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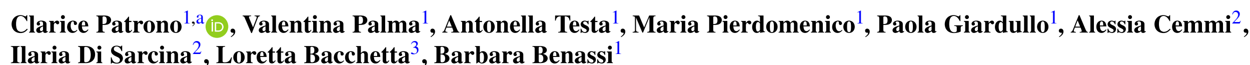

Radioprotective potential of *Opuntia ficus-indica* mucilage extract in human peripheral blood lymphocytes

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Abstract Ionizing radiation is widely used in medicine but can cause genotoxic and cytotoxic effects in healthy tissues. Natural radioprotectors represent a promising strategy to mitigate the radiation-induced damage. This study investigates the radioprotective efficacy of a mucilage extract from *Opuntia ficus-indica* (OFI) cladodes in human peripheral blood lymphocytes (PBLs). PBLs were treated with two concentrations of OFI extract (0.01 and 0.05 µg/mL) and assessed for cytotoxicity, viability and chromosomal integrity. Cytokinesis block micronucleus (CBMN) assay and cytokinesis block proliferation index (CBPI) were used to evaluate genotoxicity and cytotoxicity, respectively. For radioprotection assays, PBLs were pretreated with OFI extract prior to exposure to ^{60}Co γ-rays (0.5 and 1 Gy) and the frequency of micronuclei was determined. OFI extract per se did not induce cytotoxic or genotoxic effects. Exposure to gamma radiation significantly increased micronuclei frequency in a dose-dependent manner. Pretreatment with OFI extract resulted in a significant reduction in radiation-induced micronuclei formation at 1 Gy total absorbed dose, indicating a protective effect against chromosome damage. In conclusion, OFI mucilage extract is non-toxic to human lymphocytes and exhibits radioprotective potential, likely due to its bioactive phytocompounds. These preliminary findings support further investigation of OFI as a natural radioprotective agent in clinical or environmental settings.

1 Introduction

Ionizing radiation (IR) plays a pivotal role in cancer treatment, with radiotherapy representing one of the primary therapeutic modalities alongside surgery and chemotherapy [1]. The cytotoxic efficacy of IR primarily stems from its ability to induce DNA damage through both direct ionization of nucleic acids and indirect mechanisms involving the radiolysis of water, which generates free radicals and reactive oxygen species (ROS) [2]. In addition, IR can trigger non-targeted effects, such as the radiation-induced bystander effect, wherein irradiated cells release signaling molecules that affect adjacent non-irradiated cells, contributing to genomic instability and altered cellular responses [3]. Although these mechanisms underpin the therapeutic benefit of radiotherapy in eradicating malignant cells, they may also lead to collateral damage in surrounding normal tissues. Technological advances such as intensity-modulated radiotherapy and image-guided radiotherapy have significantly enhanced the precision of radiation delivery, thereby minimizing off-target effects and preserving healthy tissue integrity [4]. Nonetheless, adverse effects in non-tumor tissues remain a significant clinical concern, particularly in long-term cancer survivors who may experience late-onset toxicities or secondary malignancies [5].

In this context, the use of radioprotective compounds that can mitigate radiation injury in normal tissues has extensive and important applications [6]. Many natural and synthetic molecules have been tested to identify effective radioprotectors; nevertheless, ideal radioprotective agents, with high stability, efficacy, selectivity for normal tissues and low side effects, have not yet been identified [7]. According to recent reviews, different natural extracts as well as several purified natural compounds (including caffeine, apigenin, curcumin, chlorogenic acid, quinic acid, bergenin, lycopene and lutein) have demonstrated radioprotective properties in both in vitro and in vivo studies [7–9]. These compounds exert their effects through various mechanisms, including enhanced DNA repair, free radical scavenging, inhibition of lipid peroxidation as well as anti-inflammatory, antioxidant, immunomodulatory, anticytotoxic and antigenotoxic activities [8].

The *Opuntia ficus-indica* (L.) Mill. (OFI) is an edible plant, member of the Cactaceae family; it is widely distributed worldwide and adapted to dry climatic conditions [10]. The importance of OFI fruits and cladodes as a dietary source, rich in minerals, vitamins

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and antioxidants, has increased due to their potential nutraceutical properties. The use of extracts obtained from different parts of the plant, moreover, has been gaining interest due to their promising beneficial health effects. In this context, extracts obtained from OFI cladodes were tested for their cytoprotective, antioxidant, antigenotoxic, anticancer and anti-inflammatory activities [11], with the benefit of exploiting OFI waste products for health protection applications. Specifically, our group has recently demonstrated that a mucilage extracted from OFI cladodes significantly attenuates lipopolysaccharide (LPS)-induced inflammation in HepG2 liver cells by downregulating pro-inflammatory gene expression and inhibiting the inflammatory signaling pathway mediated by Toll-like receptor 4 (TRL4), with direct interference in TLR4-LPS binding (showed via in silico molecular docking) [12]. Moreover, OFI cladode extract was preliminarily tested for its ability to protect DNA plasmids from γ-radiation [13]; OFI extract proved to be effective in inhibiting DNA damage, in terms of single-strand breaks (SSB) and double-strand breaks (DSB) formation and DNA base damage.

The current study aimed to investigate the radioprotective potential of an OFI cladode mucilage extract in human peripheral blood lymphocytes (PBLs) undergoing exposure to ^{60}Co γ-rays. The OFI extract was deeply characterized in terms of total polysaccharide, protein and polyphenol content and profiled by metabolomic characterization through gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–high-resolution mass spectrometry (LC-HRMS) analysis [12]. To assess the efficacy of OFI pretreatment on decreasing γ-radiation-induced cell damage, cytotoxicity and chromosome damage were evaluated in samples irradiated in the presence or absence of OFI extract.

2 Methods

2.1 Extract from *Opuntia ficus-indica* cladodes

OFI cladodes were collected in central Italy, in winter. OFI mucilage was extracted from cladodes by maceration in distilled water and filtration and then characterized, as previously described and detailed [12]; the total content of proteins, polysaccharides and polyphenols, as well as its metabolomic characterization—through GC–MS and LC-HRMS analysis—was recently reported [12]. OFI mucilage was stored at − 20 °C at a concentration of 1 g/mL. Dilutions of the extract in sterile distilled water were used in subsequent experiments.

2.2 Human peripheral blood lymphocyte culture

Before the study began, we submitted the research protocol, including the participation of healthy volunteers, to ENEA Ethics Committee for evaluation and approval. The Committee carefully reviewed all ethical and methodological aspects of the protocol, particularly those concerning the use of biological material, and approved it on December 12, 2023 (Prot. No. 7/2023).

Human blood samples were obtained with informed consent from four non-smoking healthy donors (two males and two females, mean age 44 ± 5 years), without any history of chronic diseases, major radiological examination or drug treatment. Blood samples were taken at the Medical Service of ENEA Casaccia Research Centre (Rome, Italy), using heparinized tubes, and processed immediately thereafter. For each peripheral blood lymphocyte culture, 0.5 mL of whole blood was added in a sterile 15 mL conical tube containing 4.5 mL of RPMI-1640 Dutch Modification medium (Merck KGaA, Darmstadt, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Corning, Glendale, AZ, USA), 1% penicillin/streptomycin solution and 2 mM L-glutamine solution (S.I.A.L. Srl, Rome, Italy) [14].

2.3 Treatments with OFI extract

In preliminary experiments, PBL cultures were treated with OFI extract in a range from 0.01 µg/mL to 10 µg/mL for 3 h and 24 h, at 37 °C. After treatment, samples were kept for two more hours at 37 °C to unify experimental conditions (see Sect. 2.4), and then they were cultured in incubator at 37 °C in fresh medium supplemented with 2% of phytohemagglutinin (PHA) M form (Thermo Fisher Scientific Inc., Waltham, MA, USA) to stimulate lymphocyte proliferation. Cell viability and genotoxicity assays were performed 72 h after stimulation with PHA. Two concentrations of the extract (0.01 µg/mL and 0.05 µg/mL) and 3-h treatment were selected for combined treatments with ionizing radiation.

2.4 Irradiation with gamma rays

PBL cultures were exposed to two total absorbed dose values of γ-rays (0.5 Gy and 1 Gy) at Calliope ^{60}Co gamma irradiation facility (ENEA Casaccia Research Centre, Rome, Italy) [15]. Samples were placed in the center of a glass beaker containing preheated water at 37 °C. The depth of the samples in water was 4 cm. Gamma irradiation was performed at a distance of 340 cm from the rack housing the 25 ^{60}Co sources, at a mean dose rate of 11 Gy/h. The total absorbed dose was accurately measured using Fricke dosimetry [16], by irradiating the dosimetric solution under the same conditions as the sample (identical test tubes immersed in the

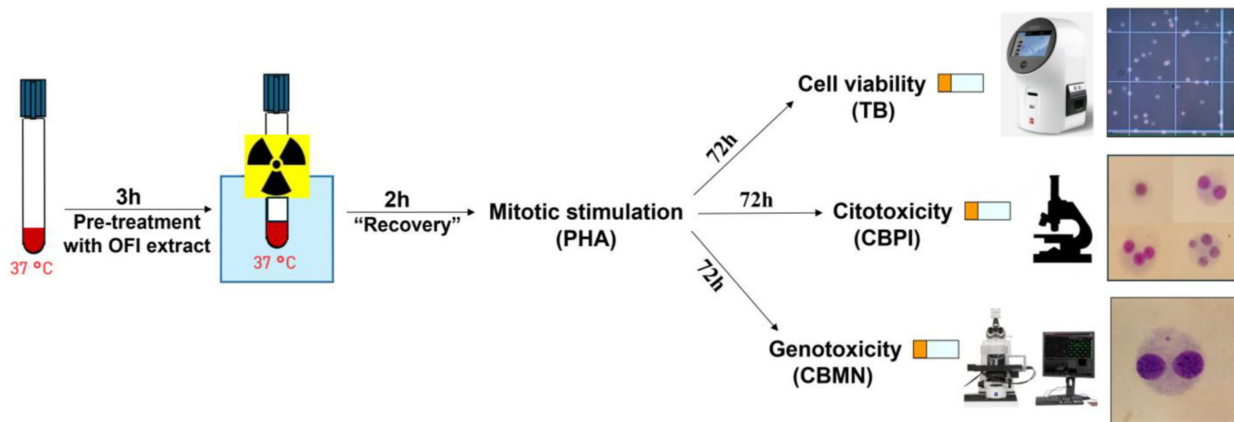

Fig. 1 Experimental design of the combined treatment with OFI extract and ionizing radiation in human lymphocyte cultures. The scheme illustrates the sequential steps of pretreatment with OFI extract, irradiation protocol, mitotic stimulation and subsequent analysis. PHA, phytohemagglutinin; TB, trypan blue; CBPI, cytokinesis block proliferation index; CBMN, cytokinesis block micronucleus assay

same beaker containing preheated water, same irradiation position). To assess dose uniformity, dosimetry was performed using two dosimeters containing Fricke solution, positioned to simulate the spatial arrangement of the PBL samples.

To evaluate the possible radioprotective potential of OFI extract in human PBLs, combined treatments with OFI and ionizing radiation were performed, according to the experimental schedule reported in Fig. 1. Human blood samples were pretreated with each concentration of OFI extract (either 0.01 μg/mL or 0.05 μg/mL) for 3 h and then exposed to 0.5 Gy and 1 Gy of gamma rays. A wider range of concentrations (0.01–5 μg/mL) and a longer pretreatment time (24 h) had been tested in preliminary experiments.

All irradiated samples were held at 37 °C for 2 h to allow DNA repair. Then, they were cultured in incubator at 37 °C in fresh medium supplemented with 2% PHA. Cell viability, cytotoxicity and genotoxicity assays were performed 72 h after stimulation with PHA.

2.5 Cell viability assessment

PBL viability was determined by trypan blue staining. 10 μL of trypan blue 0.4% (Logos Biosystems, South Korea) was mixed with equivalent volume of lymphocyte suspension diluted 1:10. The number of live/dead cells and the percentage of viability were measured using LUNA-II Automated Cell Counter (Logos Biosystems). A specific protocol was used to exclude red blood cells from the count, modifying some parameters (live cell sensitivity, roundness, minimum and maximum cell size) of PBMC (peripheral blood mononuclear cell) protocol, provided by LUNA-II. This unique protocol was initially validated by comparing the automatic count with the manual PBL count using Bürker chamber.

2.6 Applications of cytokinesis block micronucleus (CBMN) assay

2.6.1 CBMN assay

Cytotoxicity and radiation-induced chromosome damage on human lymphocytes were evaluated via the CBMN assay using cytochalasin-B (cyt-B), following standard procedures [14, 17]. In brief, cyt-B (Merck KGaA, Darmstadt, Germany) was added to all treated PBL cultures and to controls, 24 h after stimulation with PHA, to give a final concentration of 6 μg/mL. At 72 h after PHA stimulation, PBL cultures were centrifuged at 200 g for 10 min and the supernatant was removed. Then, a short hypotonic treatment with 0.075 M potassium chloride solution, preheated at 37 °C, was performed. The cells were fixed with cold fixative (5:1 methanol/acetic acid) and washed several times with fixative solution. Then, cells were resuspended in a small volume of fixative, a few drops of cell suspension were dispensed on clean glass slides and air-dried overnight. Slides were stained with 5% Giemsa stain (Carlo Erba Reagents srl, Milan, Italy) prepared in Gurr phosphate buffer, pH 6.8 (VWR International srl, Milan, Italy). The presence of micronuclei (MN) was evaluated by scoring about 1000 binucleated (BN) cells for each subject and experimental point; three experiments were performed for each individual, for a total of approximately 3000 cells analyzed per individual. Micronuclei were scored by using Metafer platform (MetaSystems, Altlussheim, Germany). Semi-automated scoring was used: the automatically scored cells with micronuclei, obtained with Metafer MNScore system, were visually assessed by the operator. The frequencies of binucleate cells with micronuclei (BNMN), micronuclei per cell and MN distribution (number of cells with one, two and multiple MN) were determined.

Table 1 Cell viability and proliferation index in PBLs of four subjects treated with OFI extract for 3 h

Exp. Pt	Subject	Age	Sex	Viability (%)	CBPI
CN	S1	39	F	60.0 ± 3.0	1.7 ± 0.03
	S2	41	M	70.5 ± 2.5	1.6 ± 0.03
	S3	46	M	61.4 ± 2.4	1.6 ± 0.02
	S4	50	F	64.2 ± 3.6	1.8 ± 0.04
	Mean	44		64.0 ± 2.3	1.7 ± 0.04
OFI 0.01 μg/mL	S1			70.0 ± 4.4	1.6 ± 0.01
	S2			73.9 ± 2.9	1.7 ± 0.03
	S3			60.6 ± 2.6	1.8 ± 0.03
	S4			68.4 ± 4.9	1.8 ± 0.03
	Mean			68.2 ± 2.8	1.7 ± 0.04
OFI 0.05 μg/mL	S1			64.9 ± 3.6	1.7 ± 0.01
	S2			71.7 ± 1.3	1.6 ± 0.03
	S3			65.6 ± 2.9	1.7 ± 0.03
	S4			63.9 ± 5.5	1.7 ± 0.05
	Mean			66.5 ± 1.8	1.7 ± 0.02

Data are expressed as mean of three experiments and mean of the four subjects ± standard error of the mean
CBPI = cytokinesis block proliferation index

2.6.2 Cytokinesis block proliferation index (CBPI)

Cytotoxicity was evaluated 72 h after PHA stimulation, by classifying 500 cells for each donor and for each experimental point, according to the number of nuclei. The cytokinesis block proliferation index (CBPI) was applied, using the following formula: CBPI = [MI + 2MII + 3 (MIII + MIV)]/N, where MI, MII, MIII and MIV represent the number of cells with one, two, three and four nuclei, while N is the total number of cells [18].

2.7 Statistical analysis

All data were obtained through three independent experiments for each subject and expressed as mean ± standard error of mean (SEM). In Table 2 and in figures (see Sect. 3), data are presented as the average of the means of the four subjects ± SEM. Normal distribution of data was tested using Shapiro–Wilk test. Since data followed the normal distribution, unpaired t test was applied to compare treated and exposed cells to control ones; the level of significance was set at $p < 0.05$. Statistical analysis of data was performed using GraphPad Prism software version 9.0 (GraphPad Software, Boston, MA, USA).

3 Results

3.1 OFI extract does not alter viability and has no cytotoxic effect in human PBLs

Cell viability and cytotoxicity were evaluated in PBLs after treatment with the two concentrations of OFI extract selected for combined treatments with ionizing radiation (0.01 and 0.05 μg/mL, 3 h), performed on non-stimulated blood samples. Analyses were carried out 72 h after stimulation with phytohemagglutinin. Viability data were expressed as percentage of viable cells, using trypan blue staining. As reported in Table 1 and in Fig. 2a, cell viability was not affected in treated cells compared to untreated controls. Higher concentrations of the extract (0.1–10 μg/mL) and longer treatment time (24 h) were tested in preliminary experiments and none of these conditions altered PBL viability (data not shown). Cytotoxicity was also determined using the cytokinesis block proliferation index (CBPI). CBPI values remained unchanged in treated samples when compared to the controls (Table 1, Fig. 2b).

3.2 OFI extract does not induce chromosome damage in human PBLs

To analyze the effect of OFI extract on chromosome damage induction in PBLs, micronucleus test (CBMN) was performed. Non-stimulated blood samples were treated with OFI (0.01 and 0.05 μg/mL, 3 h). Either treatment did not induce micronuclei formation, measured 72 h after stimulation with PHA and expressed as frequency of binucleated cells with micronuclei (BNMN) on total binucleated cells (BN) and as total micronuclei per cell (MN/BN). As reported in Table 2 and in Fig. 3, BNMN/BN, MN/BN values and MN distribution did not undergo any statistically significant change in OFI-treated PBLs when compared to untreated controls ($p > 0.05$, t test). Higher concentrations of the extract (0.1–5 μg/mL) and longer treatment time (24 h) were tested in preliminary experiments and none of these was genotoxic for PBLs (data not shown).

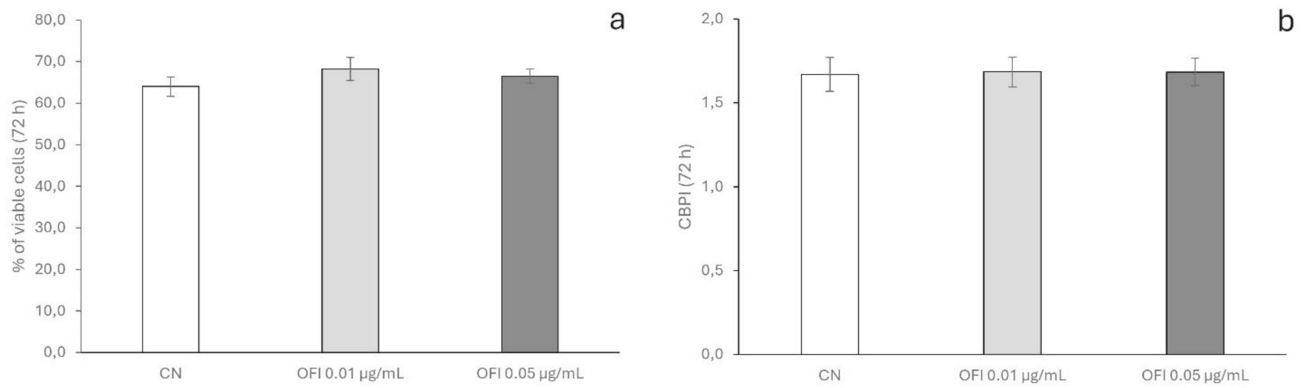

Fig. 2 Percentage of viable peripheral blood lymphocytes (**a**) and cytokinesis block proliferation index (CBPI) (**b**) following treatment with two concentrations of OFI mucilage extract, compared to untreated controls (CN). Data are presented as mean of the four subjects ± SEM

Table 2 Chromosome damage in PBLs treated with OFI extract for 3 h, ionizing radiation (0.5 and 1 Gy) and OFI extract before irradiation

Exp. Pt	BNMN/BN	MN/BN	1MN/BN	2MN/BN	3–4MN/BN
CN	0.033 ± 0.004	0.035 ± 0.004	0.031 ± 0.004	0.002 ± 0.001	0.000
OFI 0.01 µg/mL	0,028 ± 0.002	0,031 ± 0.003	0,026 ± 0.001	0,002 ± 0.001	0.000
OFI 0.05 µg/mL	0,027 ± 0.003	0,029 ± 0.002	0,024 ± 0.003	0,003 ± 0.001	0.000
0.5 Gy	0.049 ± 0.005[a]	0.055 ± 0.006[a]	0.045 ± 0.004	0.004 ± 0.002	0.000
1 Gy	0.079 ± 0.011[b]	0.090 ± 0.013[b]	0.069 ± 0.011[a]	0.009 ± 0.003[a]	0.001 ± 0.0006
0.5 Gy + OFI 0.01 µg/mL	0,041 ± 0.006	0,045 ± 0.009	0,037 ± 0.004	0,003 ± 0.001	0000
0.5 Gy + OFI 0.05 µg/mL	0,041 ± 0.008	0,044 ± 0.008	0,039 ± 0.007	0,002 ± 0.001	0000
1 Gy + OFI 0.01 µg/mL	0,048 ± 0.004[c]	0,053 ± 0.005[c]	0,043 ± 0.003	0,005 ± 0.002	0000
1 Gy + OFI 0.05 µg/mL	0,046 ± 0.005[c]	0,048 ± 0.006[c]	0,044 ± 0.004	0,002 ± 0.001	0000

Data are expressed as average of the means of the four subjects ± standard error of the mean
BNMN, binucleated cells with micronuclei; MN, micronuclei; BN binucleated cells; 1MN, binucleated cells with 1 MN; 2MN, binucleated cells with 2 MN; 3–4MN, binucleated cells with 3 or 4 MN
[a] $p < 0.05$, [b] $p < 0.01$, compared to the control; [c] $p < 0.05$ compared to 1 Gy (t test)

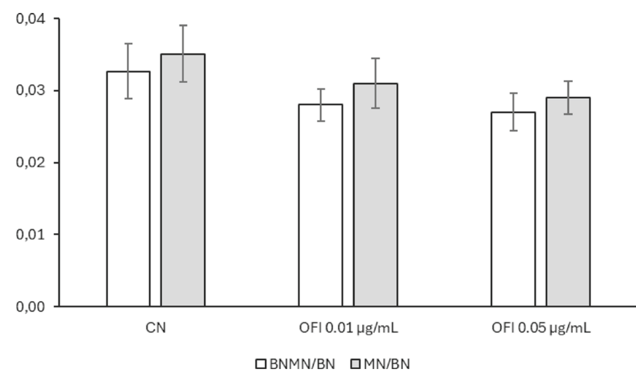

Fig. 3 Frequencies of micronuclei in peripheral blood lymphocytes treated with OFI extract at concentrations of 0.01 and 0.05 µg/mL, compared to untreated control samples (CN). Data are presented as mean of the four subjects ± SEM. BNMN/BN = binucleated cells with micronuclei/total binucleated cells); MN/BN = total micronuclei per cell

3.3 OFI extract radioprotective effect in cells exposed to gamma rays

To evaluate the potential radioprotective effect exerted by OFI extract in human PBLs exposed to gamma rays, cells were given the following administration schedule: pretreatment with OFI extract for 3 h, followed by exposure to 0.5 Gy and 1 Gy gamma total absorbed dose. Cell viability, cytotoxicity and chromosome damage were determined 72 h after stimulation with PHA (Fig. 1). Cells were also screened in response to either OFI or gamma rays, given as single agents.

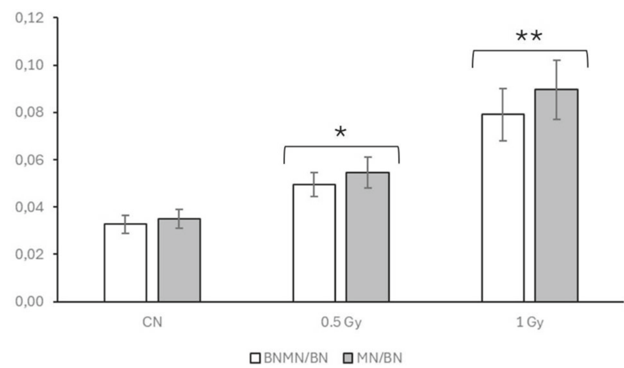

Fig. 4 Micronuclei frequency in peripheral blood lymphocytes exposed to gamma radiation at doses of 0.5 Gy and 1 Gy, compared to untreated controls (CN). Data are presented as mean of the four subjects ± SEM. BNMN/BN = binucleated cells with micronuclei/total binucleated cells); MN/BN = total micronuclei per cell. Statistical significance refers to the respective control. *$p < 0.05$; **$p < 0.01$ (t test)

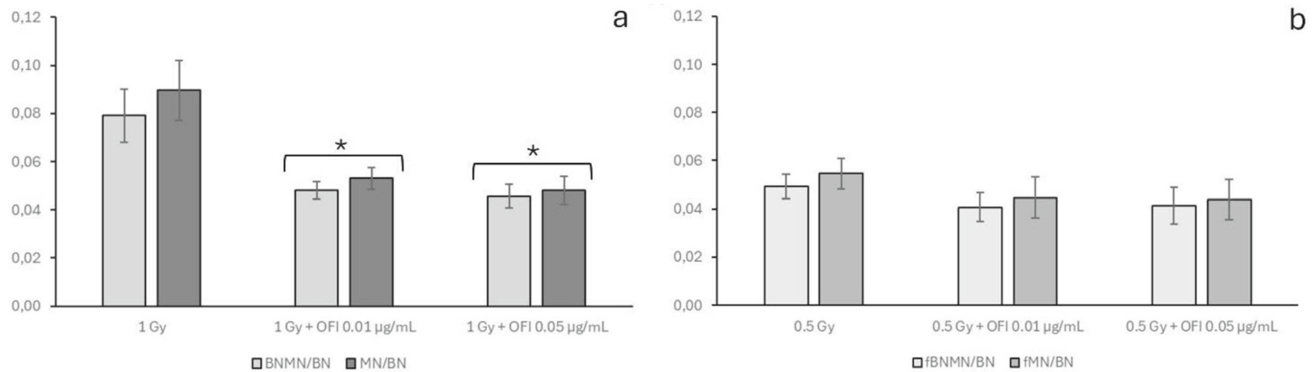

Fig. 5 Micronuclei frequency in peripheral blood lymphocytes pretreated with OFI extract at 0.01 and 0.05 µg/mL, followed by exposure to gamma radiation at doses of 1 Gy (**a**) and 0.5 Gy (**b**), compared to samples exposed to gamma radiation alone. Data are expressed as mean of the four subjects ± SEM. BNMN/BN = binucleated cells with micronuclei/total binucleated cells; MN/BN = total micronuclei per cell. Statistical significance refers to the respective control. *$p < 0.05$ (t test)

In samples treated with OFI in combination with gamma radiation, cell viability was not significantly altered, compared to samples treated only with IR (data not shown).

CBPI values decreased, albeit not significantly, in response to gamma rays, evidencing radiation-induced cytotoxicity. Pretreatment with OFI extract combined with gamma-ray exposure showed no significant effect on CBPI, compared to treatment with gamma radiation alone (data not shown).

OFI per se did not induce chromosome damage (see Sect. 3.2). Conversely, gamma-ray treatment significantly increased MN induction (Table 2, Fig. 4), as expected. Exposure to 1 Gy dose also significantly increased the frequency of cells with 1 and 2 MN (Table 2). Conversely, in response to 1 Gy exposure, the frequency of micronuclei was significantly reduced in OFI-pretreated samples, at both tested OFI concentrations, compared to gamma-ray treatment alone. (Fig. 5a). After exposure to the lower dose of gamma radiation (0.5 Gy), BNMN/BN and MN/BN frequencies decreased, although not significantly, in samples pretreated with OFI (Fig. 5b). In addition, OFI treatment was effective in reducing the frequencies of cells with 2 and multiple MN produced by both radiation doses (Table 2). This effect on MN distribution was more evident at 1 Gy dose, albeit not significant.

Preliminary experiments with 24-h pretreatment yielded results similar to those shown in Fig. 5; the 3-h treatment was chosen to meet experimental requirements, considering the restricted access time to the irradiation facility. On the other hand, higher concentrations of OFI mucilage, initially tested in combination with gamma rays, showed no mitigation of radiation-induced chromosome damage.

4 Discussion

In this study, we investigated the effects of mucilage extracted from *Opuntia ficus-indica* cladodes on human peripheral blood lymphocytes. Treatments with OFI extract were performed on unstimulated PBL cultures to mimic physiological condition, in which lymphocytes are in a quiescent state (G0 phase of the cell cycle). In combined treatments with gamma-ray exposure, PBLs were stimulated with PHA after irradiation, followed by a recovery time to allow DNA repair. The effects of radiation damage and the potential protective activities were evaluated in dividing cells, 72 h after PHA stimulation (see Fig. 1).

Our results demonstrated that the OFI extract, at the concentrations tested, exerts neither cytotoxic nor genotoxic effects in PBLs. Furthermore, we provided evidence that OFI mucilage exhibits an antigenotoxic effect in PBLs exposed to gamma radiation.

Pretreatment with OFI extract resulted in a significant reduction of radiation-induced chromosomal damage following exposure to a total absorbed dose of 1 Gy, although a slighter protective effect was observed in samples exposed to 0.5 Gy. The radioprotective activity of the extract, more evident at the higher radiation dose, might be attributable to a threshold effect: at lower radiation doses, such as 0.5 Gy, radiation damage could be not relevant enough to efficiently activate the cellular pathways involved in the radioprotective mechanism of action of OFI, which is significantly triggered at 1 Gy. Inter-individual variability may also contribute to the observed dose-dependent effect, masking the overall extent of radioprotective efficacy, which is less evident at low doses. In this regard, the dose-dependent effect would be attenuated by extending the study to a larger number of healthy subjects. In the present study, OFI extract also showed efficacy in reducing the number of cells with 2 and multiple MN induced by gamma rays. This protective effect was not significant at the radiation doses tested, but it could be evidenced by exposing PBLs to doses higher than 1 Gy.

Previous studies have reported the antigenotoxic activity of OFI extracts in both in vitro and in vivo models [10, 19–21]. In contrast, extracts from prickly pear peels, obtained using different solvents, in some cases have shown cytotoxic effects on human lymphocytes, suggesting that biological activity may depend on extract composition and concentration [20].

The nutritional composition, bioactive constituents and biological activities of OFI and other *Opuntia* species have been recently reviewed, highlighting their anti-inflammatory, antiulcerative, neuroprotective, antimicrobial and antiviral properties and encouraging further investigation of their therapeutic potential [22, 23].

To date, the potential protective effect of OFI cladode extract against radiation-induced genotoxicity in normal human cells has not been explored, despite previous evidence supporting its ability to protect plasmid DNA from gamma irradiation [13].

Here, we demonstrated that very low concentrations of OFI cladode mucilage (0.01 and 0.05 μg/ml) effectively reduce the formation of micronuclei in irradiated PBLs. Higher concentrations of the extract, preliminarily tested, did not show the same mitigating effect. This phenomenon was already observed in PBLs treated with different bioactive natural compounds, such as resveratrol, more effective in reducing DNA damage at the lowest dose tested [24], or genistein, acting as a potential radiomitigator only at a very low concentration [25].

The OFI extract, previously characterized by our group [12], is rich in polysaccharides, polyphenols and various metabolites including monosaccharides, disaccharides and organic acids. Its antioxidant activity is primarily attributed to abundant compounds such as citric acid, along with phenolic acids like piscidic and eucomic acid, and minor bioactive compounds such as quinic acid (a polyphenol) and rutin (a flavonoid). These components likely contribute synergistically to the inhibition of oxidative stress and the mitigation of radiation-induced genotoxic damage in PBLs.

Plant polysaccharides such as *Aloe* acetylated mannan or *Astragalus* and *Angelica* polysaccharides have been shown to exhibit protective effect against radiation. Although the exact mechanism of their radioprotective activity is not clear, it is thought to involve free radical scavenging, immunomodulation, enhancement of DNA repair and inhibition of apoptosis [7, 26]. Polyphenols (both flavonoid and non-flavonoid) such as apigenin, genistein, quercetin, resveratrol and curcumin, are also known to have biological properties underlying radiation protection mechanisms [7]. In particular, two phenolic compounds present in our extract, quinic acid and rutin, have been studied for their potential as radioprotectors [8, 27]. Quinic acid, along with chlorogenic acid, proved effective in protecting human blood lymphocytes against X-ray induced DNA damage [28]; moreover, methods using analogs of quinic acid for the treatment of radiation-exposed humans and animals, are currently subject of a patent [29]. Similarly, rutin, in combination with other phenolic compounds, has been shown to mitigate radiation-induced chromosome damage in human PBLs [30], whereas in vivo studies have demonstrated its ability to reduce DNA damage and to enhance DNA repair after whole body exposure to IR. Interestingly, in silico analyses identified a functional hydroxyl group in the rutin that may be involved in free radical scavenging [31].

Additionally, the anti-inflammatory potential of OFI cladode extract has been demonstrated through its ability to suppress the inflammatory response in lipopolysaccharide (LPS)-stimulated hepatocarcinoma cells. In silico analyses have identified a possible mechanism, involving direct molecular interactions between the extract's monosaccharides and the TLR4 receptor, thereby interfering with the LPS-triggered inflammatory signaling pathway [12]. Such interactions may also suggest a possible role of OFI extract in the attenuation of radiation-induced inflammatory responses, further supporting its radioprotective efficacy.

Taken together, these preliminary findings highlight the promising potential of OFI cladode mucilage as a natural radioprotector. In this perspective, further studies will be conducted to investigate the possible mechanisms of action of the OFI extract. Free radical scavenging assays are planned to determine OFI antioxidant activities and the kinetics of γ-H2AX expression will be evaluated to assess its effects on the induction and repair of radiation-induced DNA double-strand breaks. The levels of apoptosis and necrosis will also be measured to study the involvement of OFI treatments in cell death mechanisms associated with ionizing radiation.

Moreover, the current study presents limited statistical power, due to the small number of samples and inter-individual variability. Further investigations, extended to a greater number of subjects and to a wider range of radiation doses, are warranted to confirm the efficacy of OFI pretreatment in reducing chromosome damage induced by IR and to clarify its potential applications in clinical, occupational and environmental contexts.

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Data availability Data will be made available on reasonable request. The manuscript has associated data in a data repository.

Declarations

Competing interests The authors declare no conflicts of interest.

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