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Research Article

Cytoprotective effect and antioxidant activity of *Sambucus nigra* L. after gamma radiation exposure

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Abstract

Radiation exposure triggers harmful effects such as free radical surges, oxidative stress, and genetic and cellular toxicity, ultimately leading to cell death. In this study, a freeze-dried fruit extract of *Sambucus nigra* L. (*S. nigra*), rich in flavonoids, anthocyanins was tested at a concentration of 4 μL.mL^{-1} for its potential protective effects against radiation-induced genetic and cellular damage in human lymphocyte cells. Key indicators such as chromosomal aberrations (CA), levels of reactive oxygen and nitrogen species (ROS/RNS), lipid peroxidation (malondialdehyde - MDA), and antioxidant enzymes- including glutathione (GSH), glutathione peroxidase (GPx-1), superoxide dismutase (SOD), and catalase (CAT) - were analyzed in cells exposed to 2 Gy gamma radiation, with or without *S. nigra* pretreatment. These measurements were conducted using ELISA assays and electron paramagnetic resonance (EPR) spectroscopy. The results showed that pretreatment with *S. nigra* at 4 μL.mL^{-1} provided notable protection against 2 Gy radiation, reducing chromosomal aberrations by approximately 27%. Compared to irradiated cells without treatment, those pretreated with *S. nigra* exhibited significantly lower MDA levels and reduced ROS/RNS production ($p<0.05$). Additionally, the pretreatment helped maintain significantly higher levels of the antioxidant enzymes SOD, CAT, GPx-1, and GSH ($p<0.05$). In conclusion, *S. nigra* extract pretreated lymphocyte cells helped activate anti-cytotoxic and radioprotective mechanisms, effectively reducing oxidative stress caused by radiation exposure.

Keywords

Sambucus nigra L., γ-radiation, chromosome aberrations, oxidative stress, antioxidant enzymes, lipid peroxidation

Abbreviations

S. nigra – *Sambucus nigra* L. lyophilized fruit extract; OS – oxidative stress; CA – chromosomal aberrations; ROS – reactive oxygen species; RNS – reactive nitrogen species; NO – Nitric Oxide; GPx– glutathioneperoxidase–1; GSH – antioxidant enzymes glutathione - reduced glutathione; SOD – superoxide dismutase; CAT– catalase; MDA Malondialdehyde ; EPR– Electron Paramagnetic Resonance spectroscopy; PBN – N-tert-butyl-alpha-phenylnitrone; carboxy-PTIO – potassium salt – 2-(4 Carboxyphenyl) 4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazol-1-yloxy-3-oxide; DMSO – dimethyl sulfoxide; NO – Nitric Oxide; LPO – lipid peroxidation; DNA – Deoxyribonucleic acid; LOOH – lipid hydroperoxides.

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Introduction

Chemotherapy and radiotherapy remain the primary treatment strategies for cancer, while immunotherapy - using either natural or synthetic agents - is typically employed as a secondary option. Ionizing radiation (IR), including X-rays and alpha (α), beta (β), and gamma (γ) rays, disrupts immune system homeostasis, elevates free radical levels, and induces oxidative stress (OS). These effects impair cellular repair mechanisms, prolong cell cycle arrest, and trigger apoptosis (Kesarwani et al. 2013; Vallier 2015; Malhotra et al. 2018).

Among these, γ-radiation is particularly damaging as it leads to mutations, chromosomal abnormalities, and cellular injury (Shaban et al. 2017). Additionally, it promotes the formation of reactive oxygen and nitrogen species (ROS/RNS), including hydroxyl radicals (•OH), superoxide anions ($•O_2^-$), and hydrogen peroxide (H_2O_2) (Kelachayeh et al. 2020; Saini and Gurung 2025). These reactive species can damage cellular membranes, deactivate protective serum proteins, and cause DNA strand breaks or base modifications, ultimately contributing to mutagenesis (Kelachayeh et al. 2020).

Effective radioprotection requires mechanisms that repair single- and double-stranded DNA breaks and restore the function of key enzymes such as DNA ligase, thereby limiting DNA damage (Saini and Gurung 2025; Sun et al. 2025). Structural alterations in DNA and proteins caused by γ-radiation also disrupt the cellular redox balance by increasing ROS/RNS accumulation. IR reduces the activity of major antioxidant enzymes - including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), which heightens OS. This is reflected in elevated levels of malondialdehyde (MDA), nitroxyl radicals (NO•), and intracellular calcium (Stasiłowicz-Krzemień et al. 2024).

Prolonged radiation exposure exacerbates OS, leading to mitochondrial DNA mutations that disrupt the epigenetic regulation of nuclear DNA. This initiates a feedback loop of increased ROS/RNS production and OS damage (Stasiłowicz-Krzemień et al. 2024; Szumiel 2015). Breaking this cycle is essential.

As such, the development and evaluation of natural or synthetic radioprotective agents- capable of preserving DNA integrity, supporting methyltransferase function, preventing DNA hypomethylation, neutralizing ROS/RNS, and protecting membranes and micronuclei- are critical (Saini and Gurung 2025; Sun et al. 2025).

Radioprotective compounds often come from plant secondary metabolites, which help minimize γ-radiation-induced damage in healthy cells while enhancing the body's resilience (Zhang et al. 2015; Stasiłowicz-Krzemień et al. 2024). These compounds facilitate stress adaptation and activate additional defence mechanisms. Notable protective metabolites include terpenes (mono-, di-, and sesquiterpenes), phenolic compounds (e.g., coumarins), flavonoids (e.g., quercetin, kaempferol), tannins (e.g., catechins), stilbenes (e.g., resveratrol), as well as nitrogen-containing compounds (e.g., alkaloids, cyanogenic glycosides, and non-protein amino acids) (Zhang et al. 2015; Reshi et al. 2023; Stasiłowicz-Krzemień et al. 2024).

Sambucus nigra L. (*S. nigra*; black elderberry) is a natural source, rich in polyphenols, flavonols, phenolic acids, anthocyanins, and proanthocyanidins, which are known for their potent antioxidant activity (Liu et al. 2022). These constituents help reduce OS injuries and have shown anticarcinogenic, anti-inflammatory, and DNA-repair properties. Furthermore, they support hematopoietic recovery and normalize the immune system following radiation exposure (Seymenska et al. 2023; Ferreira et al. 2022).

Extracts from *S. nigra* flowers have demonstrated protective effects against UV-B-induced biotic stress (Stanković et al. 2017; Petkova-Parlapanska et al. 2023), although there is some debate regarding the cytotoxic and genotoxic potential of certain *S. nigra* components (Ferreira-Santos et al. 2022). The current study investigates the radioprotective potential of *S. nigra* fruit extract at a concentration of 4 $\mu L.mL^{-1}$ in γ-irradiated human lymphocytes. The focus is on evaluating its ability to reduce ROS/RNS levels, mitigate genotoxic effects, and support immune regulation, underscoring its potential as a natural radioprotective agent.

Materials and Methods

Extract preparation. Fresh *S. nigra* (black elderberry) fruits were hand-pressed and strained using sterile gauze, then processed using ultrasonic treatment (80 kHz) in a bath equipped with a piezoceramic emitter (Elmasonic P 30 H, Elma, Singen; 2.8 L capacity; operating frequency range: 37 - 80 kHz; temperature: 22°C) to obtain a uniform juice. The resulting extract was filtered sequentially through cotton and a 0.8 μm nylon membrane syringe filter (Acrodisc, Sigma-Aldrich). It was observed that anthocyanin content diminished with prolonged storage or increased pH. The juice was then lyophilized- initially shaken at 300 rpm for 10 min at a 1 cm layer thickness and - 45°C followed by vacuum freeze-drying in a TG 16.50 unit (Hochvacuum, Germany) for 24 ± 1 h. The qualitative and quantitative profiles of phenolic bioactive compounds in the resulting lyophilized extracts were previously characterized using high-performance liquid chromatography with diode-array detection (HPLC–DAD; G1315B). These findings are detailed in: Petkova-Parlapanska et al. (2025).

As protocol, rutin (782.6 ± 0.68 μg.g^{-1}), epigallocatechin (314.15 ± 0.59 μg.g^{-1}), myricetin (136.06 ± 0.84 μg.g^{-1}), quercetin (37.9 ± 1.27 μg. g^{-1}) were the main flavonoids in *S. nigra* extract, with recorded 89.25% DPPH-R scavenging, *in vitro*.

Human lymphocytes in vitro and irradiation exposure. For the preparation of lymphocyte cultures (10 mL), peripheral venous blood from three healthy donors (nonsmoking/nondrinking women) aged ~ 45.8 years, was used. The procedures followed the Declaration of Helsinki, approved by the Ethics and Academic Integrity Committee of Trakia University (№ 7.5.1 OD_4.1.5.9/protocol 18/15.04.2022). Each lymphocyte culture contained: RPMI-1640 medium supplemented with 20 mM HEPES, L-glutamine, 2 mL fetal bovine serum, 0.2 mL phytohemagglutinin P (2%), 1% antibiotic antimycotic solution (10,000 U penicillin, 10 mg streptomycin, 25 μg amphotericin B·mL^{-1}), peripheral venous blood (1 mL), with/without *S. nigra* extract (4 μL.mL^{-1}) (Evans 1984). The cells were irradiated with γ-radiation at a dose rate of 2 Gy/ 1.37 Gy . min^{-1} with Terabalt 80, in air, at 25 ± 2°C. After 47 h, 0.5 mL of the cell culture was analyzed for radioprotective effect. Two cultures are made from each donor: both lymphocyte culture was 2 Gy irradiated; then first lymphocyte culture was treated with *S. nigra* extract; the second lymphocyte culture – without *S. nigra* extract treatment. Untreated lymphocytes were used as a control.

Chromosome metaphases and aberrations. The remaining lymphocyte cultures were treated with: 100 μL colcemid solution·90 min^{-1}; 0.075M KCl; 4× methanol/acetic acid (3:1, v/v) at 23°C; and the clastogenic effect was examined (Evans 1984). Microscopy of chromosomal metaphases (10 % Giemsa (Merck)) and analysis of chromosomal aberrations were performed at ×1000 magnification (Olympus BX-41, Germany), with a minimum of 100 metaphases. Untreated lymphocytes were used as controls.

ELISA assay. Cells from different treatment groups were subjected to enzyme activity and lipid peroxidation experiments: catalase (CAT; No. ab83464), superoxide dismutase (SOD; No. ab65354), glutathione (GSH; No. ab142044) glutathione peroxidase 1 (GPx1; No. ab41464), and malondialdehyde (MDA; No. ab233471).

Electron paramagnetic resonance (EPR) study. The ROS production: 100 μL of cell cultures was mixed with 900 μL (50 mM) N-tert-butyl-alpha-phenylnitrone (PBN) dissolved in DMSO. The mixture was centrifuged at 4000×g, 10 min at 4°C, by Shi et al. (2005).

The nitric oxide (•NO) generation: 50 μM spintrap carboxy 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl (CPTIO.K) was dissolved in a mixture of 50 mM Tris (pH = 7.5), and DMSO (9:1) was centrifuged at 4000×g for 10 min at 4°C. Then, 100 μL cell cultures were mixed in 100 μL CPTIO.K, and spin–adducts/•NO were recorded (Yoshioka et al. 1996; Yokoyama et al. 2004).

All EPR analyses were performed with fivefold measurement in recorded EPR spectra, with the following characteristics: 3503–3515 G center field; 6.42–20.00 mW microwave power; 5 - 10 G modulation per sample, 2.5 × 10^2 gain; the results are presented in arbitrary units (a.u.).

Statistical analysis. EPR (EPR-X-Bruker-EMX^{micro}, BioSpin-GmbH, Ettlingen, Germany/ standard resonator) spectral processing was performed using WIN-EPR-2019 and Simfonia software, version 1.2. Statistical analysis was performed with

Statistica 8, StaSoft, Inc. (Madrid, Spain), and the results were expressed as means ± SE. One-way ANOVA and LSD post hoc test ($p < 0.05$) were considered statistically significant. LSD post hoc tests were used to define which groups were different from each other. For categorical data, cells with/without chromosomal aberrations, a $\chi 2$ test was used.

Results and Discussion

The chromosomal aberrations frequency in lymphocyte cultures treated with *S. nigra* extract can provide valuable information about its potential genotoxic effects (Fig.1). By conducting this experiment, we demonstrated the safety and biological effects of *S. nigra* extract, especially with regard to its therapeutic applications.

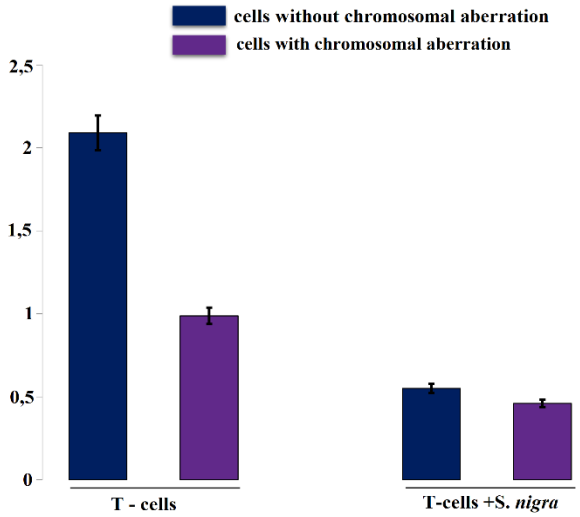


Figure 1. The chromosomal aberrations frequency in lymphocyte cultures treated with *S. nigra* extract

The aberrance and aberrant cell degrees were processed by one-way ANOVA followed by $\chi 2$-Cramer's V test (GraphPadPrism 6/Windows; GraphPad Software, Inc., San Diego, CA, USA). Data are presented as mean ± SD; $p < 0.05$ was considered significant.

The CA frequency demonstrates the cytoprotective effect of *S. nigra* extract in cell cultures exposed to 2 Gy irradiation (Fig. 2A). The $\chi 2$-analysis indicated a weak but significant relationship ($p = 0.018$; $p < 0.05$) between treatment with *S. nigra* extract and the occurrence of aberrant cells, including one or more dicentric chromosomes, ring chromosomes, and fragments (Cramer's V = 0.097, $p < 0.2$). In the 2 Gy irradiated cells, the percentage of aberrant cells after treatment with *S. nigra* extract was approximately 26.7% lower, compared to 32.3% observed after exposure to 2 Gy irradiation alone.

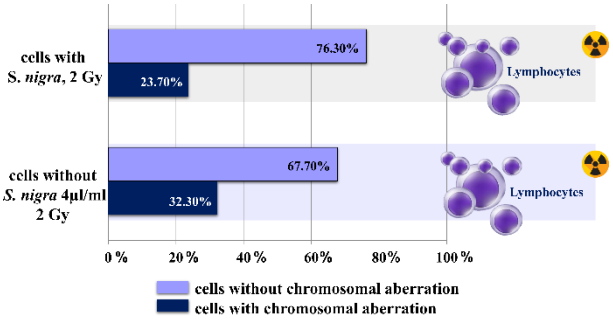


Figure 2A. The aberrance and aberrant cell degrees in 2Gy- irradiated and *S. nigra* + 2Gy- irradiated groups

They were processed by $\chi 2$-Cramer's V test (Stat. 8, Madrid, Spain). Data are presented as mean ± SE; $p < 0.05$ was considered significant (Fig. 2B).

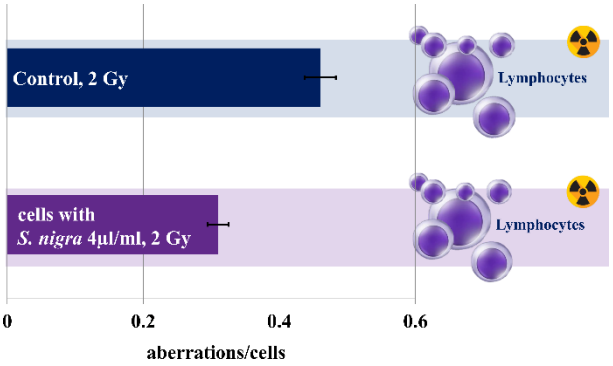


Figure 2B. Differences in chromosome aberrations (mean values) in 2Gy irradiated cells versus *S. nigra* + 2Gy- irradiated

One-way ANOVA analysis reported differences presented as means ± SE. *The difference in the mean aberration values is significant ($p = 0.005$, < 0.05). Treatment with 2 Gy irradiation showed a significant increase in ROS products and cumulatively increased oxidative damage ($p < 0.05$) in irradiated cells versus non-irradiated cells (Fig. 3A). However, pretreatment of irradiated cells with *S. nigra* significantly reduced ROS levels compared to 2 Gy irradiation alone ($p < 0.05$). Therefore, pretreatment with *S. nigra* effectively reduced •OH and superoxide ($\bullet O_2^-$) radical in the cell wall of the plant cell.

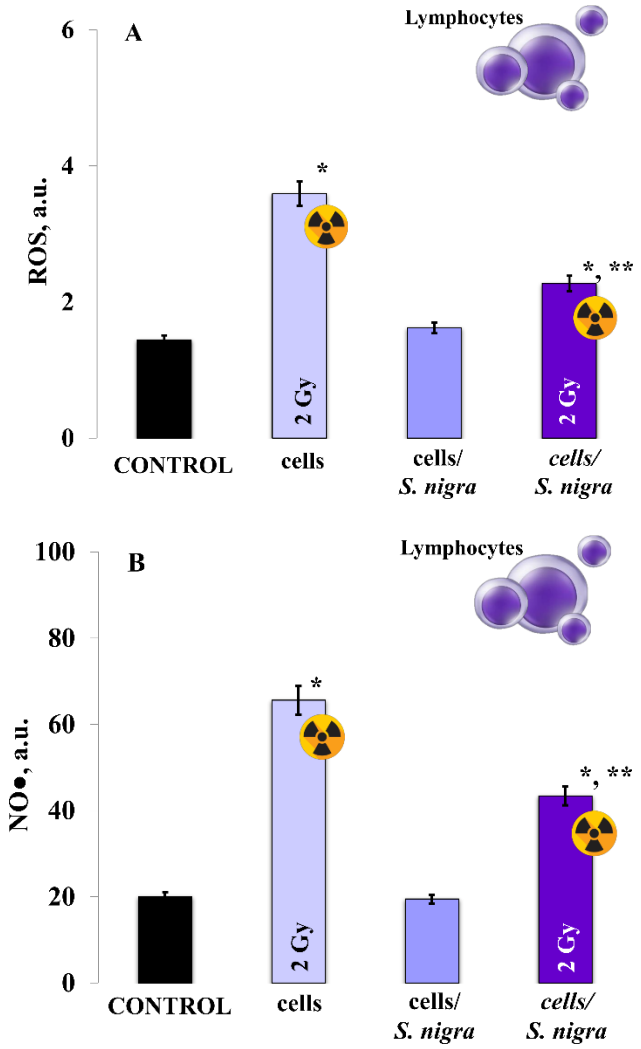
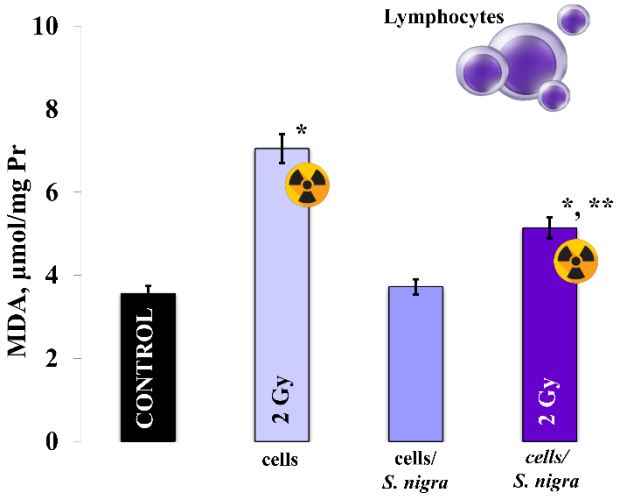


Figure 3. ROS production (3A) and NO scavenging activity (3B) in control, 2 Gy-irradiated cells, *S. nigra* cells and pre-treated with *S. nigra* + 2 Gy-irradiated cells

LSD post hoc test, * $p < 0.05$ vs. control; ** $p < 0.005$ vs. irradiated cells.

Cellular induction of 2 Gy radiation statistically significantly increased the •NO radicals levels, compared to the control group ($p < 0.05$), and to the group treated with *S. nigra* alone ($p < 0.05$). Treatment with *S. nigra* resulted in a significant reduction in •NO production ($p < 0.005$) (Fig. 3B). To confirm the radioprotective role of *S. nigra* extract against 2 Gy-induced oxidative stress, the lipid peroxidation degree was assessed. MDA levels (Fig. 4) in the 2 Gy irradiated cells were twofold higher compared to the untreated cells ($p < 0.05$), and to those pretreated with *S. nigra* ($p < 0.04$). In contrast, un-irradiated cells treated with *S. nigra* only, exhibited MDA levels comparable to the controls (3.56 ± 0.2 vs 3.72 ± 0.11 μmoL.mgPr^{-1}). Notably, the *S. nigra* extract co-administration with 2 Gy irradiation alleviated lipid peroxidation and likely promoted enzymatic defence mechanisms by restoring the redox-homeostatic imbalance, when



compared to the group treated with 2 Gy irradiation.

Figure 4. Lipid peroxidation changes (MDA, μmol. mL^{-1}) in control, irradiated cells, *S. nigra* and pre-treated with S. nigra irradiated cells

LSD post hoc test, * $p < 0.05$ vs. control; ** $p < 0.05$ vs. irradiated cells.

The values of SOD, CAT, GPx-1, GSH are significantly diminished, after 2 Gy-induced radiation (Fig. 5). A 2.4- fold decreased activity of SOD ($p < 0.05$), CAT ($p < 0.05$), GPx-1 ($p < 0.005$) and a reduced content of reduced GSH ($p < 0.05$) were reported in 2 Gy-irradiated cells. A statistically significant preservation of the levels of antioxidant enzymes SOD, CAT, GPx, GSH was observed in cells treated with *S. nigra* alone and after pretreatment with *S. nigra* + 2 Gy-irradiated ($p < 0.05$) cells. LSD post hoc test, * $p<0.05$ vs. control; ** $p <0.05$ vs. irradiated cells.

S. nigra (elderberry) extract at 4 μL.mL^{-1} (Fig. 1) was found to be non-toxic to lymphocyte cultures and did not exhibit clastogenic effects. When administered one hour before exposure to 2 Gy γ-radiation, the extract reduced chromosomal aberrations by 26.6% (Cramer's $V = 0.097$, $p < 0.2$) (Fig. 2).

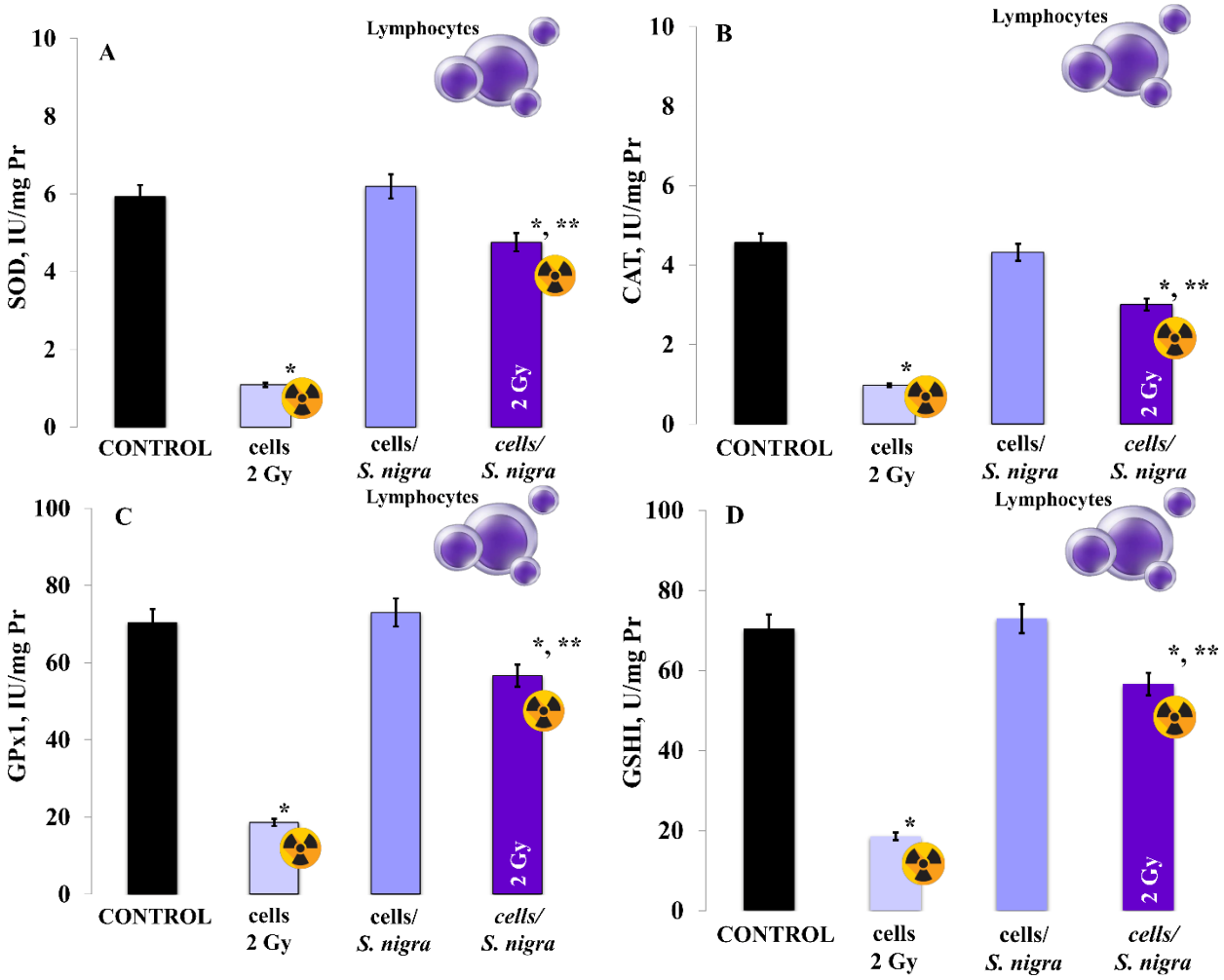


Figure 5. The activity of antioxidant enzymes like (A) superoxide dismutase (SOD, IU.gPr^{-1}), (B) catalase (CAT, IU.gPr^{-1}), (C) glutathioneperoxidase-1 (GPx^{-1}, IU. gPr^{-1}), (D) reduced glutathione (GSH; µmol.gPr^{-1})

This protective effect likely arises from multiple mechanisms: modulation of genotoxic mutation rates; stabilization of cell membranes; enhanced cellular reproductive capacity; suppression of carcinogenic processes; and inhibition of ROS/ RNS generation (Malhotra et al. 2018; Stasiłowicz-Krzemień et al. 2024).

In our current study, 2 Gy irradiation provoked a statistically significant rise in ROS production ($p < 0.05$). However, lymphocytes pretreated with 4 µL.mL^{-1} *S. nigra* extract exhibited a marked reduction in ROS generation (Fig. 3A). We propose that this protective effect is due to the extract's antioxidant activity, metal-chelation capabilities, and inhibition of hydroxyl radical (•OH) initiation.

The beneficial effects of elderberry are thought to stem from its rich content of polyphenols and flavonoids- such as rutin, quercetin, and epigallocatechin- which possess ROS-scavenging, metal-chelating, and DNA-repair properties. These compounds have been shown to limit H_2O_2-induced mitochondrial Fe^{2+}/Fe^{3+} mobilization and modulate both innate and adaptive immune responses, facilitating DNA lesion repair (Stasiłowicz-Krzemień et al. 2024; Petkova-Parlapanska et al. 2025).

NO/•NO radicals activation are potent radiosensitizers that directly react with damaged macromolecules regions, thus modulating the oxidative damage that occurred (Yang et al. 2022). Extremely increased NO/•NO bioavailability caused the opposite effect, and the NO/•NO release from the irradiated cells is a side IR effect. Increased •NO production after 2Gy-activated cells additionally reacts with $•O_2$- radicals, and causes the harmful peroxynitrite (ONOO-) radicals' formation, responsible for the modified macromolecular damages (Pérez de la Lastra et al. 2022). In line with these studies, our study confirmed that 2 Gy-irradiation cellularly modifies the immune response and enhances •NO formation ($p < 0.05$). Cellular induction of 2 Gy radiation significantly increased the •NO radicals levels, compared to the control group ($p < 0.05$), and the group treated with *S. nigra* alone ($p < 0.05$). Treatment with *S. nigra* resulted in a marked reduction of •NO radical production ($p < 0.005$) (Fig. 3B). To further confirm the radioprotective effects of *S. nigra* extract against 2 Gy-induced oxidative stress, the lipid peroxidation degree was examined. MDA levels (Fig. 4) in the 2 Gy irradiated cells were found to be twofold higher compared to untreated cells ($p < 0.05$) and also higher than those pretreated with *S. nigra* ($p < 0.04$). Conversely, unirradiated cells treated with *S. nigra* demonstrated MDA levels comparable to the controls (3.56 ± 0.2 vs 3.72 ± 0.11 μmol/mgPr). Notably, the co-administration of *S. nigra* extract with 2 Gy irradiation alleviated lipid peroxidation, likely promoting both exogenous and endogenous enzymatic defences by restoring redox-homeostatic balance, compared to the group treated with 2 Gy alone.

Ionizing radiation (IR) significantly increases lipid peroxidation, as measured by malondialdehyde (MDA) levels, while simultaneously depleting antioxidant defences such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx-1), and glutathione (GSH). In our experiments, exposure to 2 Gy irradiation triggered a marked increase in MDA levels ($p < 0.05$) (Fig. 4), indicative of oxidative membrane damage, accompanied by decreased activity of SOD, CAT, GPx-1, and reduced GSH. This reflects an acute inflammatory response and excessive ROS/RNS production in the affected cells. However, pre-treatment of the cells with *S. nigra* extract at 4 $\mu L.mL^{-1}$ nearly halved the MDA levels and restored the SOD, CAT, and GPx1 activities (Fig. 5).

Furthermore, the extract replenished intracellular GSH (Fig. 5D), facilitating the detoxification of hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and lipid hydroperoxides (L-OOH). This aligns with existing evidence that natural radioprotective agents upregulate antioxidant proteins, limit oxidative damage (Przybylska Balcerek et al. 2021; Dominguez et al. 2020; Gonçalves et al. 2024). Results from a similar study showed that the tested mixture of elderberry, chokeberry, and blackcurrant exerted significant radioprotective effects by reducing oxidative damage, modulating inflammation and immune responses, and preventing cell death *in vivo* (Niska et al. 2025; Abrashev et al. 2025).

Key takeaways

- *S. nigra* extract (4 $\mu L.mL^{-1}$) is non-toxic, non-clastogenic, and reduces radiation-induced chromosomal aberrations by ~26.6 %.
- It significantly lowers ROS and •NO levels in irradiated lymphocytes, likely through antioxidant and metal-chelation mechanisms.
- The extract's ability to neutralize •NO/ $ONOO^-$ may help maintain mitochondrial integrity and reduce apoptosis.
- 2Gy radiation elevates MDA and suppresses antioxidant and enzymatic activity of SOD, CAT, GPx-1, GSH.
- *S. nigra* extract pretreatment (containing rutin, quercetin, etc.) inhibits 2Gy-induced lipid peroxidation, preserves antioxidant enzymes, and maintains GSH levels.

These findings reinforce the potential of *S. nigra* extract and elderberry-based compounds as natural radioprotectors, with multifaceted mechanisms that range from genetic protection to OS mitigation.

Conclusions

The current study indicates that *S. nigra* extract pretreated human lymphocytes stabilizes the immune response following γ radiation exposure. Rather than accelerating chromosomal aberrations, *S. nigra* mitigates lipid peroxidation and cytotoxicity by activating the cells' intrinsic

antioxidant enzymes. These findings support the potential of *S. nigra* as a potent radioprotective agent, safeguarding macromolecules integrity and countering oxidative damage induced by gamma radiation.

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Author Contributions

Conceptualization, K.P.-P., Y.K. and V.P.-T.; methodology, G.N., Y. K. and V.P.-T.; formal analysis K.P.-P, Y.K. and V.P.-T.; investigation, K.P.-P, Y.K. and V.P.-T.; resources, G.N. and P.V.; data curation, G.N., E.G. and P.V.; writing - original draft preparation, K.P.-P. and N.K.; writing - K.P.-P., Y.K. and G.N., review and editing, E.G., and V.P.-T., visualization, E.G.; supervision, E.G. and P.V.; project administration, G.N. and Y.K.; funding acquisition, G.N. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

For the preparation of lymphocyte cultures (10 mL), peripheral venous blood from three healthy donors (nonsmoking/ nondrinking women) aged ~ 45.8 years, was used. The procedures followed the Declaration of Helsinki, approved by the Ethics and Academic Integrity Committee of Trakia University (№ 7.5.1 OD_4.1.5.9/protocol 18/15.04.2022)

Informed Consent Statement

Applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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