycopene at 50 Gy radiation dosage by ImageJ analysis (Figure 6). Without lycopene extract, intensity of linear band was higher due to radiation damage rather than with lycopene extract sample lane. Gradually higher lycopene concentration gives more protection at irradiation, but much higher concentration showed poor protection at both 50 and 100 Gy radiation doses.

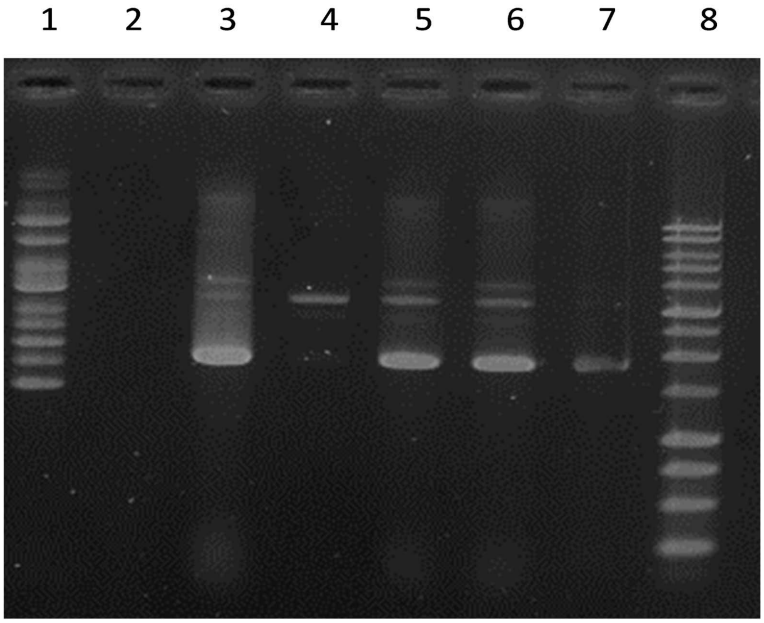

Figure 5. Agarose gel electrophoretic pattern showing forms of plasmid pUC19 at 100 Gy. Lane 1: Supercoiled plasmid ladder, Lane-2: Only lycopene, lane-3: Control non-radiated, Lane 4: Control irradiated without lycopene Lane 5 - 7: Irradiated with lycopene at different concentration (150, 260 and 500 µg/ml), Lane 8: 1 kb DNA ladder.

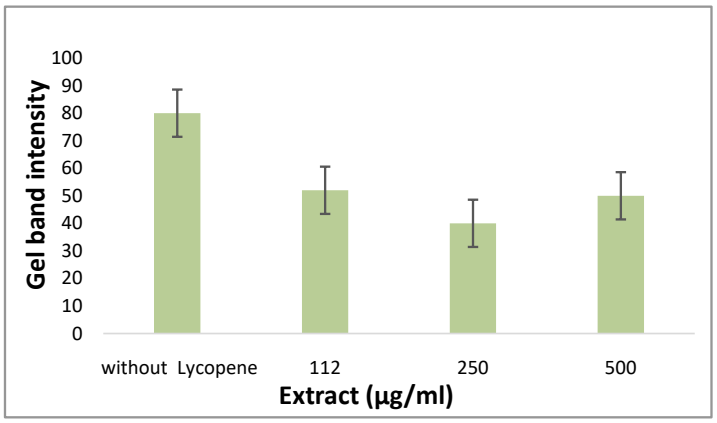

Figure 6. Percentage gel band intensity as assessed by the induction of plasmid linear form is plotted against concentrations (150 - 500 µg/ml) of the extract, in pUC19 DNA assay after exposure to 50 Gy dose.

4. Discussion

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) in biological systems, such as superoxide, hydroxyl, and nitric oxide radicals, can oxidize proteins and lipids in cells and damage DNA [23]. Antioxidants from food and medicinal plants must be properly extracted and evaluated in order to be used in functional foods, medications, and food additives. This process also helps to identify new sources of antioxidants. Natural antioxidants derived from plant materials mostly consist of carotenoids (xanthophylls, lycopene, and carotenes), vitamins (vitamin E and C), and polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes) [24]. The three-peak spectrum that results from lycopene's maximal absorption at three wavelengths (440, 470 and 505 nm) is consistent with the findings of the previous investigation [25]. The local tomato is a good source of natural lycopene, which shows promise for use as a functional nutrient in the food industry [26]. Epidemiologic research indicates that diets high in tomatoes may decrease the risk of lung, stomach, prostate, cervix, breast, oral cavity, pancreatic, colorectal, and esophageal cancers, among other cancer types [27]. It's possible to propose that lycopene, which was extracted from local tomato paste, acts as an easily accessible natural source of lipophilic antioxidants. However, in addition to lycopene, other polyphenols and ascorbic acid contents may also contribute to tomatoes' antioxidant activity [28]. So we should focus on the improvement of lycopene extraction protocol. While chemically manufactured radio-protectors, such as butylated hydroxytoluene, reduce these adverse effects, they can cause a host of unintended side effects in humans, including changes in blood pressure, nausea, vomiting, and localized and widespread cutaneous reactions [29]. Edible plants are currently at the forefront of the quest for natural antioxidants that are both safe and effective. Because of their supposed health benefits, lycopene is now commercially available and referred as supplemental medications [30]. The results of the DPPH scavenging method demonstrated a high level of antioxidant activity. Lycopene is a cheap, natural,

and effective antioxidant that may also have radioprotective properties at the chromosomal level [31]. Lycopene significantly decreased the organism's sensitivity to γ-radiation. One study showed that the antioxidant containing spices (chili, Turmeric and black pepper) added to a food could significantly alter the susceptibility of its microflora to γ-radiation. These findings also suggested that the protection of microorganisms that was seen might primarily be attributed to the antioxidant of spices ability to protect their DNA [32]. This study results is similar to one study that the fenugreek seed extracts also protect the pBR322 DNA assay after exposure to 60 Gy dose [33]. One study found that higher sugarcane juice concentration (5% and more) and higher dose rate of gamma radiation exhibit less protection of DNA [34]. One useful biomarker of the oxidative state and the antioxidant defense system is the examination of the degree of DNA damage. According to research, lycopene can prevent radiation-induced DNA mutations and modulate DNA repair when applied at the proper times before and after exposure to ionizing radiation [35].

5. Conclusion

Radioprotectants shield DNA from ionizing radiation-induced damage by scavenging free radicals. Natural radioprotectants have improved as a result of the side effects of chemical radioprotectants, providing an alternative. Relevant studies indicate that lycopene is a potent scavenger that can be used with minimal side effects in treatment, suggesting that it could be a promising radio-protective for our radiation exposure anxiety. Lycopene supplementation, particularly at low doses, may help prevent oxidative damage induced by radiation. In the case of radiation emergency accidents and radiotherapy treatment, this supplement may help lessen DNA disorders or side effects.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data and Materials Availability

All data associated with this study are available from the corresponding author upon reasonable request.

Author Contribution

S. Islam conceived the idea of the study. S. Islam and A. H. M. Kamal, contributed to data analysis and interpretation. A.Y.K. M. M. Rana supervised the study. A.Y.K. M. M. Rana, M. Z. Rahman, and P. K. Roy contributed to drafting and revising the manuscript. All authors reviewed and approved the final version.

References

[1] Lü, J.M., Lin, P.H., Yao, Q. and Chen, C. (2010) Chemical and Molecular Mechan-

isms of Antioxidants: Experimental Approaches and Model Systems. *Journal of Cellular and Molecular Medicine*, **14**, 840-860. https://doi.org/10.1111/j.1582-4934.2009.00897.x

[2] Kawamura, K., Qi, F. and Kobayashi, J. (2018) Potential Relationship between the Biological Effects of Low-Dose Irradiation and Mitochondrial ROS Production. *Journal of Radiation Research*, **59**, ii91-ii97. https://doi.org/10.1093/jrr/rrx091

[3] Hall, E.J. and Giaccia, A.J. (2005) Radiobiology for the Radiologist. 6th Edition, Lippincott Williams & Wilkins, Philadelphia.

[4] Ravanat, J.L. and Douki, T. (2016) UV and Ionizing Radiations Induced DNA Damage, Differences and Similarities. *Radiation Physics and Chemistry*, **128**, 92-102. https://doi.org/10.1016/j.radphyschem.2016.07.007

[5] Fabre, K.M., Saito, K., DeGraff, W., Sowers, A.L., Thetford, A., Cook, J.A., Krishna, M.C. and Mitchell, J.B. (2011) The Effects of Resveratrol and Selected Metabolites on the Radiation and Antioxidant Response. *Cancer Biology and Therapy*, **12**, 915-923. https://doi.org/10.4161/cbt.12.10.17714

[6] Halliwell, B. (1995) How to Characterize an Antioxidant: An Update. *Biochemical Society Symposia*, **61**, 73-101. https://doi.org/10.1042/bss0610073

[7] Jayakumar, S., Pal, D. and Sandur, S.K. (2015) Nrf2 Facilitates Repair of Radiation Induced DNA Damage through Homologous Recombination Repair Pathway in a ROS Independent Manner in Cancer Cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **779**, 33-45. https://doi.org/10.1016/j.mrfmmm.2015.06.007

[8] Mai, A.A., Al Dajah, S., Murad, A.A., El-Salem, A.M. and Khafajah, A.M. (2019) Extraction, Purification, and Characterization of Lycopene from Jordanian Vine Tomato Cultivar, and Study of Its Potential Natural Antioxidant Effect on Samen Baladi. *Current Research in Nutrition and Food Science*, **7**, 532-546. https://doi.org/10.12944/CRNFSJ.7.2.22

[9] Papas, A.M. (1999) Diet and Antioxidant Status. *Food and Chemical Toxicology*, **37**, 999-1007. https://doi.org/10.1016/S0278-6915(99)00088-5

[10] Rao, A.V., Young, G.L. and Rao, L.G. (2018) Lycopene and Tomatoes in Human Nutrition and Health. CRC Press, Boca Raton. https://doi.org/10.1201/9781351110877

[11] Gajowik, A. and Dobrzyńska, M.M. (2017) The Evaluation of Protective Effect of Lycopene against Genotoxic Influence of X-Irradiation in Human Blood Lymphocytes. *Radiation and Environmental Biophysics*, **56**, 413-422. https://doi.org/10.1007/s00411-017-0713-6

[12] Pathak, S., Akhade, P., Gajanan Bhojane, K., Sadar, D., Folane, P., Biyani, K.R. and Pathak, S.S. (2020) A Review on Recent Techniques of Extraction and Isolation of Lycopene from Tomato. *International Journal of Research and Review*, **7**, 487-490.

[13] Imran, M., Ghorat, F., Ulhaq, I., Urrehman, H., Aslam, F., Heydari, M., Shariati, M.A., Okuskhanova, E., Yessimbekov, Z., Thiruvengadam, M., Hashempur, M.H. and Rebezov, M. (2020) Lycopene as a Natural Antioxidant Used to Prevent Human Health Disorders. *Antioxidants*, **9**, Article 706. https://doi.org/10.3390/antiox9080706

[14] Islamian, J.P. and Mehrali, H. (2015) Lycopene as a Carotenoid Provides Radioprotectant and Antioxidant Effects by Quenching Radiation-Induced Free Radical Singlet Oxygen: An Overview. *Cell Journal*, **16**, 386-391.

[15] Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Improved M13 Phage Cloning Vectors and Host Strains: Nucleotide Sequences of the M13mpl8 and pUC19 Vec-

tors. *Gene*, **33**, 103-119. https://doi.org/10.1016/0378-1119(85)90120-9

[16] Roldán-Gutiérrez, J.M. and de Castro, M.D.L. (2007) Lycopene: The Need for Better Methods for Characterization and Determination. *TrAC Trends in Analytical Chemistry*, **26**, 163-170. https://doi.org/10.1016/j.trac.2006.11.013

[17] Brand-Williams, W. (1999) Use of a Free Radical Method to Evaluate Antioxidant Activity. *Food Science and Technology*, **28**, 1231-1237.

[18] Green, M.R. and Sambrook, J. (2012) Molecular Cloning: A Laboratory Manual. 4th Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

[19] Dalgaard, P., Ross, T., Kamperman, L., Neumeyer, K. and McMeekin, T.A. (1994) Estimation of Bacterial Growth Rates from Turbidimetric and Viable Count Data. *International Journal of Food Microbiology*, **23**, 391-404. https://doi.org/10.1016/0168-1605(94)90165-1

[20] Madigan, M.T., Martinko, J.M. and Parker, J. (1997) Brock Biology of Microorganisms (Vol. 11). Prentice Hall, Upper Saddle River.

[21] Alda, L.M., Gogoa, I., Bordean, D., Gergen, I., Alda, S., Moldovan, C. and Ni, L. (2009) Lycopene Content of Tomatoes and Tomato Products. *Journal of Agroalimentary Process and Technologies*, **15**, 540-542.

[22] Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. (1998) Hplc Method for Evaluation of the Free Radical-Scavenging Activity of Foods by Using 1, 1-Diphenyl-2-Picrylhydrazyl. *Bioscience*, *Biotechnology and Biochemistry*, **62**, 1201-1204. https://doi.org/10.1271/bbb.62.1201

[23] Fang, Y.Z., Yang, S. and Wu, G. (2002) Free Radicals, Antioxidants, and Nutrition. *Nutrition*, **18**, 872-879. https://doi.org/10.1016/S0899-9007(02)00916-4

[24] Xu, D.P., *et al.* (2017) Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International Journal of Molecular Sciences*, **18**, 20-31. https://doi.org/10.3390/ijms18010096

[25] Bunghez, I.R., Raduly, M., Doncea, S., Aksahin, I. and Ion, R.M. (2011) Lycopene Determination in Tomatoes by Different Spectral Techniques (UV-VIS, FTIR and HPLC). *Digest Journal of Nanomaterials and Biostructures*, **6**, 1349-1356.

[26] Rodriguez-Amaya, D.B. and Kimura, M. (2004) Harvest Plus Handbook for Carotenoid Analysis. International Food Policy Research Institute (IFPRI), Washington DC.

[27] Ganesan, M., Rajesh, M., Solairaj, P. and Senthilkumar, T. (2012) Tomato as a Pioneer in Health Management. *International Journal of Pharmaceutical*, *Chemical and Biological Sciences*, **2**, 210-217.

[28] Borguini, R.G., Bastos, D.H.M., Moita-Neto, J.M., Capasso, F.S. and Torres, E.A.F.D.S. (2013) Antioxidant Potential of Tomatoes Cultivated in Organic and Conventional Systems. *Brazilian Archives of Biology and Technology*, **56**, 521-529. https://doi.org/10.1590/S1516-89132013000400001

[29] Viuda-Martos, M., Sanchez-Zapata, E., Sayas-Barberá, E., Sendra, E., Pérez-Álvarez, J.A. and Fernández-López, J. (2014) Tomato and Tomato Byproducts. Human Health Benefits of Lycopene and Its Application to Meat Products: A Review. *Critical Reviews in Food Science and Nutrition*, **54**, 1032-1049. https://doi.org/10.1080/10408398.2011.623799

[30] Duthie, S.J., Ma, A., Ross, M.A. and Collins, A.R. (1996) Antioxidant Supplementation Decreases Oxidative DNA Damage in Human Lymphocytes. *Cancer Research*, **56**, 1291-1295.

[31] Wertz, K., Siler, U. and Goralczyk, R. (2004) Lycopene: Modes of Action to Pro-

mote Prostate Health. *Archives of Biochemistry and Biophysics*, **430**, 127-134. https://doi.org/10.1016/j.abb.2004.04.023

[32] Sharma, A., Gautam, S. and Jadhav, S.S. (2000) Spice Extracts as Dose-Modifying Factors in Radiation Inactivation of Bacteria. *Journal of Agricultural and Food Chemistry*, **48**, 1340-1344. https://doi.org/10.1021/jf990851u

[33] Kaviarasan, S., Naik, G.H., Gangabhagirathi, R., Anuradha, C.V. and Priyadarsini, K.I. (2007) *In Vitro* Studies on Antiradical and Antioxidant Activities of Fenugreek (*Trigonella foenum graecum*) Seeds. *Food Chemistry*, **103**, 31-37. https://doi.org/10.1016/j.foodchem.2006.05.064

[34] Kadam, U.S., Ghosh, S.B., De, S., Suprasanna, P., Devasagayam, T.P.A. and Bapat, V.A. (2008) Antioxidant Activity in Sugarcane Juice and Its Protective Role against Radiation Induced DNA Damage. *Food Chemistry*, **106**, 1154-1160. https://doi.org/10.1016/j.foodchem.2007.07.066

[35] Srinivasan, M., Sudheer, A.R., Pillai, K.R., Kumar, P.R., Sudhakaran, P.R. and Menon, V.P. (2007) Lycopene as a Natural Protector against γ-Radiation Induced DNA Damage, Lipid Peroxidation and Antioxidant Status in Primary Culture of Isolated Rat Hepatocytes *in Vitro*. *Biochimica et Biophysica Acta* (*BBA*)-*General Subjects*, **1770**, 659-665. https://doi.org/10.1016/j.bbagen.2006.11.008