



Moderate radioprotective role of zeolite in rats

Umerena radioprotektivna uloga zeolita kod pacova

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Abstract

Background/Aim. Exposure of living organisms to γ -radiation results in the overproduction of free radicals. The aim of the study was to test if the subacute administration of micronized zeolite (MZC) accomplishes radioprotective role based on the evaluation of the status of oxidative stress (OS) in the brain and 8-hydroxyguanosine (8-OH-dG) in the plasma of the rats exposed to the single γ -ray irradiation of 2 and/or 10 Gray (Gy). **Methods.** Wistar rats were on a four-week normal or 5% MZC supplemented diet and afterward exposed to the single γ -ray irradiation of 2 and 10 Gy. Groups of rats were: a) on a normal diet (the control group, and 2Gy and 10Gy groups); b) on 5% MZC supplemented diet (the control group – MZC, MZC + 2Gy, and MZC + 10Gy groups). We measured malondialdehyde (MDA), glutathione (GSH) total, and activity of total superoxide dismutase (tSOD) and manganese superoxide dismutase (MnSOD) in vulnerable brain regions (cerebellum, hippocampus, and cerebral cortex) and 8-OH-dG in plasma. **Results.** Lower MDA was found in the MZC+2Gy and MZC+10Gy groups compared to the 2Gy and 10Gy groups. Activity of total SOD was higher in the MZC+10Gy group than in the 10Gy one. GSH was the highest in the 10Gy group. Compared to the control group, 8-OH-dG was extremely higher in groups radiated with 10 Gy regardless of a diet, but slightly lower in the MZC+2Gy and 2Gy groups. **Conclusion.** Subacute MZC pretreatment accomplished partial radioprotective effect in irradiated rats compared to non-irradiated rats, based on suppressed SOD activity at 2 Gy, and reduced brain MDA when exposed to 2 Gy and 10 Gy.

Key words:

brain; oxidative stress; plasma; radiation, ionizing; rats; zeolites.

Apstrakt

Uvod/Cilj. Izlaganje živih organizama gama zračenju rezultira hiperprodukcijom slobodnih radikala. Cilj istraživanja je bio da se ispita da li subakutna ishrana dopunjena sa 5% mikronizovanog zeolita (MZC) ispoljava radiozaštitnu ulogu na osnovu statusa oksidativnog stresa (OS) u mozgu i 8-hidroksiguanozina (8-OH-dG) u plazmi pacova izloženih pojedinačnim dozama jonizujućeg zračenja od 2 i 10 Gray (Gy). **Metode.** Wistar pacovi su bili na četvoronedelnoj normalnoj ishrani ili ishrani obogaćenoj sa 5% MZC, posle čega su bili izloženi pojedinačnom jonizujućem zračenju od 2 Gy, odnosno 10 Gy. Grupe pacova bile su: a) grupa pacova na normalnoj ishrani (kontrolna grupa i grupe 2Gy i 10Gy); b) grupa pacova na ishrani obogaćenoj sa 5% MZC (kontrolna grupa – MZC i grupe MZC+2Gy i MZC+10Gy). Meren je malondialdehid (MDA), glutation (GSH) i aktivnost ukupne (tSOD) i mangan superoksid dizmutaze (MnSOD) u osetljivim strukturama mozga (cerebelum, hipokampus i cerebralni korteks), a 8-OH-dG u plazmi. **Rezultati.** Biomarker MDA je bio niži u MZC+2Gy i MZC+10Gy grupama, u odnosu na grupe 2Gy i 10Gy. Aktivnost ukupne SOD je bila viša u grupi MZC+10Gy, u odnosu na grupu 10Gy. Najviši nivo GSH bio je u grupi 10Gy. U pređenju sa kontrolnom grupom, 8-OH-dG je bio izuzetno viši u grupama ozračenim sa 10 Gy, bez obzira na dijetetski režim i niži u grupama MZC+2Gy i 2Gy. **Zaključak.** Pacovi koji su bili na režimu ishrane obogaćene sa 5% MZC bili su delimično zaštićeni od zračenja, shodno redukovanoj moždanoj aktivnosti SOD pri 2 Gy i sniženom nivou MDA pri izlaganju zračenju od 2 i 10 Gy.

Ključne reči:

mozak; stress, oksidativni; plazma; zračenje, jonizujuće; pacovi; zeoliti.

Introduction

Exposure of living organisms to γ -radiation results in the overproduction of free radicals through water radiolysis¹. Generated reactive oxygen and nitrogen species develop oxidative stress (OS) and/or nitrosative stress, reflected in deteriorated cell morphology and physiology, including oxidation/nitration of proteins, lipids, and deoxyribonucleic acid (DNA)²⁻⁴. Insufficient cell antioxidative defense and adaptive mechanisms against free radicals (FRs) mostly ended with energy devastation and apoptosis³.

Zeolite, a natural clinoptilolite, is a strong, nonselective adsorbent, ion/exchanger, catalyst, detergent or anti-diarrheic with a wide range of uses for the treatment of stomach poisoning, poisoning by some harmful agents, alkaloids, mycotoxins, some strains of bacteria, dyspepsia or flatulence (endogenous gasses generated either by products of digesting particular food or of incomplete digestion in the stomach or small intestine, such as oxygen, ammonium, nitrogen oxide), in humans and animals^{5,6}. No selective adsorption and ion/exchange by zeolite limits its prolonged use, because it may decrease the overall bioavailability of nutrients, including essential metals, etc. and so it can endanger health. Zeolite does not pass into the systemic circulation after oral intake and remains within the gastrointestinal tract⁷.

The extent of zeolite ion/exchange and adsorption (binding) capacity depends on the size of its surface area; thus, we used micronized zeolite (MZC) in our study⁷.

The brain is particularly vulnerable to oxidative injury compared to other organs because it spends 15–20% of the entire body energy⁸. If oxidative stress is going to happen in the body affected by some stimulus (xenobiotics, inflammation, etc.) it may be expected that lipid peroxidation would be developed more in the brain than in other organs, since the brain has a higher lipid content compared to other body organs and that omega-three polyunsaturated fatty acids are susceptible to oxidation⁹. Also, the content of transition metals (especially iron and copper) is pretty high in the brain tissue, which additionally contributes to OS development and generation of reactive oxygen species (ROS), concretely hydroxyl radical (HO[•]), through Fenton-like reactions^{8,10}. Vulnerable brain regions such as the pyramidal neurons of CA1 and CA3 sectors of the hippocampus, the third layer of the cerebral cortex, and striatum are particularly vulnerable to free radicals toxicity¹¹.

Gamma rays induce metabolic oxidative stress and prolonged cell injury by oxidative damage of biomolecules, including DNA, chromatin materials, lipids, and proteins. 8-hydroxyguanosine (8-OH-dG) is a well-known biomarker of DNA oxidation^{2,3}. Looking for the systemic effect of the applied γ -radiation of rats in terms of OS, we measured the content of 8-OH-dG in plasma.

In this study, we used rats as animal models to define if the pretreatment of a four-week diet supplemented with 5% MZC could change radiation responses to applied single γ -ray irradiation of 2 and/or 10 Gray (Gy), based on the evaluation of the tested end-points referring to OS status in

the cerebellum, hippocampus and cortex, and plasma 8-OH-dG, after 5 postirradiation days.

Methods

Experimental animals

Adult male Wistar rats (weights of 220–250 g) were kept under standardized housing conditions (temperature 23 ± 2 °C, lighting 12:12 light: dark, light on from 8:00 to 20:00 h) with free access to tap water and a custom pellet rat diet. Suspension of MZC was administered daily by gavage. The Ethical Committee for Experimental Animals of the Institute of Nuclear Science “Vinča” approved this experimental protocol (No. 6/12), which follows the “Guide for the Care and Use of Laboratory Animals”.

Experimental design

Wistar rats on normal diet were randomly subdivided into three groups (n = 6): control (not treated) group, and 2Gy and 10Gy groups (rats subjected to the single doses radiation of 2 Gy or 10 Gy, respectively); and accordingly, rats on 5% MZC supplemented diet covered three groups (n = 6): MZC, MZC + 2Gy, and MZC + 10Gy groups. The MZC amount was calculated concerning the quantity of ingested food and rat body mass. The suspension of 0.85–1 g of MZC/day (corresponds to 5% of 17–20 g of custom pellet/day) was administered orally, by gavage, during four-weeks^{12,13}. Rats from the control group gained 133.85 ± 24.7 g body weight, and from the MZC group, 126.28 ± 31.42 g during the four-week diet. No statistically significant differences between them were observed.

⁶⁰Co gamma source was used and designed for radiobiological and radiation chemistry experiments in the Laboratory of Radiation Chemistry and Physics, Institute of Nuclear Sciences “Vinča”. The animals were confined in custom-made individual cages, made of wire, sideways positioned, and subjected to the γ -ray irradiation.

The results of our previous pilot study, designed in line with the study of Kassayová et al.¹⁴, ascertained maximal lethal dose (LD₁₀₀) of ≥ 12.5 Gy within five postirradiation days in rats. Also, Alya et al.¹⁵ reported LD₁₀₀ of 9 Gy for male and female Wistar rats within 16 postirradiation days, whereas the observed peak (mediana) was between 6th to 10th days. However, Mason et al.¹⁶ established total-body irradiation sublethal dose of 4 Gy for rats and 5 Gy for mice (ranking radiosensitivity of the organs as follows: lung > hematopoietic system > gastrointestinal tract) within a reasonable time.

According to the abovementioned, we applied a total body irradiation of 0.167 Gy/min for 12 min, that corresponds to a total non lethal dose of 2 Gy, i.e. 200 rad, and for 60 min, that corresponds to a sublethal dose of 10Gy, i.e. 1,000 rad, in order to study chosen end-points within the appropriate period of postirradiation time of 5 days^{16,17}.

After the applied treatments, animals continued with the same diet (normal or 5% MZC supplemented diet) for

the next 5 five postirradiation days, when they were sacrificed by decapitation (previously anesthetized with an injection of 50 mg of sodium pentobarbital/kg). The brains were removed immediately and stored at -80°C until analyses were performed.

We measured OS parameters, including malondialdehyde (MDA), total superoxide dismutase – SOD (tSOD) activities and manganese superoxide dismutase (MnSOD) activities, and glutathione (GSH) in the cerebellum, hippocampus, and cerebral cortex and 8-OH-dG in the plasma of the rats.

Measurements of oxidative status

The cerebellum, hippocampus, and cerebral cortex were dissected from each frozen brain, and a crude mitochondrial fraction was prepared from each region¹⁸. Slices of brain structures were transferred separately into a saline solution (0.9% w/v). Aliquots (1 mL) were placed into a glass tube homogenizer (Tehnica Zelezniki Manufacturing, Slovenia). Homogenization was performed twice with a Teflon pestle at 800 rpm (1,000 g) for 15 min at 4°C . The supernatant was centrifuged at 2,500 g for 30 min at 4°C . The resulting precipitate was suspended in 1.5 mL of deionized water. The subcellular membranes were constantly mixed in the hypotonic solution for one hour, using a Pasteur pipette. Then, homogenates were centrifuged at 2,000 g for 15 min at 4°C , and the resulting supernatant was used for the analysis.

The Lowry method was used to measure protein concentrations in the homogenates of the tested brain regions of the rats¹⁹.

The activity of SOD (EC 1.15.1.1.; SOD) was measured spectrophotometrically. The principle of the method is related to the sequestration of superoxide anion radical ($\text{O}_2^{\bullet-}$) by SOD, which disables spontaneous epinephrine auto-oxidation (recorded at 480 nm). The kinetic of sample enzyme activity was followed in a carbonate buffer (50 mM, pH = 10.2, containing 0.1 mM EDTA), after the addition of 10 mM epinephrine²⁰. The results were expressed as U tSOD per mg of protein.

The principle of MnSOD activity measurement (which applies to tSOD, as well) assumes the addition of cyanide anions to block CuZnSOD activity. Samples were prepared in a carbonate buffer (50 mM, pH = 10.2) with the addition of 8 mM KCN, containing 0.1 mM EDTA, and 10 mM of epinephrine²⁰. The results were expressed as U MnSOD per mg of proteins.

Lipid peroxidation was measured as the quantity of MDA produced. Upon reaction with thiobarbituric acid, MDA forms a fluorescent red-complex at a ratio of 2:1, whose absorbance is measured at 532 nm²¹.

The amount of GSH present within the tissues was determined by using 5,5-dithiobis-2-nitrobenzoic acid (DTNB, 36.9 mg in 10 mL of 100% methanol) in Tris-HCl buffer (0.4 M, pH = 8.9). The intensity of produced yellow-colored p-nitrophenol anion (corresponds to GSH concentration) was spectrophotometrically measured at 412 nm.

Brain tissue was prepared in 10% sulfosalicylic acid for GSH determination²².

8-OH-dG was measured in the plasma of the rats by using commercial HT 8-OH-dG ELISA Kit II (R&D Systems, Inc. 614 McKinley Place NE Minneapolis, USA).

Kruskal-Wallis, *post hoc* Dunn's tests and Spearman's nonparametric correlations were used for the statistical data analysis using GraphPad Prism, version 5.01. Differences were considered statistically significant at $p < 0.05$. Values were presented graphically as average \pm standard deviation using GraphPad Prism.

Results

A decrease of MDA was obtained in the hippocampus ($p < 0.05$) and the cortex ($p < 0.0001$) of the rats in the MZC group and in the cortex ($p < 0.0001$) of the rats in the MZC + 2Gy and MZC + 10Gy groups compared to the C group; and in the cerebellum and the hippocampus ($p < 0.05$) and the cortex ($p < 0.0001$) of the rats in the MZC + 2Gy group compared to the 2Gy group; and in the cerebellum and the hippocampus ($p < 0.001$) and the cortex ($p < 0.0001$) of the rats in the MZC + 10Gy group compared to the 10Gy group (Figure 1A).

The increase of tSOD was documented in the cortex of the rats in the 2Gy ($p < 0.0001$), MZC + 2Gy ($p < 0.05$) and MZC + 10Gy ($p < 0.0001$) groups compared to the C group. In the radiated groups (2Gy, 10Gy), lower tSOD activity was documented in the cortex of the rats in the MZC + 2Gy group ($p < 0.05$) and higher in the MZC + 10Gy group ($p < 0.0001$), respectively. Also, tSOD activity was lower in the cortex of the rats in the 10Gy group ($p < 0.0001$) compared to the 2Gy group (Figure 1B).

A reduced MnSOD activity ($p < 0.05$) was observed in all the examined tested brain regions of rats in the 10Gy group and the cortex of the rats in the MZC + 2Gy group ($p < 0.05$) compared to the C group. Compared to the 2Gy group, a decrease of MnSOD activity was observed in the cortex of the rats in the MZC + 2Gy group ($p < 0.0001$) and 10Gy [in the cerebellum ($p < 0.05$) and the cortex ($p < 0.0001$)] groups. In contrast, MnSOD activity was higher in the cortex of the rats ($p < 0.05$) in the 2Gy group compared to the C group and in the MZC + 10Gy group [in the hippocampus ($p < 0.05$) and the cortex ($p < 0.0001$)] compared to the 10Gy group (Figure 1C).

Higher GSH contents were documented in the MZC group [in the cerebellum ($p < 0.05$)]; the MZC + 2Gy and MZC + 10Gy groups [in the cortex ($p < 0.0001$)]; the 2Gy group [in the hippocampus ($p < 0.05$) and the cortex ($p < 0.0001$)] and the 10Gy group in all examined brain structures ($p < 0.0001$) compared to the C group. GSH decrease was observed in the MZC + 10Gy group [in the cerebellum ($p < 0.05$) and the cortex ($p < 0.0001$)] compared to the 10Gy group. Also, the GSH level was profoundly higher in the cortex ($p < 0.0001$) of the rats in the 10Gy group than in the 2Gy group (Figure 1D).

Significantly higher values of 8-OH-dG were obtained in the plasma of the rats radiated with 10 Gy, regardless of

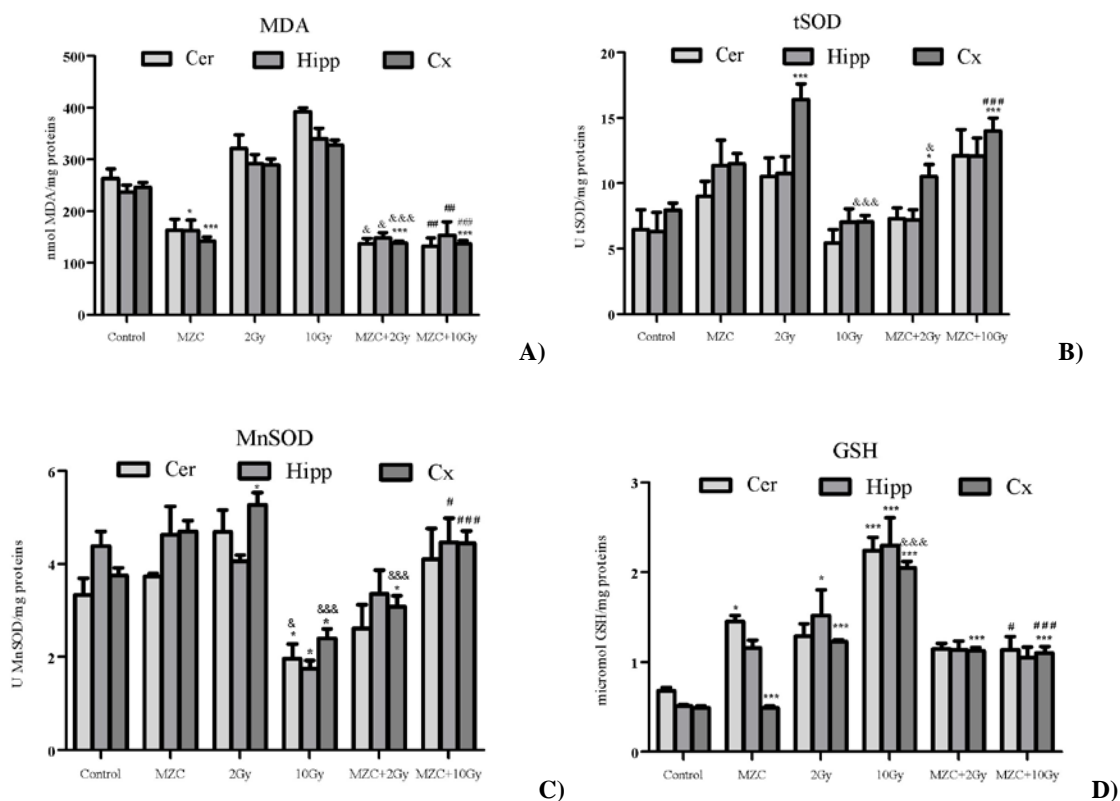


Fig. 1 – Oxidative stress (OS) in selectively vulnerable brain regions of rats on normal and the 5% micronized zeolite (MZC) supplemented diet, lately subjected to single 2 or 10 Gy γ -irradiation. Measured OS parameters in the cerebellum (Cer), hippocampus (Hipp) and cortex (Cx) were: (A) nmol malondialdehyde (MDA)/mg proteins; (B) total superoxide dismutase (tSOD): units of tSOD/mg proteins; (C) manganese superoxide dismutase (MnSOD): units of MnSOD/mg proteins) and (D) nmol glutathione (GSH)/mg proteins. Values are presented as means \pm standard deviation ($n = 6$). Differences were considered statistically significant at: $p < 0.05$ (*, #, &), $p < 0.001$ (, ##, &&) and $p < 0.0001$ (***, ###, &&&). Labeling: * – compared to control, # – compared to 10Gy group and & – compared to 2Gy group). Kruskal-Wallis and *post hoc* Dunn's tests were used for statistical analysis.**

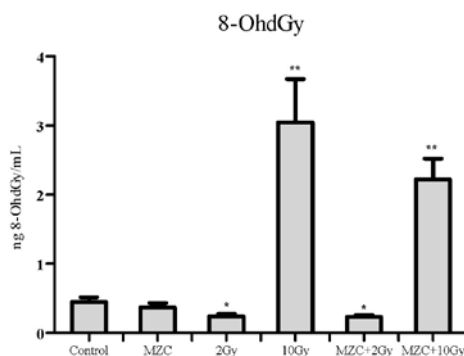


Fig. 2 – The plasma 8-hydroxyguanosine (8-OH-dGy) of rats on the four-week normal and the 5% micronized zeolite (MZC) supplemented diet, lately subjected to single 2 or 10 Gy γ -irradiation. The concentration of 8-OH-dGy was expressed as ng 8-OH-dGy/mL plasma. The values are presented as means \pm standard deviation ($n = 6$). Differences were considered statistically significant at $p < 0.05$ (*) and $p < 0.01$ () compared to control. Kruskal-Wallis and *post hoc* Dunn's tests were used for statistical analysis.**

the diet type (MZC + 10Gy, 10Gy) ($p < 0.001$), while the decrease was documented in the MZC + 2Gy and 2Gy groups ($p < 0.05$) compared to the C group (Figure 2).

Spearman's nonparametric correlation data analysis for the tested brain regions and OS parameters is presented in tabular form (Table 1).

Table 1

Spearman nonparametric correlations of the tested oxidative stress parameters across the hippocampus (Hipp), cerebellum (Cer), and cortex (Cx) of rats on normal and the 5% micronized zeolite (MZC) supplemented diet, lately subjected to single 2 Gy or 10 Gy γ -irradiation

Group	Parameter/Structure	<i>r</i>	<i>p</i>
MZC + 10Gy	MDA/Hipp vs. MDA/Cer	+ 0.943*	0.017
	MDA/Hipp vs. MDA/Cx	+ 0.886*	0.033
	GSH/Cx vs. GSH/Cer	+ 0.886*	0.033
10Gy	tSOD/Hip vs. tSOD/Cer	+1.000**	0.003
2Gy	MDA/Cx vs. MDA/Cer	+ 0.886*	0.033
	MnSOD/Cx vs. MnSOD/Cer	-0.943*	0.017

Wistar rats on four-week normal and diet supplemented with 5% MZC, lately subjected to the single 2 or 10 Gy γ -irradiation. Nonirradiated groups (n = 6) were C and MZC. Radiated groups (n = 6) were 2Gy, 10Gy, MZC + 2Gy, and MZC + 10Gy. Oxidative stress parameters [malondialdehyde (MDA), manganese superoxide dismutase (MnSOD), total superoxide dismutase (tSOD), and glutathione (GSH)] were correlated across the Hipp, Cer, Cx. Spearman's correlation coefficient (*r*) > \pm 0.70 was the criterion for the segregation of the results. Differences were considered statistically significant for *p* < 0.05.

Discussion

We showed that a four-week diet supplemented with 5% MZC *per se* resulted in a significant systemic shift of redox homeostasis towards a reductive potential in the tested brain regions susceptible to OS, based on the decreased MDA (in the hippocampus and cortex) and elevated GSH (in the cerebellum, hippocampus, and cortex). The diet supplemented with MZS did not realize protection against DNA oxidation in plasma. Regarding the radioprotective effect of the applied diet supplemented with zeolite in the rats, partial results were achieved in the tested brain regions, referring to suppressed SOD activity at 2 Gy and reduced brain MDA levels at 2 Gy and 10 Gy. The zeolite supplemented diet achieved no radioprotection of DNA against oxidation (Figures 1 and 2).

Assumingly, a decline of OS in the brain tested regions occurred due to reduced bioavailability of some nutrients by zeolite, not because of boosted antioxidant defense system: a) zeolite binds metals and gases (by adsorption or through ion/exchange reactions) from food and remains within the alimentary tract after oral intake; b) transition metals may induce ROS overproduction *via* Fenton reactions, thus, zeolite significantly reduced the yield of this reaction; c) some essential metals are constituents of many antioxidative metalloenzymes, so, zeolite may decrease the availability of the certain metals to be incorporated in the enzymes and make them functional; d) some gasses (nitrogen monoxide and oxygen) liberated from food within the intestine, may contribute to the formation of ROS or reactive nitrogen species⁴.

An irradiated body surface and the applied dose of γ -ray irradiation are equally important and influential factors for the induced postirradiated effects. Short term outcomes (such as an acute radiation syndrome) depend on the exposure dose, while low doses are found to be associated with possible late somatic and long-term genetic effects, unlike large

doses of radiation with immediate somatic effects on the body²³. The human body can probably absorb up to 200 rads (2 Gy) acutely without a fatality. Also, the human population can be exposed to 1–10 Gy of γ -irradiation during radiation therapy treatment or radiation accidents or nuclear/radiological terrorism^{23, 24}. Upon absorption of ionizing radiation, many chemico-biological changes occur in the living cells, including direct structural disruption or indirectly, through interaction with products of water radiolysis³. Often, 30 postirradiated days are taken to determine lethality in mice or rats. Hence, we selected oxidative damage in the brain and plasma DNA, as the end-points provoked by the irradiation imposed a prerequisite – which was to have the rats alive; therefore, 5 postirradiated days were concluded to be the appropriate period²³.

Reduced lipid peroxidation was documented by lower brain MDA levels in the tested brain regions in irradiated MZC pretreated rats, compared to irradiated rats on a normal diet (Figure 1 A). Our previous results have indicated that MZC treatment decreased the levels of O₂^{•-} and nitrates in the brain²⁵. One-electron reduction reactions between free radicals and unsaturated fatty acids resulted in cell membrane degradation and elevated production of MDA. Though, O₂^{•-} can initiate lipid peroxidation in its protonated form. However, lipid peroxidation can be triggered more easily by HO[•], which is the most potent free radical [easily generated by homolytic cleavage of hydrogen peroxide (H₂O₂) (the product of SOD catalyzed reactions) or generated through Fenton-like reactions that occur between transition metals (in their reduced form) and H₂O₂]⁴. According to Hill and Switzer²⁶, certain brain regions, such as the cortex, striatum, and hippocampus, are highly enriched with nonheme iron, which is catalytically involved in the production of ROS²⁶. Water radiolysis occurs within cells during radiation¹. In such circumstances (radiation disease), the overproduction of H₂O₂ occurs. In Fenton-like reactions, H₂O₂ easily reacts

with low valent transition metals, such as iron- Fe^{+2} , copper- Cu^{+1} , manganese- Mn^{+2} , etc. to form OH^{\cdot} , which spontaneously triggers free radical chain reactions with all kinds of biomolecules in a body. Assumingly, lowered lipid peroxidation in the group of the rats on a 5% MZC diet is related to the lower bioavailability of transition metals.

Cellular antioxidant defense system, including antioxidative metalloenzymes [tSOD (CuZnSOD, MnSOD), catalase, GSH peroxidase, GSH reductase, etc.] is responsible for free radicals scavenging/neutralization^{27,28}. The first-line antioxidant enzyme, SOD [catalyzes $\text{O}_2^{\cdot-}$ dismutation into H_2O_2 and molecular oxygen] involves cytosolic and extracellular (CuZn-SOD) and mitochondrial (MnSOD) isoforms²⁹. The fraction of MnSOD in tSOD is extremely small, thus observed changes in tSOD are mainly due to CuZn-SOD.

The 5% MZC 4-week diet, *per se*, did not affect the activity of MnSOD and tSOD (i.e., CuZn-SOD). Herein, we showed that the activity of SOD isoforms was not affected by zeolite subacute intake, but lipid peroxidation in rats.

We showed that the activities of t-SOD were significantly elevated only in the cortex (not in the cerebellum and the hippocampus) of the rats on normal diet at both doses of γ -ray irradiation, while the activity of MnSOD was significantly elevated only in the cortex at the dose of 2 Gy, and lowered in all three tested brain regions at 10 Gy, compared to the controls. Our results are similar to those of the study of Lee et al.³⁰, who observed no changes for SOD and catalase, but GSH in mouse spleen induced by doses of γ -ray irradiation from 0.02 to 0.2 Gy. The disparities in the content of SOD-isoenzymes across the tested brain regions may explain their unequal responses associated with oxidative stress against applied treatments in the rats. Also, brain regions are not equally susceptible to a variety of neuronal injuries associated with oxidative stress, for the same reason (Table 1).

Different distribution of the SOD isoforms within the brain and spinal cord tissues and cells is confirmed by confocal laser scanning microscopy and digital photoimaging according to the study reported by Lindenau et al.³¹. Cu/Zn-SOD is predominantly localized in astrocytes of the CNS and the motor neurons of the spinal cord (much more than in brain neurons). In lower amounts, Cu/Zn-SOD is present in the nucleus sparing the nucleolus, neuronal perikarya, and in the structures of the neutrophil.

Mitochondrial MnSOD is more abundant in the brain and the spinal cord neurons than in astroglial cells. The higher susceptibility of the cortex to OS in the rats observed in our study (the activity of SOD isoforms) is in accordance with the study of Melov et al.³², who demonstrated that transgenic MnSOD knockout mice easily develop neuronal phenotype and a spongiform degeneration of the cortex and specific brain stem nuclei. Additionally, the cerebral cortex and striatum are more prone to oxidative damage due to a higher oxygen consumption rate in those regions³³. The phenomenon of selective neuronal vulnerability is related to the difference in susceptibility of neuronal populations in the CNS to different kinds of stressors (including oxidative and nitrosative stress) that induce neurodegeneration. This phenomenon is not limited to cross-regional differences in the

brain, as within a single brain region – such as the hippocampus or the entorhinal cortex – it also manifests in internal, subregional differences in relative susceptibility to OS (as it was confirmed by the correlation analysis, Table 1). While most brain neurons can tolerate OS, some of them (small pyramidal neurons and the third layer of the cerebral cortex and striatum, the hippocampal CA1 region and cerebellar granule cell layer) are particularly vulnerable to OS^{11,34,35}. The susceptibility of the cerebellar neurons to OS and NS might play an important role in the significant loss of these neurons in the aging process³⁶.

Activities of both SOD isoforms were significantly higher in rats on the normal diet after radiation by 2 Gy than in those on zeolite supplemented diet and the opposite for 10 Gy. The type of initiated antioxidant defense mechanistic pathway depends on the dose of γ -irradiation, which was confirmed by our results for the 2Gy and 10Gy groups³⁷. Additionally, $\text{O}_2^{\cdot-}$ can act both as an initiator and a terminator of free radicals chain reaction mediated³.

For 2 Gy radiation, only in the cortex, the activity of tSOD and MnSOD was higher in the rats on the normal diet than in the control ($p < 0.001$ and $p < 0.05$, respectively) and those on the zeolite supplemented diet ($p < 0.05$ and $p < 0.001$, respectively) (Figures 1B and 1C). These results follow the Pathak et al.³⁸ study, showing that lower doses of γ -radiation induce the activity of SOD in various organs in rats. According to our results, the dose of 2 Gy of radiation increases the activity of tSOD (in the cortex statistically significant), which implies the increased production of H_2O_2 as well, and which may consequently explain higher MDA levels (Figure 1B).

Contrary to that, for 10 Gy radiation, the activity of MnSOD were lower in the rats on normal diet compared the control (in all tested brain regions, $p < 0.05$) and those on the zeolite supplemented diet (in hippocampus, $p < 0.05$ and in cortex, $p < 0.001$), while the activity of tSOD did not differ from the controls and was lower from the rats on the zeolite supplemented diet only in the cortex ($p < 0.001$) (Figures 1B and 1C).

The *in vitro* study of Wu and Navrotsky³⁹ showed that zeolite binds metals in the following descending order $\text{Mn} > \text{Zn} > \text{Mg} > \text{F} > \text{Cu}$. Also, the *in vitro* study of Jacobs and Waite⁴⁰ confirmed the strong zeolite affinity for Mn. Our results indicate that zeolite indirectly affects the activity of SOD by binding essential metals (Mn, Cu, and Zn) that are cofactors and constituents of metalloenzymes.

Brain GSH is a confirmed neuromodulator, neurotransmitter, and neurohormone. The sulfhydryl group of cysteine of the tripeptide GSH is responsible for its antioxidant (donor of reducing equivalents) and metal-binding abilities. We showed significantly elevated brain GSH levels after exposure to γ -ray irradiation at both doses (Figure 1D), which is in agreement with Ballatori et al.⁴¹ study. These results adhere to the literature, showing that lower doses of radiation cause an increase of GSH levels in many organs, including of GSH synthesis-related proteins via the *de novo* synthesis⁴². According to Kawakita et al.⁴³, it is known that changed cellular redox signalisation leads to

phosphorylation of various serine/threonine mitogen-activated protein (MAP) kinases that further activate different redox-sensitive transcription factors like nuclear factor- κ B (NF- κ B) and activating protein-1 (AP-1), resulting in the gene expression of various antioxidant defense proteins (enzymatic and no-enzymatic) to overcome the effect of OS-mediated cellular damage.

Yamaoka et al.⁴⁴ reported that persistent radiation increases SOD activity in the rat liver and spleen up to 8–12 weeks after exposure. Additionally, the increased induction of GSH by low-dose γ -rays appears to activate immune function, according to Kojima et al.⁴² Seven decades ago, Avti et al.⁴⁵ and Patt et al.⁴⁶ reported that cysteine (the key amino acid in tripeptide GSH) administered to rats before 800 R of X-rays, significantly increased survival. However, Teshima et al.⁴⁷ found that the increase of intracellular GSH induced by low-dose gamma-radiation occurs because of higher expression of mRNA for γ -glutamylcysteine synthetase (γ -GCS), a rate-limiting enzyme of the *de novo* GSH synthesis pathway, rather than because of glutathione reductase. The study of Lee et al.³⁰ confirmed that the elevation of GSH at low-dose γ -ray irradiation (0.02 and 0.2 Gy) is accompanied with the elevated expression of glutamate-cysteine ligase modifier (not catalytic) subunit, emphasizing that no changes in the expression of thioredoxin occurred in *de novo* GSH synthesis.

We showed that 10 Gy dose of radiation causes oxidative DNA damage in plasma no matter what diet the rats were on (Figure 2). Prooxidants such as HO \cdot , excited oxygen, photosensitizers, or ONOO $^-$, produce 8-OH-dG in the reaction with DNA. The study of Floyd and Carney⁴⁸ underlined that iron and reactive oxygen-free radical intermediates are involved in the oxidative damage of proteins and DNA. Very high plasma 8-OH-dG concentrations obtained in the rats subjected to 10 Gy (regardless of the diet) are in accordance with Cuttler and Pollycove⁴⁹ study, affirming that damaging or lethal cellular effects are observed following high radiation doses, while cellular stimulatory effects happened following low-dose-short-term exposures in the range 0.01–0.50 Gy.

Based on our results, the applied 5% MZC diet in rats appeared to have no radioprotective effect against oxidative damage of DNA in plasma (Figure 2).

The correlation analysis confirmed differences across the hippocampus, cerebellum, and cortex responses to ap-

plied treatments in rats (Table 1). Correlation of the data related to lipid peroxidation (MDA) and GSH changes, within the MZC + 10 Gy group, showed that both the hippocampus/cerebellum and hippocampus/cortex, and cortex/cerebellum similarly responded, respectively. Also, changes of tSOD activity upon radiation of 10 Gy were similar in the hippocampus/cerebellum, while the 2 Gy radiation caused similar changes in MDA in the cortex/cerebellum, but the MnSOD activities in the cortex/cerebellum. Anatomical and physiological characteristics of the tested brain regions (such as localization of antioxidant enzymes, an abundance of transition metals, richness with polyunsaturated free fatty acids, etc.) dictate a profile of OS response to zeolite diet and against gamma radiation, in rats^{31, 33–35}.

Conclusion

Gamma-ray irradiation of 2 and 10 Gy changes brain redox homeostasis and causes oxidative alternations of plasma DNA at higher doses, in rats. Subacute MZC pretreatment accomplished partial radioprotective effect in rats based on reduced brain MDA and activity of SOD, compared to the rats on a normal diet. The cortex appears to be the most susceptible to OS induced by γ -ray irradiation.

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Conflict of interest

The authors declare no conflict of interest.

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