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Free radical generation in post-irradiation period

Dr. Dhankesh Meena

Assistant professor

SCRS Government College Sawai Madhopur

Abstract

One of the most important sources of free radicals in biological systems is the xanthine oxidoreductase (XOR) system. This system is comprised of xanthine dehydrogenase (XDH) and xathine oxidase (XO). In normal tissues, the XOR system is almost exclusively found in the XDH form, but in injured tissues, it changes into the free radical producing XO form. Therefore, it is anticipated that the XO-form of the XOR system will predominately be found in tissues that have been radiolytically injured. In the case that this occurs, XO may stimulate the formation of free radicals and intensify the effects of radiation in the time period after the radiation exposure. EPR spectrum of the reference (non-irradiated) sample represents a broad singlet line with unresolved hyperfine splitting, which can be attributed to Mn(II) ions. On top of this, an additional sharp EPR signal (g = 2.0022, Bpp 1 mT) is superimposed, which can be attributed to stable semiquinone radicals produced by the oxidation of polyphenolic compounds that are present in plants. The gamma-irradiated samples were found to include additional paramagnetic structures with varying origins (mainly consisting of cellulose and carbohydrates), varying degrees of thermal stability, and varying lifetimes. These structures were identified. Immediately after the irradiation, the values of TBARS and the total content of phenolic compounds in the oregano extract made with methanol and water were found to have increased in a manner that was statistically significant. Alterations in the antioxidant capabilities of oregano extracts were also tracked as time passed after radiation therapy was administered. It was clear from the ferric reducing power test and the amount of total phenolic substances that the oregano samples that had been irradiated and those that had not been irradiated experienced a significant time-dependent decrease in their antioxidant activity over the course of storage. This was likely the result of the storage process.

keywords: *Free radical, post-irradiation*

Introduction

Ionizing radiation is damaging to life. The biological effects of radiation are the end results of a lengthy sequence of phenomena that are put in motion by the passage of radiation through the cell. These phenomenan are set in motion by the passage of radiation through the cell (Table 1). Damage caused by radiation can be either direct or indirect; this depends on whether the energy is absorbed by the molecules of the tissue itself or by the water that is around it. Following the absorption of radiation's energy, the processes of excitation/ionization (lOt5 _10-13 sec) and free radical formation (10- 13_10-11 sec) take place. Absorption of radiation's energy takes occur within 10-;8_10-16 seconds. As a result of the fact that cells are composed of around 60–80% water, the action of radiation on water (reaction I) is primarily responsible for mediating the majority of the biological effects:

$$H_2O \rightarrow e^-_{aq}, HO^{\bullet}, H^{\bullet}, O_2^{\bullet-}, H_2O_2, H_2 \text{ etc.} \quad \ldots (1)$$

In reaction I, radiolytically generated free radicals and molecular products react with biomolecules and bring about changes in the structure and function of the biomolecules. This reaction takes place as a result of the breakdown of water. As a result, the mechanisms that result in the creation of free radicals are thought to play a very essential part in the cellular death that is caused by radiation. The free radical species in question are highly reactive and have a very short lifetime. Table 2 presents information on the biological half lives of some of the free radicals. It is essential that the majority of free radical reactions in irradiation cells be rapid and finished within a fraction of a second (Table 1). This leads to the repair of damage, which may be manifested right away, within hours, days, or years depending on the nature and severity of the damage. Because of these facts, it is generally accepted as a given that free radicals do not arise during the post-irradiation phase or during the late effects of radiation, nor do they have any part to play in these processes. It is now well documented that free radicals are involved in the damage done to cells and the death of cells in non-radiolytic systems. It is important to note that certain species have been demonstrated to be responsible for not only the causation of numerous diseases but also their consequences. Pathophysiological circumstances are, in fact, one of the most important producers of free radicals. In the majority of illnesses, an increase in the generation of free radicals is thought to be the result of the activity that is associated with that disease. It has been discovered that damaged tissue goes through the process of peroxidation at a far faster rate than healthy tissue does t.2. It is now abundantly obvious that the damage done to the tissues themselves may be a significant source of free radicals. As a result, radiolytically injured cells should also act as a source of free radicals in the post-irradiation era, which should add to the damage and problems associated with the late effects of radiation. In order to investigate this possibility, the xanthine oxidoreductase (XOR) system was installed in the livers of mice that had been exposed to radiation.

Radiation damgae and XOR system

XO is found in living organisms almost exclusively as NAD+-dependent XDH, which does not contribute to oxidative cell damage. 7 R XDI-l can be converted to XO in many pathophysiological situations. There's evidence that ischemiaireperfusion9 converts XDH to XO. Injured cells convert XDH into XO 10, it's hypothesized. These studies and others confirmed that tissue injury is required to convert XDH to XO. 2. Glutathione (GSH) and thiols

Table 2 - Intra cellular half life (T Y2) in seconds of some import ant frec radicals

Free radical	T ½
$^1O_2$	1×10^{-6}
$O^{\bullet}{}_2^-$	1×10^{-6}
$^{\bullet}OH$	1×10^{-9}
$ROO^{\bullet}$	1×10^{-2}
$RO^{\bullet}$	1×10^{-6}

$RO^{\bullet}$ and $ROO^{\bullet}$ represent alkoxyl and peroxyl free radicals

be crucial for preserving XDH in Vi V0 13. 14 and converting XO to XDH IS. Radiation-induced oxidation of XDH sulfhydryl groups to disulfides may convert XDH to XO by changing its structure and depleting GSH and other thiols. Ionizing radiation disrupts Ca2+ homeostasis. Ca2+ homeostasis disruption may trigger XDH to X06 conversion. Radiation damage and the XOR system are linked. In radiolytically injured

cells, XDH may be transformed into XO during the post-irradiation phase. The XOR system seems to be an excellent model for confirming post-irradiation free radical production in injured cells/tissues.

Free Radical Isolation

Gomberg's synthesis of the triphenylmethyl radical, (C6H5)3C•, at the beginning of the previous century showed that organic free radicals with a measured lifespan could be isolated.

In (C6H5)3-C-C-(C6H5)3, a byproduct of synthesising hexaphenylethane, the core carbon is trivalent because it has three instead of four substituents and an unshared electron. Triphenylmethyl free radicals are only stable in specific organic solvents; they are destroyed by air, water, or strong acids.

Molecular weight data supported Gomberg's assertion that hexaphenylethane disintegrated into two free radicals. This finding involves stable free radicals. Free radicals are reactive and short-lived. The high chemical reactivity of free radicals is due to the odd electron's combining energy and reactions that complete electron pairs. The considerable stability of triphenylmethyl and its analogues made isolating them difficult for theoretical chemists. The introduction of wave mechanics to organic chemistry led to a broader idea of resonance inside complex molecules. It was understood that the domain of the odd electron of triphenylmethyl, like that of benzene's aromatic sextet, may extend over a wide intramolecular area. The free valence electron in the triphenylmethyl molecule has less inherent energy than in simpler molecules. Triphenylmethyl is a rather stable species, but the technology in that year couldn't handle transient entities with a short life, therefore free radical research had to wait.

Moses Gomberg's report of triaryl methyl free radicals was regarded with incredulity or apathy in 1930. Von Richter noted in 1915, "The idea of free radicals, able to live alone and play a specific function in chemical processes, has long been abandoned." A decade later, Porter at Berkeley observed, "Negative results progressively cemented the concept that a free carbon radical was incapable of independent existence," despite growing evidence of free radicals.

Vapour densities of gases at high temperatures indicated that diatomic molecules could dissociate into free atoms, but the possibility of the independent existence, at normal temperatures, of free atoms such as hydrogen, oxygen, or chlorine was almost never considered until 1913, when Bohr showed that the spectrum emitted from a hydrogen discharge tube could be interpreted as an atomic form and not a molecular form.

Wood initially separated and reported atomic hydrogen's characteristics in 1922. Bonhoeffer studied hydrogen's chemical characteristics in 1924.Paneth and Hofeditz synthesised methyl (•CH3) by pyrolyzing tetramethyl lead utilising Bonheoffer's technique to examine atomic hydrogen. In succeeding years, Gomberg and other writers who continued in his field, together with Paneth's work on gas-phase free radicals, led to experimental evidence for stable as well as short-lived free radicals, so free radicals received acceptable reputation in chemical circles.

Free Radical in Chemical Reactions

Given the advancements in free radical isolation, it's not unexpected that chemists are incorporating free radical theories into reaction processes. Kharasch and Mayo proposed a free radical technique for adding hydrogen bromide to olefins in 1933. Before 1933, adding hydrogen bromide to alkenes was confusing.

Markovnikow's rule states that the acidic proton contributes to the double bond's less substituted carbon. Because electron-repellent alkyl groups stabilise the resulting carbocation:

$$CH_3 - CH = CH_2 + H^+ \longrightarrow CH_3 - \overset{+}{C} - CH_3 \xrightarrow{Br^-} CH_3 - CHBr - CH_3 \quad (1)$$

On the other hand, there were cases when addition occurred in the reverse direction, with the hydrogen being added to the carbon that had the most substitutes. Through their study in 1933, Kharasch and Mayo were able to decipher the riddle and describe how an anti-Markovnikow orientation could be created through the addition of free radicals. This allowed the enigma to be solved. It was determined that the organic peroxides that were present in the alkenes and that had been created as a result of the action of ambient oxygen on the alkenes were the factors that were able to explain this process. In point of fact, Kharasch and Mayo discovered that an anti-Markownikoff addition of hydrogen bromide took place when alkenes that included peroxides or hydrogen peroxide interacted with hydrogen bromide. Therefore, they hypothesised that the anti-Markownikoff addition of HBr occurred because of the presence of peroxides. They referred to this phenomenon as the "peroxide effect," and they believed that it occurred as a result of a chain reaction involving free radicals in which the intermediate carbon-centered radical was stabilised by the adjacent alkyl group(s):

$$R - O - O - R \longrightarrow 2R - O^{\cdot} \xrightarrow{2HBr} 2R - OH + 2Br^{\cdot}$$
$$CH_3 - CH = CH_2 + Br^{\cdot} \longrightarrow CH_3 - \overset{\bullet}{C}H - CH_2 - Br \xrightarrow{HBr} CH_3 - CH_2 - CH_2 - Br + Br^{\cdot} \quad (2)$$

Kharasch also proposed that radical intermediates and chain reactions could play a significant part in a wide variety of organic reactions. In the years that followed, he was successful in developing synthesis reactions, such as the sulfonation, chlorination, and carboxylation of hydrocarbons and paraffin.

His research laid the groundwork for the creation of synthetic materials such as rubber and plastics and cleared the path for their production. The traditional polymerization method of condensation was utilised to continue the production of nylon and a variety of other goods. Free radical polymerization, on the other hand, offered a number of benefits, including a high tolerance of chemical contaminants and severe temperatures, as well as the capability of being employed with a diverse assortment of monomers (organic molecules). Today, over half of the polymers that we use are produced using free radicals. Polymers are materials that are utilised in a wide variety of applications, including food packaging, paint, adhesives, film, carpets, pipes, and more. Within the confines of this discussion, the contributions that Semenov has made to the fields of chemical kinetics and the processes of combustion stand out as particularly noteworthy. The findings of these studies have led to the conclusion that free radicals, which are formed throughout the process, play an important role in the success of many chemical processes. The work of Semenov not only paved the way for a new understanding of the connection between the reactivity and the structure of particles entering a chemical reaction, but it also made it possible to rationally regulate the rate at which chemical changes occur and the direction in which they take place. This, in turn, has significant repercussions for the enhancement of established industrial processes as well as the creation of new processes, such as in the

domains of polymerization and direct oxidation in addition to hydrocarbon cracking. It is interesting to note that some of Semenov's ideas about chain reactions were also articulated by the well-known English kinetic chemist C. N. Hinshelwood, who shared the Nobel Prize in Chemistry with Semenov in 1956. Hinshelwood was given the prize jointly with Semenov.

Free Radicals in Biological Systems

The abundant work done by Leonor Michaelis is usually thought to be the impetus behind the interest in free radicals as naturally occurring biochemical intermediates in biological systems. In research that Michaelis began in the 1930s, he observed that the curves of oxidation-reduction potentials obtained by adding increasing amounts of an oxidant to hydroquinone initially showed the loss of an electron and then, in a subsequent stage, the loss of another electron. These results were obtained by adding increasing amounts of an oxidant to the hydroquinone. He was under the impression that the departure of the first electron was directly proportional to the advent of the free semiquinone radical. The creation of the totally oxidised form, known as the quinone, resulted in the semiquinone contributing a second electron to the compound. Because the intermediate radicals are extremely unstable and soon lose the second electron, the concentration of these radicals in the medium in which the reaction takes place is very low; as a result, identifying them is extremely challenging, if not impossible. Therefore, it is not always possible to differentiate between oxidation reactions, which proceed in a single step and involve radicals, and those that proceed by transferring two electrons at a time, relying solely on the products that are formed. This is because oxidation reactions proceed in a single step and involve radicals. However, Michaelis proposed a notion that all oxidations of organic molecules, which entail the exchange of two electrons, take place in two phases one after the other, with the intermediate step being made up of a free radical. Michaelis is credited with the development of this idea. We now know that this idea is flawed since there are biological processes in oxidative reactions that result in the loss of two electrons but only require a single step to complete and do not entail free radicals. These reactions can take place in a single step. However, Michaelis's ideas gave rise to research that indicated that some intermediate reagent can sometimes be identified in the enzymatic oxidation-reduction reactions of biological molecules. This was the case despite the fact that there was no evidence to suggest that the intermediate was a free radical. Michaelis's ideas are credited with having inspired the research.

However, beginning in 1954, using sensitive methods for detecting free radicals, such as electronic spin resonance (ESR), which uses the paramagnetism of free radicals, some researchers demonstrated that it was possible to identify a paramagnetic intermediate compound in certain enzyme-substrate systems. These researchers used the fact that the paramagnetism of free radicals was used. The investigation of enzymatic oxidation was one of the domains in which the ESR was successfully applied. During these types of reactions, an electron is first taken from the substrate by an enzyme, and then it is transferred to a coenzyme. The formation of a semiquinone during the riboflavin oxide-reduction (vitamin B2) is an important example. This reaction results in the formation of flavin coenzymes, including flavin mononucleotide (FMN) and flavin adenine dinucleotide, which are both involved in a variety of oxidation-reduction reactions.

After some time, it was discovered that free radicals were far more common in biological systems than had been previously believed. Free radicals were discovered not only in the case of oxidation-reduction processes, but also in numerous reactions of biological significance such as photochemical reactions,

photosynthesis, and bioluminescence. Specifically, free radicals were identified in the situation of oxidation-reduction processes.

The path, which has led to the beginning and acceptance by the scientific community of the idea that free radicals are continuously formed in the cell as collateral products of normal metabolic reactions, can be initiated by the discovery of the mechanisms that are behind the effects of oxygen toxicity and ionising radiation. This idea has led to the path that has led to the beginning and acceptance by the scientific community of the idea that free radicals are continuously formed in the cell as collateral products of normal metabolic reactions.

MATERIALS AND METHODS

Solid sample characterisation. All EPR studies and extract preparations utilised Cambidi (Izmir, Turkey) powdered oregano. The samples were packed in 75 g polyethylene bags and irradiated according to Artim's (Prague, Czech Republic) commercial practices, using 60Co source at various average doses from 5 to 30 kGy, using average gamma-radiation dose rate, 2 kGy/h, on June 10, 2004. After irradiation, the average dry matter content was 90.1%; after four months, it was 90.6% (w/w).

Oregano extracts preparation. Preparing oregano extracts for antioxidant testing: 2 g solid oregano was mixed with 50 ml 80% (v/v) water/methanol solution and shaken for 1 hour at 200 rpm. The solid phase was separated by filtration, and the final extract was stored at 25°C and 40% relative humidity.

EPR measurements. Each 100 mg oregano sample was inserted in a thin-walled quartz EPR tube (internal diameter 3 mm, length 150 mm, wall thickness 0.1 mm) to generate a cylindrically shaped sample with homogeneous dimensions (sample column height 5.2 0.2 cm). The material was placed in a rectangular cavity of an EMX X-band EPR spectrometer (Bruker, Germany) and EPR spectra were collected at various temperatures. Bruker ER 4111 VT temperature control was used. Careful EPR cell filling led to high consistency between samples, with a 5% standard variation in relative EPR intensity for five separate experiments. EPR spectrometer parameters were: 9.45 GHz, 0.63–31.73 mW, 335.4 mT centre field, 20–500 mT sweep width, 5 105 gain, 0.05 mT modulation amplitude, 100 kHz modulation frequency, 84 s scan, 40.96 ms time constant, 5 scans, 298–373 K. The g-values were measured simultaneously with a reference sample containing DPPH immobilised on EPR cell. DPPH studied the quantitative EPR instrument settings. WIN EPR and SimFonia were used to simulate experimental EPR spectra (Bruker). Double spectrum integration yielded EPR integral intensities. Multi-component experimental EPR spectra were analysed as a linear combination of separate simulations utilising Scientist's least-squares minimization approach (MicroMath). The statistical characteristics of the calculation technique (R2, coefficient of determination, and correlation) determined simulation quality, or the correlation of experimental and simulated spectra. After double integration, the relative concentrations of various paramagnetic substances were estimated from simulation contributions to the experimental spectrum. They assessed the thermal stability and lifetime of individual radical formations.

Spectrophotometric measurements. The antioxidant characteristics were monitored using a doublebeam UV-VIS spectrophotometer (Carl Zeiss Jena, Germany). All tests were done in quartz UV-VIS cells (path length, 1 cm). Spectral bandwidth 20 cm–1, integration time 1 s, gain 3. The antioxidant behaviour was characterised by averaging three independent simultaneous absorbance measurements for each oregano

extract in each experimental setting. One-factor ANOVA at 0.05 was used to determine statistical significance.

DPPH radical scavenging assay.

DPPH radical scavenging assay was performed according to Bandonienė (2002). Oregano methanol/water extract (0.65 ml) was placed into 25 ml of DPPH methanolic solution c0 (DPPH) = 6 × 10–5 mol/dm3 , and the absorbance at 515 nm was measured after 15 min. Radical scavenging ability of the individual extracts was expressed as:

% = (absorbance of control – absorbance of sample) × 100/absorbance of control.

Thiobarbituric acid number. In accordance with the methodology described by Zin, the levels of thiobarbituric acid reactive compounds were analysed (2002). The following solutions were added to 1 ml of oregano methanol/water extract: 20% (w/w) aqueous trichloroacetic acid solution (two millilitres), and 0.67% (w/w) aqueous thiobarbituric acid solution (two millilitres). After that, the mixture was heated in a container containing boiling water for ten minutes. After allowing the mixture to reach room temperature, it was centrifuged at a speed of 3000 rpm for a period of 20 minutes. The thiobarbituric acid number was computed by relating the absorbance of the blank to the absorbance of the supernatant when measured at 532 nm.

Ferric reducing power. The determination of ferric reducing power was realised according to Chyau et al. (2002). Oregano methanol/water extract (2 ml) was mixed with 2 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2 ml of 1% (w/w) potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Subsequently, 2 ml of 10% (w/w) trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 minutes. Then 1 ml of the upper layer was mixed with 1 ml of distilled water and 0.2 ml of 0.1% (w/w) ferric chloride. The absorbance at 700 nm was monitored after 1 min and related to the absorbance of the blank.

Total phenolic compounds. Using a modified version of the Folin-Ciocalteau method, we were able to determine the amount of total phenolic compounds. After diluting the methanolic oregano extracts with 15.9 millilitres of distilled water, one millilitre of Folin-Ciocalteau reagent manufactured by Merck in Germany was added to the mixture. After waiting three minutes, three millilitres of sodium carbonate solution containing twenty percent was added, and the contents were combined. A colour was produced as a consequence of the reaction, and the absorbance at 755 nm was determined after sixty minutes of observation. This value was then compared to the absorbance of the blank. The same process was repeated with a gallic acid solution that was considered to be standard. The findings were reported in terms of milligrammes of gallic acid per litre of extract.

RESULTS AND DISCUSSION

EPR investigation

To begin, the EPR spectra of all of the oregano samples that were analysed were determined by applying a magnetic field strength of 500 mT. All of the samples' spectra revealed the presence of a broad EPR signal along with unresolved hyperfine splittings, as was demonstrated by the observations.

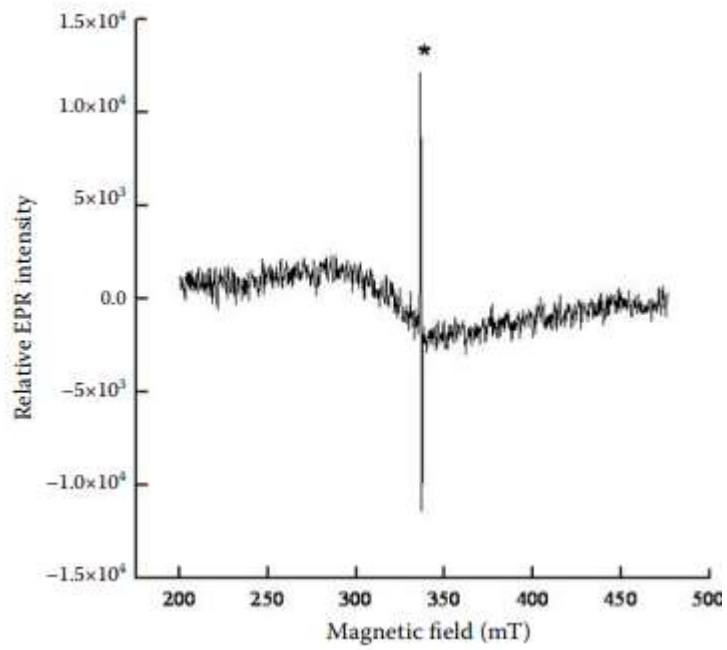

Figure 1. X-band EPR spectrum of non-irradiated dry oregano sample measured using 0.633 mW microwave power at 298 K

Due to the fact that oregano comes from a plant, this line was thought to be caused by paramagnetic Mn(II) ions, as was stated in the introduction. Manganese ions, in their capacity as cofactors of proteins and enzymes, play an important part in the biochemical activities that take place in green plants. In addition to this, they are a crucial component in the photosystem's catalytic splitting of water as well as the production of oxygen through the evolution of oxygen (II). It is anticipated that the characteristic feature of the Mn(II) EPR spectra will be a six-line component centred at geff 2.0, flanked by shoulders with a weak feature centred at geff 4.3 and a measurable absorption at zero field. This characteristic feature is expected to be present in the spectrum. The six-line multiplet spectrum is the consequence of a hyperfine interaction between the ground state of the 6 S5/2 and the nucleus of the 55Mn atom (I = 5/2). (Griscom 1990). We have demonstrated in the past (Polovka et al. 2003) that the less resolved EPR spectrum of solid tea samples can be attributed to comparatively undisturbed bonding of Mn(II) in the protein complex. According to our hypothesis, the same physical process is responsible for the form of the EPR spectra of solid oregano samples. In addition to this, a characteristically narrow EPR signal (g = 2.0022, B pp 1 mT) is overlaid on this broad line. This signal is allocated to stable radical structures and may be seen as a * in Figure 1. The absorbed gamma-radiation dosage is a crucial factor that plays a role in determining the relative EPR strength of this strong signal.

Figure 2 depicts the complete simulation analysis of the experimental EPR spectra (20 mT magnetic field sweep width) of both the reference oregano sample and the sample that was irradiated at a dosage of 30 kGy. The spectra of the non-irradiated sample were simulated as a sharp single line that was characterised by g = 2.0044, g= 2.0010, and pp = 0.285 mT. This line's characteristics can be attributed to semiquinone radicals, which are produced by the oxidation of polyphenolic compounds that are present in plants.

Antioxidant activity of oregano extracts

Several different antioxidant testing methodologies were utilised in order to study the oregano samples that had been irradiated for their potential antioxidant qualities. Irradiation and subsequent storage of oregano samples had very no impact on the plant's capacity to scavenge DPPH radicals; in fact, the effect was almost nonexistent. The examination into the antiradical activity of dessert spices came to the same conclusion as the previous inquiry. The examination of the impact that gamma-irradiation has on ferric reducing power yielded comparable findings. The FRP values of the extract that was obtained from the sample that was subjected to 30 kGy of radiation treatment.

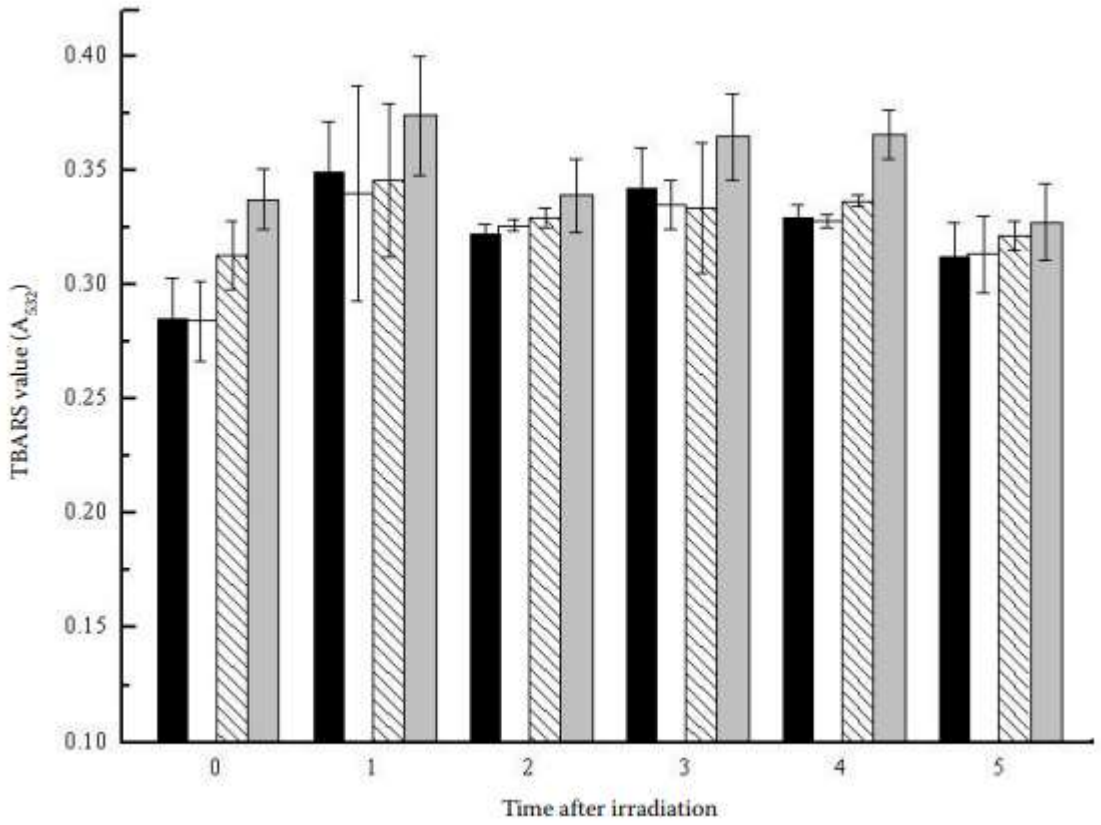

Figure 5. Effect of irradiation and storage time on thiobarbituric reactive substances content (TBARS) of oregano), 10 kGy≤) and from oregano samples irradiated at 5 kGy (′methanolic extracts prepared from reference sample and 30kGy .

was indistinguishable from either the samples used as a reference or the sample that had been irradiated at lower dosages. The ferric reducing ability of the sample irradiated at 5 kGy and monitored after 4 months of storage was slightly increased, which is to say it was increased by about 16% in comparison to the values that were determined immediately after the irradiation. This was the only change that occurred in the FRP values after post-irradiation storage. (The data are not displayed.) On the other hand, it was discovered that the TBARS values of oregano extracts had a substantial influence on the amount of gamma radiation that they have taken in. Irradiation led to a significant rise in the TBARS values of the oregano extract that was prepared from the sample that was irradiated at 30 kGy, as shown in Figure 5. This rise was approximately 18% higher than the TBARS values of the oregano extract that was prepared from the reference sample immediately after the radiation process. This difference is maintained during the storage period of five

months. In addition to this, the post-irradiation storage for one month resulted in a rise of TBARS levels that was statistically significant in all of the oregano sample extracts. The difference that was most substantial reached around 20%. The constant storage causes a marginal drop in the value of each and every TBARS parameter as time passes. The radiation treatment and post-irradiation storage of oregano were found to result in changes that were statistically significant in the case of total phenolic compounds expressed as gallic acid equivalent (Figure 6). These changes were found to have occurred as a direct consequence of the radiation. After being stored for a month, the TPC level in the reference sample reached its highest point, and then it began to progressively drop. The variations in the extracts that were made from the reference are closely tied to the variations in the amount of dry matter that occurred over the course of time. A treatment with gamma radiation led to a discernible rise in the total phenolic content of all oregano extracts. The TPC values of the extracts prepared from the samples irradiated at lower doses (up to 10 kGy) gradually increased with the absorbed dose of gamma-radiation. These values reached their highest point in the extract prepared from the sample that had been treated with 10 kGy. On the other hand, a decrease in TPC values was seen in the extracts that were generated from the samples that were irradiated at 20 and 30 kGy respectively. After being stored for one month after irradiation, the polyphenolic content of each extract experienced a slight increase (approximately 10%). The disparities between the samples became less pronounced.

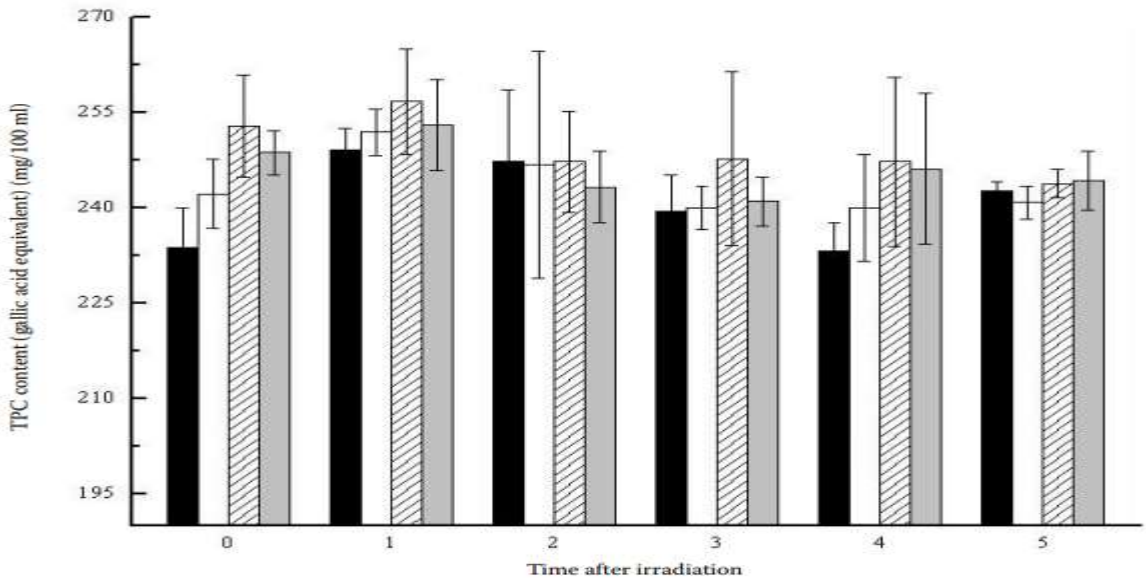

Figure 6. Irradiation and storage time had different effects on the total phenolic content (TPC) of oregano methanolic extracts. TPC) and from oregano□values are represented as gallic acid equivalent. The reference sample was irradiated at 10 kGy, and samples irradiated at 30 kGy were used to prepare extracts. 5 kGy

after being sent away for a period of 5 months Even though the absolute values of TPC went down while the sample was being stored, when the investigation period came to a close, they were still significantly higher than the value that was found immediately after the irradiation. The findings of several recently published papers are very much in line with the effect that irradiation has on the amount of phenolics found in a substance, as was discussed above.

CONCLUSIONS

Oregano samples were subjected to gamma radiation, which, according to EPR spectroscopy, caused a dose-dependent formation of paramagnetic species with distinct structures. These structures may be attributed to cellulose and carbohydrate radicals, respectively. The temperature, the relative humidity, and the conditions in which they are stored have a considerable impact on their stability. Because radiation causes an irreversible conversion of XOH into XO, the OR system is likely to be largely present in the XO form in injured cells. This is because radiation causes the conversion. This newly produced XO is quite likely to create free radicals throughout the post-irradiation period, which would, in turn, result in the continuation of gradation effects that will increase the oxidative stress. Initiators and promoters of carcinogenesis, in addition to being connected with late consequences such as fibrogenesis, are all roles that may be attributed to reactive oxygen species that are formed from o2 radicals. This is a fact that has been well established. The discoveries that are being described here might possibly have a very major impact on the area of radiation-induced carcinogenesis as well as other late impacts. More crucially, it is possible to hypothesise, based on the facts that have been reported here, that tlH,tradi olytically damaged ceils have the capacity to create free radicals.

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