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Research Article

***Lemna minor* L. extract attenuates gamma-radiation-induced chromosomal aberrations and oxidative stress in human peripheral blood cultures**

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Abstract

Radiation exposure - whether through medical treatments, nuclear incidents, or space exploration—poses a serious threat to human health due to the significant cellular damage it can cause. *Lemna minor* L. (*LM*), plant extract rich in biologically active compounds such as polyphenols, phenolic acids, and carotenoids, has shown promise in reducing the harmful cellular effects of gamma (γ) radiation. This study aimed to evaluate the radioprotective potential of *LM* extract, focusing on its antioxidant and anticlastogenic effects in human lymphocytes cultures exposed to γ -radiation (2 Gy). Alongside the standard chromosome aberration assay, the study assessed additional markers of oxidative damage, including protein oxidation (via 5-MSL spin-labeling), lipid peroxidation (as malondialdehyde, MDA), and reactive oxygen/nitrogen (ROS/RNS) species. *LM* extract treatment at a concentration of 50 $\mu\text{g}\cdot\text{mL}^{-1}$ significantly alleviated γ -induced cellular damage. The extract exhibited significant anticlastogenic effect, markedly reducing the frequencies of total chromosomal aberrations, dicentric chromosomes, and cells with chromosomal aberrations ($p < 0.05$). *LM* treatment significantly lowered oxidative stress markers, by reducing ROS/RNS production, MDA and protein oxidation ($p < 0.05$) and restore antioxidant activities. These findings indicate that *LM* extract strengthens cellular defence mechanisms against damage from 2 Gy-radiation. Its strong antioxidant and protective properties highlight its potential as a valuable radioprotective agent.

Keywords

Lemna minor extract, γ -radiation, chromosome aberration, oxidative stress, radioprotection

Abbreviations

LM – *Lemna minor*; MDA – malondialdehyde; ROS/RNS – reactive oxygen/nitrogen species; IR – ionizing radiation; γ - gamma; Gy – Gray; CAT – catalase; SOD - superoxide dismutase; GSH – glutathione; GPx1 - glutathione peroxidase 1; ROS – reactive oxygen species; PBN – N-tert-butyl-alpha-phenylnitron; •NO – nitric oxide; CPTIO.K – carboxy 2-(4-carboxyphenyl)- 4,4,5,5-tetramethyl; 5-MSL- 3-maleimido proxyl; DMSO – dimethyl sulfoxide; •O₂⁻ – superoxide anion; OS – oxidative stress.

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Introduction

For millennia, humanity has been continuously exposed to various forms of ionizing radiation (IR) from both natural sources and human-made activities (Hadjidekova 2008). The widespread use of IR across various fields—such as energy production, industry, agriculture, and medicine—creates numerous opportunities for occupational or direct exposure. The advancement of nuclear technologies has also increased the risk of accidental radiation release, which can result in exposure to high doses of gamma (γ) radiation (Pozdaev 2015). Such radiation exposure can lead to cellular and organ damage, impairing overall human health.

The IR effects, particularly its radiosensitizing impact on the immune system, have been extensively studied, both *in vitro* and *in vivo* (Schau 2017). Components like inflammatory cytokines, granulocytes, macrophages, and lymphocytes play crucial roles in immune regulation. γ -radiation disrupts this balance by damaging antigen-presenting cells and impairing immune function. It also interferes with the Th1/Th2 immune response, creating a dysregulated and antagonistic immune environment (Frey et al. 2017).

At the molecular level, IR can cause DNA base or sugar damage, single- or double-strand breaks, DNA interstrand, intrastrand, or protein cross-links. Double-strand breaks are considered to be one of the most serious DNA lesions (Reindl et al. 2023). γ -irradiation causes structural damage to DNA, including single- and double-strand breaks, either directly through ionization or indirectly via reactive oxygen and nitrogen species (ROS/RNS). Excessive ROS/RNS production amplifies molecular damage by further ionizing surrounding molecules. If DNA repair pathways fail to activate effectively, this damage can lead to cell death or cellular senescence, often resulting in cell cycle arrest (Frey et al. 2017; Rückert et al. 2021).

In conventional γ -radiation therapy for non-cancerous conditions, a standard dose of approximately 2 Gy is typically used. However, cells exposed to this dose often struggle to repair DNA damage properly and may lose key regulatory functions that govern cell cycle progression (Mishra et al. 2014; Rückert et al. 2021; Lumniczky et al.

2018). γ -radiation also suppresses the expression of granulocytes and macrophages and triggers apoptosis in hematopoietic cells, contributing to immunosuppression. Lymphocytes, however, remain central to the body's response to γ -radiation and are considered a reliable model for monitoring radiation-induced inflammation and toxicity (Lumniczky et al. 2018; Lumniczky et al. 2021).

Efforts to prevent γ -induced toxicity have focused on developing both natural and synthetic radioprotective agents. Natural compounds rich in biologically active molecules—such as polyphenols and flavonoids—are particularly promising due to their strong antioxidant and radiomodulatory properties. These natural substances offer several advantages: inexpensive, orally administrable, generally non-toxic, and act on multiple biological targets. Natural compounds have been used traditionally in medicine and offer broad-spectrum protection. Identifying, evaluating, and validating natural products with radio-modulatory potential is essential for their use in counteracting the cytotoxic and oxidative effects (OS) of γ -radiation (Jagetia 2007; Mun et al. 2018; Montoro et al. 2023).

Lemna minor L. (*LM*), commonly known as duckweed, is a small, fast-growing aquatic plant from the *Lemnaceae* family. *LM* have rapid biomass production, ease cultivation, and adaptability to laboratory conditions. *LM* is widely used in ecotoxicological research—especially for its capacity to absorb heavy metals and detoxify harmful chemicals (ISO 20079:2005; Doğan 2025; Alkimin et al. 2019; Ziegler et al. 2016; Lakatos et al. 1993). The practical applications of *LM* (biomass; extract, etc.) in animal feed production, novel food development, biofuel and biogas generation, have various biotechnological fields (Bethancourt-Dalmasí et al. 2025; Sosa et al. 2024; Gusain and Suthar 2017; Kuznetsova et al. 2019; Vulpe et al. 2025; Yamamoto et al. 2001; Bog et al. 2019).

In traditional and homeopathic medicine, *LM* has a long history of use (Edelman et al. 2022; Makhlayuk 1967; Popov 1968; Krylov 1969; Nikolaichuk and Zhigar 1992; Petrova-Tacheva et al. 2018; Gostýnska et al. 2022). Scientific studies have confirmed a range of pharmacological properties, including antibacterial (Gülçin et al. 2010; Dafalla 2015; Tan et al. 2018), antifungal

(Nikiforov 2009; Gülçin et al. 2010), anti-inflammatory (Popov et al. 2006b; Zamoschina et al. 2011), immunomodulatory (Popov 2010; Sharma et al. 2017), and thyreostatic effects (Kravchenko et al. 2014).

Notably, *LM* has demonstrated strong antioxidant activity and the ability to scavenge free radicals (Gülçin et al. 2010; Loseva et al. 2014; Saritha and Saraswathi 2014; Bright and Kanagappan 2016; Voronov et al. 2019; Doğan et al. 2022; Karamalakova et al. 2022). Recent research has also explored its potential anticarcinogenic properties (Doğan 2025), cryoprotective effects (Svedentsov et al. 2008), and its role as an antioxidant modulator in conditions involving oxidative stress (Karamalakova et al. 2022).

Structurally, *LM* extract contains biologically active ingredients include: phytosterols (>52.8 mg. kg⁻¹), hydrocarbons (>23.1 mg. kg⁻¹), aldehydes and ketones (>20.2 mg. kg⁻¹), proteins (>21.80%), plant fibers (>17%), lipids (>11.1 mg. kg⁻¹), polysaccharides, flavonoids, amino acids, aliphatic acids, phenolic carboxylic acids, anthocyanins, triterpene compounds, vitamins (predominantly vitamin A, vitamin C and vitamins of group B), micro- and macro-elements, determining the potential antioxidant nature (Kolomiets et al. 2004; Nikiforov 2009; Nikolaichuk 1992; Solovyova 2005; Vladimirova and Georgiyants 2014). The pectin polysaccharide lemnan was isolated and characterized from freshly harvested *Lemna minor* L. mass (Ovodova et al. 2000). *LM* is a rich source of carotenoids and total flavonoids (mainly flavones and flavonols), followed by phenolic acids, low-molecular-weight phenolics and glucosinolates (Zhang et al. 2023; Dogan et al. 2022). The described pharmacological activities and bioactive compounds indicate that *LM* extract may exhibit radioprotective properties, which in combination with its rapid growth, high biomass production and high protein content would lead to its development and production as a food or nutritional supplement, with probable radio-modulatory ability, which up-to-date has not been studied completely.

The aim of the study was to evaluate the radioprotective effects and oxidative stress modulation of *LM* extract against γ -induced cellular stress, in human lymphocyte cultures, *in vitro*.

Materials and Methods

Extract preparation. The duckweed was purchased from the trading company Sirazida - Gancho Stanchev ET (Stara Zagora, Bulgaria). Initially, the dried duckweed was mechanically ground into powder. After which maceration was carried out with 70% ethanol, duration 72 h, at room temperature, hydromodule 1:10. The liquid phase was then concentrated using a vacuum evaporator to obtain 100 mL. After pressing, the obtained extract was filtered through a membrane filter – CORNING (pore size 0.20 μ m).

Cell cultures. Cell cultures were performed using peripheral venous blood collected in sterile vacutainers with lithium heparin from five healthy donors (3 women and 2 men, mean age ~47.8). The donors used had not been ill in the last 6 months, had not taken any medications or other nutritional supplements and had not been subjected to mutagenic effects. All procedures followed the Declaration of Helsinki, approved by the local Ethics and Academic Integrity Committee (No. 7.5.1 OD_4.1.5.9/protocol 18/15.04.2022). All donors were fully informed and signed written informed consent forms for the collection of venous blood and its use for scientific purposes.

The prepared cell cultures (10 mL) contained: RPMI-1640 medium supplemented with 20 mM HEPES and L-glutamine, 2 mL fetal bovine serum, 0.2 mL phytohemagglutinin P (2 %), 1% antibiotic antimycotic solution (containing 10,000 units penicillin, 10 mg streptomycin and 25 μ g amphotericin B per mL), 1 mL peripheral venous blood and with/without *LM* extract (50 μ g. mL⁻¹). All cell cultures were prepared in duplicate. One set was irradiated 60 min after the start of treatment with/without *LM* extract, and the other served as a non-irradiated control.

Cells Irradiation. The cell cultures were γ -irradiated with 2 Gy in a homogeneous medium at 37°C using a Terabalt 80 unit (cobalt-60, ⁶⁰Co source) in the Radiotherapy Department of the Clinical Oncology Center.

Experimental groups. Cell cultures were divided into four experimental groups per scheme mentioned below:

Group 1. Control: untreated cell cultures.

Group 2. LM-treated cells cultures: cell cultures treated with 50 µg. mL⁻¹ LM extract.

Group 3. Irradiation cells: cell cultures exposed to γ-radiation (2 Gy).

Group 4. LM-treated and irradiated cells: cell cultures treated with 50 µg. mL⁻¹ LM extract and exposed to γ-radiation (2 Gy). The irradiation was performed 60 minutes after the initiation of extract treatment.

Followed by all treatments and desired incubations under controlled conditions (37°C, 5% CO₂), cells from all the experimental groups were harvested at a set time point (48 h) and processed for further experimentation.

Cell culture and sampling for enzyme analyses, MDA, ROS, •NO generation. Cell cultures were incubated at 37°C in a thermostat. After 47 h, 0.5 mL cells of all different treatment groups were taken for enzyme analyses, MDA, ROS, •NO generation and protein conformation; without colcemid, hypotonic and methanol/acetic acid fixation.

Metaphase chromosome preparation. After taking 0.5 mL samples from each culture for enzyme analyses, MDA, ROS, and •NO generation, the remaining cells were treated with 100 µl colcemid solution (10 µg. mL⁻¹) for 90 min, followed by hypotonic solution (0.075 M KCl, 18 min.) and fixation with methanol/acetic acid (3:1, v/v, four repeats). The microscope slides were prepared by dropping the cell suspension onto pre-chilled glass slides, air-drying, and staining with 10% Giemsa solution. A minimum of 100 metaphases per culture (500 per experimental group in total) were analyzed under an Olympus BX41 microscope (Hamburg, Germany) at 1000× magnification (oil immersion). All observed chromosomal- and chromatid-type aberrations were recorded.

Cell cultures preparation for ELISA assay. Cells from different treatment groups were subjected to enzyme activity assays and lipid peroxidation experiments to evaluate their responses to the LM extract: catalase (CAT; No. ab83464), superoxide dismutase (SOD; No. ab65354), glutathione (GSH; No. ab142044) glutathione peroxidase 1 (GPx1; No.

ab41464) and malondialdehyde (MDA; No. ab233471).

Cell cultures preparation for electron paramagnetic resonance (EPR) estimation. The ROS experiment: 100 µl of cell cultures was mixed with 900 µl (50 mM) N-tert-butyl-alpha-phenylnitron (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 spin trap 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). The EPR settings were 3503.73-3505 G centerfield, 6.42 mW-20.00 mW microwave power, 5 G modulation amplitude, 2.5 × 102 gain.

Protein/albumin conformational (-SH) changes: prepared cell cultures (10 mg) were mixed with 20 mM 3-maleimido proxyl (5-MSL) dissolved in 900 µl dimethyl sulfoxide (DMSO). The mixture was centrifuged (1000 rpm; 15 min) at 4°C. Protein/albumin conformational (-SH) changes were recorded in triplicate, with the following parameters: 3505 G; power 6.42 MW; amplitude 5 G; 12 modulations, in arbitrary units, according to the method described previously (Takeshita et al. 2002).

Experimental replications. Five independent biological replications (N = 5) of the whole experiment were performed. At each replication, the four cell culture groups were created, studied and analysed (see the experimental groups described above).

Statistical analysis. Cytogenetic data were statistically analyzed and graphically presented with GraphPad Prism (v.8.0.1 for Windows; GraphPad Software, Inc., San Diego, CA, USA). The Mann-Whitney U test was used for comparisons between two independent groups. For multiple comparisons among three or more groups, Kruskal-Wallis test and Dunn's post hoc test were used. Statistical significance was determined at p < 0.05.

The EPR processing was performed using WIN-EPR SimFonia 1.2/6130860 software. Statistical

analysis was performed using one-way ANOVA and Student's t-test to determine differences; $p < 0.05$ was considered statistically significant.

Results and Discussion

Influence of LM treatment on cytogenetic findings in 2 Gy - irradiated cells. Using Kruskal-Wallis test and Dunn's post hoc test for multiple comparisons (Fig. 1), statistically significant increases in all studied cytogenetic parameters

(frequencies of aberrant cells, total chromosomal aberrations, dicentrics only, dicentrics plus rings, rings only and fragments) were observed in the positive control (Group 3: untreated irradiated cells) compared to both Group 1 (untreated unirradiated controls) and Group 2 (LM extract-treated samples). No significant differences were observed between the two non-irradiated groups: Group 1 (untreated unirradiated controls) and Group 2 (LM extract-treated samples).

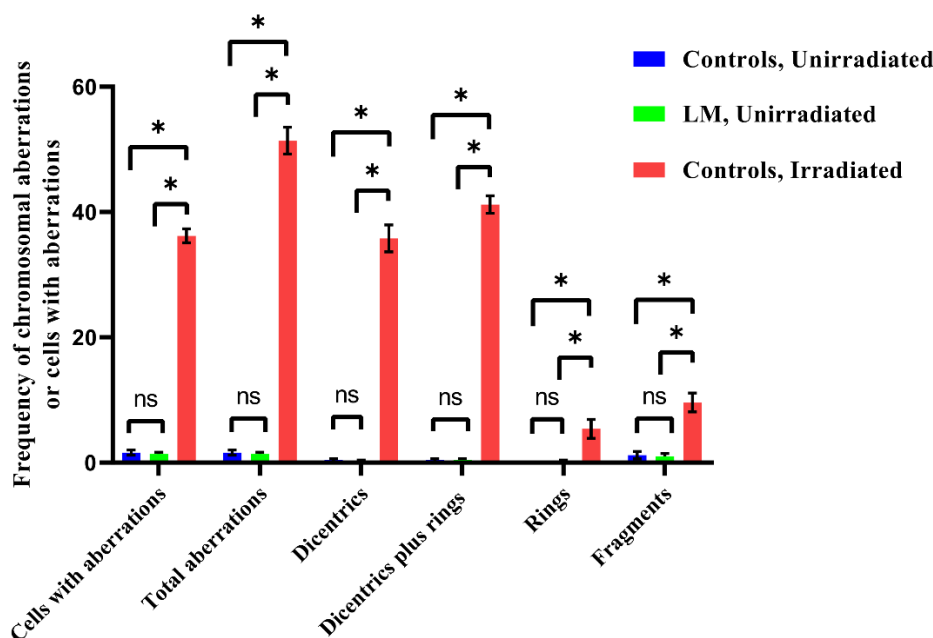


Figure 1. Frequencies of aberrant cells, total chromosomal aberrations, dicentrics only, dicentrics plus rings, rings only, and fragments in experimental groups: (Group 1) untreated unirradiated controls, (Group 2) LM extract-treated unirradiated samples and (Group 3) untreated irradiated controls. Kruskal-Wallis test was followed by Dunn's post-hoc test; Data are presented as mean \pm SEM; (*) $p < 0.05$; (ns) not significant

Using the Mann-Whitney U test (Fig. 2), the LM extract application in irradiated cell cultures resulted in a statistically significant:

- Reduction in the cells frequency with chromosomal aberrations ($p=0.0317$; $p < 0.05$) to 30% vs 36.2% in the 2 Gy irradiated group, which corresponds to a 17.13% reduction, compared to untreated cells.
- Reduction in the aberrations frequency ($p=0.0079$; $p < 0.05$) to 37.6% vs 51.4% in the 2 Gy irradiated group, which corresponds to a 26.85% reduction, compared to untreated cells.

c) Decrease in the dicentrics frequency ($p=0.0397$; $p < 0.05$) to 27.4% vs 35.8% in the 2 Gy irradiated group, which corresponds to a 23.46% reduction, compared to untreated cells.

d) Reduction in the dicentrics and rings frequency ($p=0.0159$; $p < 0.05$) to 31% vs 41.2% in the 2 Gy irradiated group, which corresponds to a 24.76% reduction, compared to untreated cells.

For both indicators, rings frequency and fragments, a decrease in the mean values was separately found when exposed to LM extract, compared to 2 Gy irradiation, without statistically insignificance ($p > 0.05$).

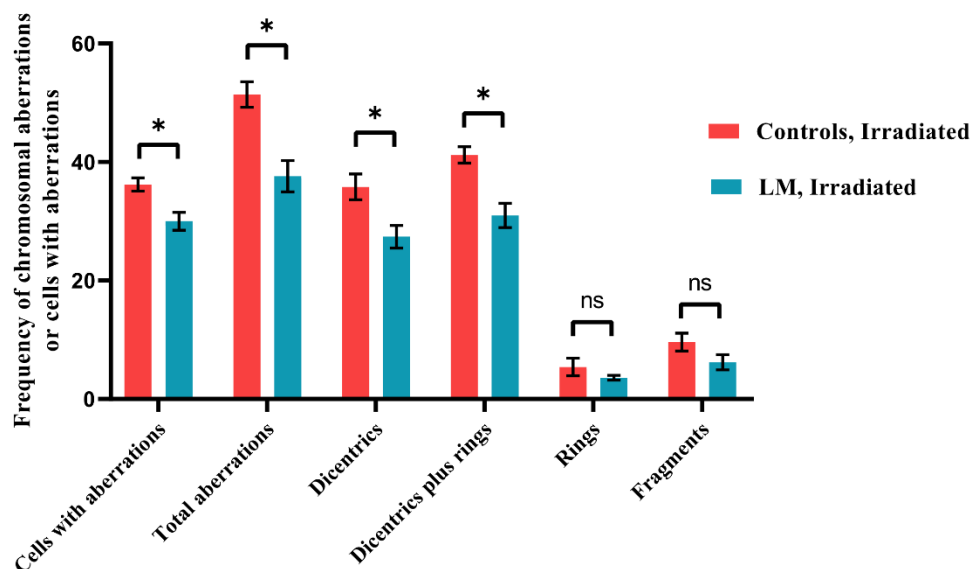


Figure 2. Frequencies of aberrant cells, total chromosomal aberrations, dicentric only, dicentric plus rings, rings only and fragments in irradiated groups: (Group 3) untreated irradiated controls and (Group 4) LM extract treated and irradiated samples. Data are presented as mean \pm SEM (Mann-Whitney U test, (*) $p < 0.05$, (ns) not significant)

LM treatment normalizes enzyme activities of 2 Gy - irradiated cells. Radiation (2 Gy) treatment of lymphocyte cultures showed a consistent decline in cellular enzyme activity (Fig. 3). CAT (1.075 ± 0.023 vs. 3.83 ± 0.947 IU. gPr^{-1} , $p < 0.003$; Fig. 3A), SOD (0.994 ± 0.036 vs. 5.234 ± 0.96 IU. gPr^{-1} , $p < 0.005$; 3B), GSH (18.478 ± 2.93 vs. 63.300 ± 12.976 nmol. gPr^{-1} , $p < 0.04$, Fig. 3C) and GPx1 (17.533 ± 13.36 vs. 70.044 ± 17.031 IU. gPr^{-1} , $p < 0.05$, Fig. 3D) decreased significantly threefold and fourfold compared to untreated control cells.

Conversely, the LM treatment at $50 \mu\text{g. mL}^{-1}$ in human lymphocyte cultures showed significant 2 Gy irradiation inhibition and substantial increase in the enzymatic defense by OS amelioration in cells. Markedly, induced activities of CAT (2.905 ± 0.54 vs. 3.83 ± 0.947 IU. gPr^{-1} , $p < 0.003$; Fig. 3A), SOD (4.726 ± 0.063 vs. 5.234 ± 0.96 IU. gPr^{-1} , $p < 0.005$; 3B), GSH (60.005 ± 13.29 vs. 63.300 ± 12.976 nmol. gPr^{-1} , $p < 0.04$, Fig. 3C), and GPx1 (47.326 ± 8.76 vs. 70.044 ± 17.031 IU. gPr^{-1} , $p < 0.05$, Fig. 3D). LM treatment at $50 \mu\text{g. mL}^{-1}$ in cell cultures showed results close to the controls.

LM treatment ameliorated ROS, NO• stress levels, protein oxidation/conformation and lipid peroxidation. To confirm the protective role of LM extracts against 2 Gy induced oxidative stress, the redox-modulated activity and protein oxidation levels were investigated (Fig. 4). Significantly, inhibition with 2 Gy irradiation increased two-fold ROS production (3.448 ± 0.35 vs. 1.54 ± 0.049 a.u., $p < 0.005$; 4A); NO• levels (45.059 ± 4.35 vs. 22.17 ± 1.95 a.u., $p < 0.002$; 4B) and protein oxidation/conformation (1.493 ± 0.49 vs. 0.516 ± 0.036 a.u., $p < 0.005$, 4D) in cell cultures compared to controls. However, 2 Gy irradiation significantly increased cellular MDA concentration (6.387 ± 0.27 vs. $2.52 \pm 0.53 \mu\text{mol. mL}^{-1}$, $p < 0.05$; 4C) compared to untreated controls. The highest protection of LM treatment against 2 Gy irradiation induction was observed in the cellular ROS reduction (2.007 ± 0.036 vs. 3.448 ± 0.35 a.u., $p < 0.005$; 4A); in NO• concentrations (39.111 ± 3.195 vs. 45.059 ± 4.35 a.u., $p < 0.002$; 4B), and in remodelled protein conformation (1.007 ± 0.021 vs. 1.493 ± 0.49 a.u., $p < 0.005$, 4D). LM treatment significantly reduced lipid peroxidation (4.691 ± 0.06 vs. $6.387 \pm 0.27 \mu\text{mol. mL}^{-1}$, $p < 0.05$) in combination with LM treatment +2 Gy irradiation (Fig. 4C).

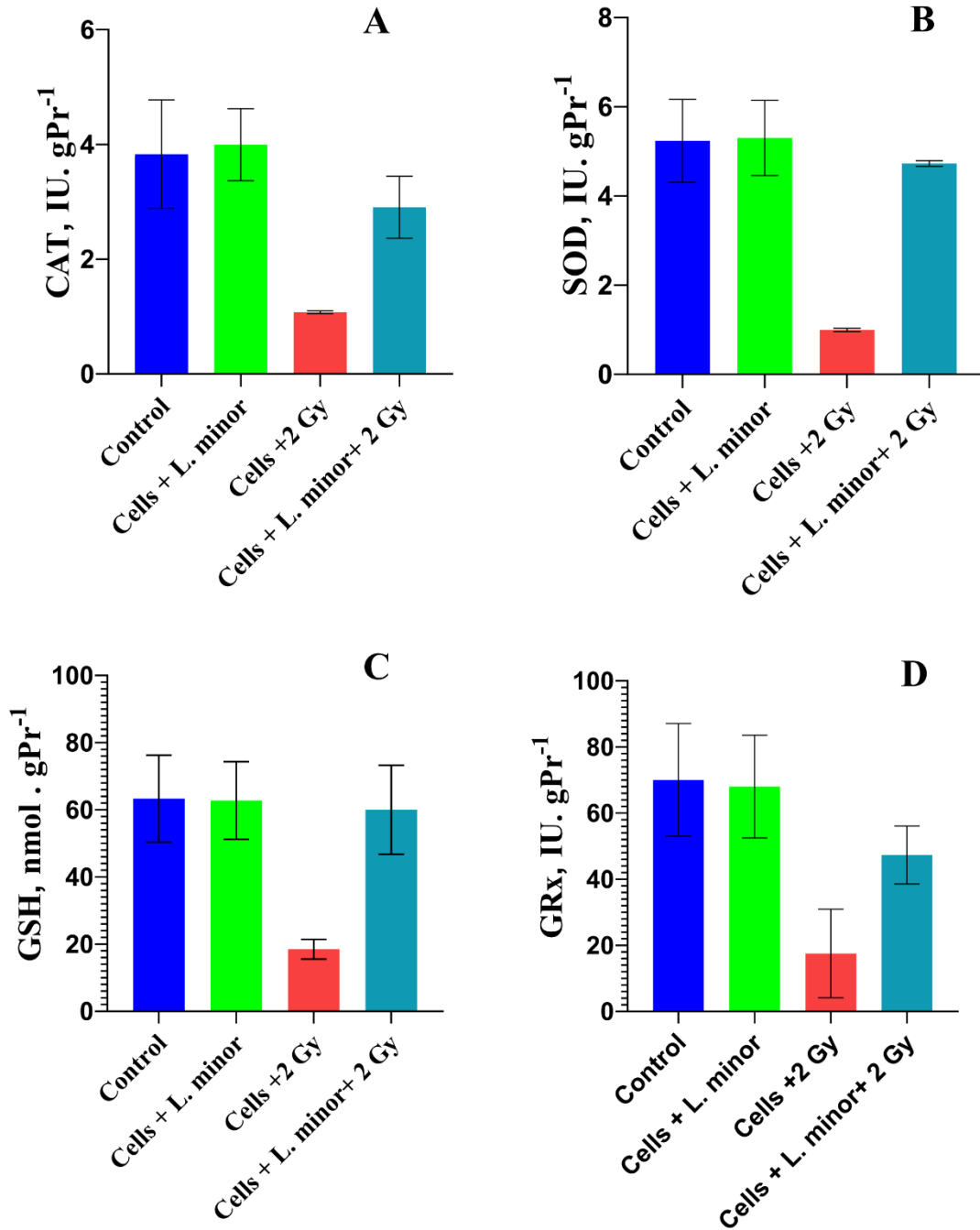


Figure 3. The *LM* effects on γ -radiation-induced oxidative changes in catalase (CAT, (A)), superoxide dismutase (SOD, (B)), glutathione (GSH, (C)), and glutathione peroxidase (GPx1, D). Statistically significance was higher than controls, $p < 0.05$, t-test. The results are presented as mean \pm S.E. $p < 0.05$. (*) vs. to controls; (**) vs. 2 Gy radiation

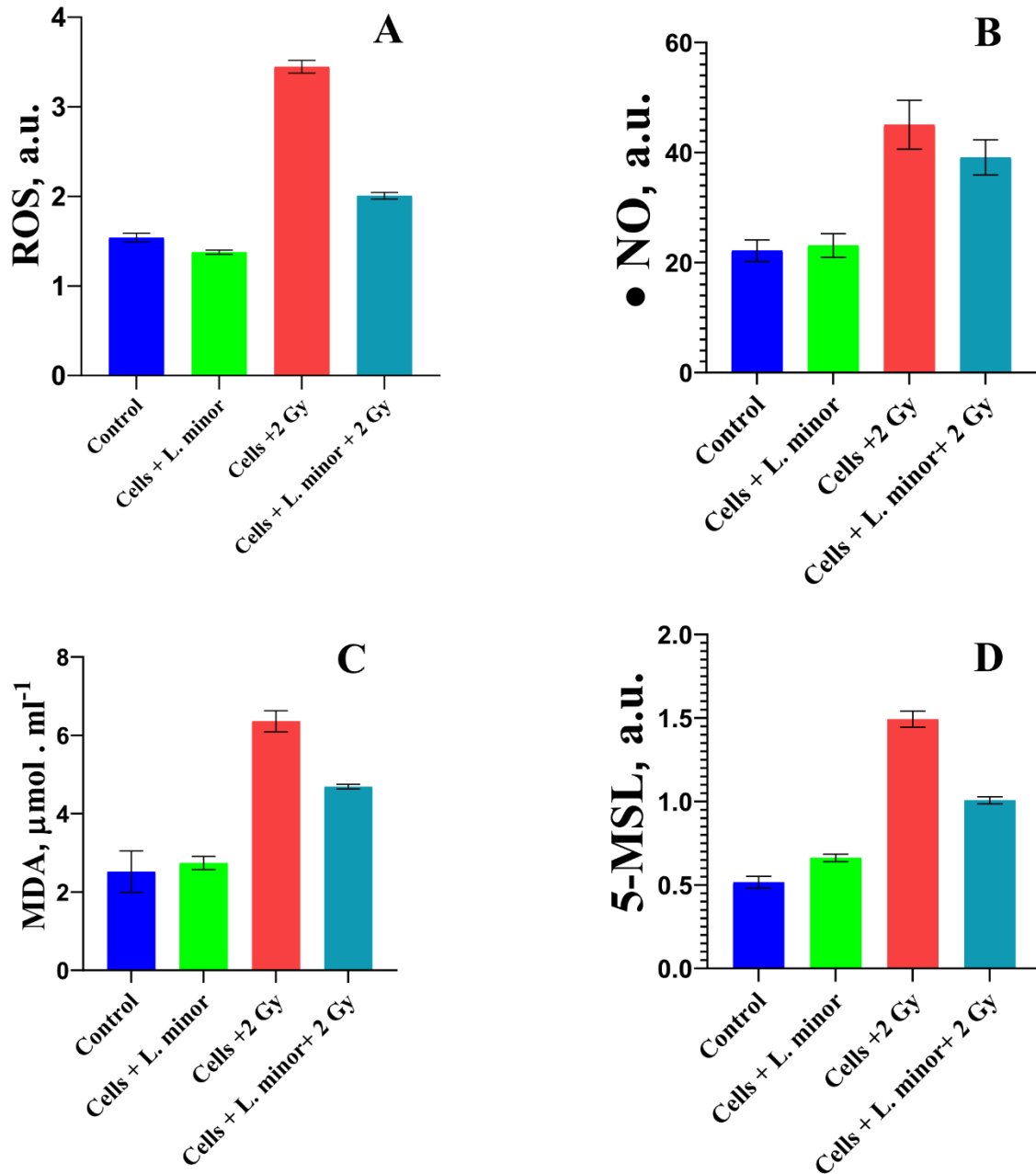


Figure 4. The *LM* effects on γ -radiation-induced oxidative changes in ROS production, (A); NO• levels, (B); lipid peroxidation (MDA, (C); and protein oxidation (D). Statistically significance was higher than controls, $p < 0.05$, t-test. The results are presented as mean \pm S.E. $p < 0.05$; (*) vs. to controls; (**) vs. 2 Gy radiation

In vitro, *LM* treatment at $50 \mu\text{g} \cdot \text{mL}^{-1}$ in cell cultures showed ROS/RNS inactive, close to the untreated controls. These findings highlight the *LM* protective effects against 2 Gy-induced OS. Moreover, *LM* treatment alleviated IR-induced oxidative stress and promoted exogenous–endogenous enzymatic defense by restoring the redox–homeostatic

imbalance and -SH conformational dysregulations versus 2 Gy malformations.

Ionizing (γ) radiation is defined as a potent mutagenic and carcinogenic factor. γ -radiation acts as an S-independent clastogen (Andersson and Kihlman 1987; Singh and Lavin 1990; International Atomic Energy Agency 2014). The peripheral

lymphocyte population is predominantly non-cycling, residing in the G₀ (non-cycling G₁) phase of the cell cycle. Consequently, γ -irradiation of these lymphocytes (as in our experiment) induces chromosomal-type aberrations (Savage 1976; International Atomic Energy Agency 1986; Singh and Lavin 1990). Chromosome-type aberrations are formed by unrepaired or incompletely repaired double-stranded DNA breaks that occur during irradiation in the G₀-G₁ phase of the cell cycle. After DNA synthesis and chromosome doubling, the aberrations that occurred in the G₀-G₁ stage are doubled and in the next metaphase, they are observed as breaks and chromosome-type exchanges (dicentrics, rings, balanced translocations). Cytogenetic findings of this type are the most sensitive biomarker for ionizing radiation exposure (Hadjidekova 2008). As the results demonstrate (Fig. 1), the significant increase in chromosome-type aberrations in irradiated positive controls (Group 3) confirms the effectiveness of our experimental model.

The absence of statistically significant differences between the non-irradiated groups (Group 1 and Group 2) indicates that *LM* extract, when applied alone at a concentration of 50 $\mu\text{g. mL}^{-1}$ in the cell cultures, does not exhibit clastogenic activity (Fig. 1). At the present, we have not found published data from similar studies.

When comparing the cytogenetic findings in the two irradiated groups (Group 3 and Group 4), the results strongly indicate that the *LM* extract (50 $\mu\text{g. mL}^{-1}$) has a pronounced anticlastogenic effect, reducing γ -induced chromosomal aberrations, especially dicentrics and rings (specific for gamma irradiation) as well as the total number of chromosomal aberrations and aberrant cells (Fig. 2). While Yefimov et al. (2004) reported radioprotection by aqueous *LM* extract in X-ray irradiated mice at dose 1 Gy, our study provides the first evidence for our extract efficacy in γ -irradiated human cell cultures at dose 2 Gy, confirming the broad-spectrum activity of *LM* extracts across distinct experimental models, radiation types and doses. The most common mechanisms of radioprotection are the scavenging of free radicals, reparation of DNA damages, inhibition of apoptosis or inflammation, increase antioxidant defenses, and modulation of growth factors, cytokines, and redox genes (Montoro et al. 2023). The radioprotective effects of

plant and herbs may be mediated by several mechanisms, as they are complex mixtures of bioactive compounds (Jagetia 2007). The studied *LM* extract may also protect cells by activating multiple mechanisms and cellular signalling pathways. In the present experimental model, *LM* extract was introduced into lymphocyte cultures -1 h before γ -irradiation, was present during irradiation and thereafter until the end of cell cultivation. Therefore, it may exhibit not only radioprotective but also radiomitigating effects. This is supported by the work of Kalmakhelidze et al. (2021), who reported that the intake of *lemnan* (a polysaccharide isolated from *LM*) in the early post-radiation period after 5 Gy γ -irradiation significantly enhances the regenerative process of the small intestinal mucosa and increases the survival rate of irradiated mice.

Ionizing radiation induces ROS/RNS generation and OS, leads to progressive lipid peroxidation, which affects complex DNA damages, and directly affects the antioxidant defense system (Reisz et al. 2014; Ping et al. 2020). Intracellular ROS/RNS deactivation is performed by endogenous and exogenous antioxidant enzymes, SOD, CAT, and GSH, etc., and possible antioxidant reaction of superoxide ($\bullet\text{O}_2^-$) anion dismutation, and ability to OS infiltration (Karamalakova et al. 2022). Our results show that the 2 Gy-application leads to a statistically significant increase in the OS parameters, i.e., increased MDA and ROS, $\bullet\text{NO}$ production, along with decreased SOD, CAT, GPx1, and GSH activity (Fig. 3) in irradiated lymphocyte cultures. In addition, there was a statistically significant restoration in the SOD, CAT, and GSH activity (Fig. 3) and a decrease in the lipid peroxidation and residual ROS/ $\bullet\text{NO}$ (Fig. 4) in the *LM* treatment + 2 Gy combination. The *LM* application to cell cultures can be explained by the radiomodulatory effect of *LM* extract. Van Hoeck et al. (2015) reported that enzyme activities remained approximately unchanged up to a dose of 62 mGy.h^{-1} . The adaptive *LM* mechanism to induced OS is due in part to an additional antioxidant response that directly reduces ROS/RNS and decompensates endogenous antioxidants, despite the reduced accumulation of soluble proteins and biomass in the plant (Huang et al. 2013). It has been found that γ -irradiation contributes to the enhancement of membrane lipid peroxidation in plants (Huang et al. 2013). Xie et al. (2019)

summarized that exposure to γ -radiation with a dose rate $> 24 \text{ mGy}\cdot\text{h}^{-1}$ and a cumulative dose of $\sim 4.27 \text{ Gy}$ significantly increased cellular ROS production in duckweed. MDA and ROS/RNS products in *LM* extract ($50 \mu\text{g}\cdot\text{mL}^{-1}$) recorded values similar to untreated lymphocyte cells. Cell cultures treatment with *LM* extract, in the combination *LM* extract+2 Gy led to a statistically ($p<0.05$) significant reduction in lipid peroxidation levels and $\bullet\text{NO}$, $\bullet\text{OH}$, $\bullet\text{O}_2^-$ concentrations (Fig. 4). Statistically, *LM* extract enhancement of lipid peroxidation and ROS/RNS production in 2 Gy cellular application suggested OS fixation. Our results are in agreement with reports to Popov et al. (2006a), showing that *lemnan*, *L. minor* isolated apiogalacturonan pectin manifests increased mucosal adjuvanticity, resulting from an intestinal epithelial lipid barrier alteration. Therefore, *L. minor* manages have the possibility to reduce cellular damages by reducing $\bullet\text{O}_2^-$ concentrations and balancing the H_2O_2 content and ROS/RNS products after lipid peroxidation. In addition, Pagliuso et al. (2020) hypothesize that C-glycosylated (luteolin-8/6-C; apigenin-8/6-C, etc.) flavonoids predominantly present in the duckweed/*Lemna* species, probably protect against induced OS disorders and prevent endogenous/exogenous action. The flavone C-glycosides from *Lemna japonica* exhibit cytotoxic activity against HepG-2, SW-620, A-549 human cell lines (Baek et al. 2021). γ -radiation induces complete protein oxidation and dysfunctions and pathologically damages cell cultures (Balasubramanian et al. 2025).

It was found that after γ -irradiation, the expression of the actin cytoskeleton was significantly reduced for various proteins related to the mitochondrial respiratory chain and the sirtuin pathway. While the protein levels responsible for OS were repeatedly increased (Balasubramanian et al. 2025). The most affected pathways after induced (chronic) γ -irradiation are protein ubiquitination, fatty acid metabolism, cytoskeletal organization, sirtuin signalling, mitochondrial dysfunction, up to OS, and impaired cardiac protein metabolism (Azimzadeh et al. 2020). Our results are in complete agreement with previously reported studies (Azimzadeh et al. 2020; Balasubramanian et al. 2025) and demonstrated that in the 2 Gy radiation cell cultures, there was a statistically significant two-fold increase in protein oxidation and $-\text{SH}$

conformation (Fig. 4A), and delayed reduction in nitroxide protein distribution. It is important to note that the combination with *LM* treatment + 2 Gy significantly reduces these parameters ($p < 0.005$, t-test), in comparison to untreated cells. *LM* extract, due to the high active proteins and amino acids content in its structure, stimulates antigen-specific immune response, which restores protein oxidation and cell-mediated cytotoxicity (Mane et al. 2017; Cox et al. 2006). Therefore, *LM* modulates cellular 2 Gy-induced inflammation by reducing OS, reducing protein dysfunction, and completely inhibiting the $\bullet\text{O}_2^-$, $\bullet\text{OH}$ radicals' concentrations. This result is consistent with the fact that *LM* has anti-inflammatory and immunosuppressive effects by reducing free haemoglobin content and antibody production in human whole blood (Baek et al. 2021). Based on these findings, we hypothesize that the *LM* anti-cytotoxic effect ($50 \mu\text{g}\cdot\text{mL}^{-1}$) is due to a modulated cellular response from the high polyphenol content (Mane et al. 2017), leading to a γ -irradiation albumin/protein reduction.

Conclusions

This study investigates the radioprotective properties of *LM* extract at a $50 \mu\text{g}\cdot\text{mL}^{-1}$ concentration in human peripheral blood cultures. For the first time, *LM* extract is reported as a potential antioxidant and radio-modulatory agent, capable to enhancing the protective response of membrane-associated proteins and mitigating oxidative stress (OS) disturbances following exposure to 2 Gy radiation. Importantly, *LM* extract did not exhibit clastogenic effect at the tested concentration. Moreover, *LM* extract demonstrated significant anticlastogenic activity against radiation-induced damage, effectively preserving DNA integrity and reducing chromosomal aberrations. These findings highlight the *LM* extract potential as a novel bioactive compound with promising applications in radiation protection and oxidative stress modulation, with cellular defense mechanisms. Our future research directions may include validation of the described effects in suitable *in vivo* models and detailed “dose-response” analysis so that the optimal effectiveness of the extract can be established. The results of these will lead to further understanding of the mechanisms of its radio-protective activity and clarifying the potential for its future therapeutic application.

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Author Contributions

Conceptualization, V.P.-T., K.P.-P. and Y.K.; methodology, B.P., Y. K. and V.P.-T.; formal analysis V.P.-T., P.V. and K.P.-P.; investigation, V.P.-T., Y.K. and T.H.; resources, G.N. and B.P.; data curation, G.N., E.G. and B.P.; writing -original draft preparation, V.P.-T., G.N. and Y. K.; writing -K.P.-P., Y.K. and G.N., review and editing, V.P.-T., B.P. and E.G., visualization, V.P.-T. and E.G.; supervision, E.G. and P.V.; project administration, G.N., Y.K. and B.P.; 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 five healthy donors (3 women and 2 men) 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

Written informed consent was obtained from all five blood donors prior to sample collection.

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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