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Low-Dose Gamma Radiation Fields Decrease Cell Viability, Damage DNA, and Increase the Expression of Hsp70 and p53 Proteins in Human Leukocytes

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Abstract

Ionizing radiations are tools in diagnosis and treatment of diseases. Leukopenia from exposure to ionizing radiation has been reported. Due to their radiosensitivity, leukocytes are a biological model to analyze cell damage. Therefore, cell viability, DNA damage, and Hsp70 and p53 expression in human leukocytes exposed to low-dose gamma radiation fields from a ^{137}Cs source were evaluated. A decrease in cell viability, DNA damage and an increase in the expression of Hsp70 and p53 proportional to the radiation dose received was found, which was 0.2, 0.4, 0.6, 0.8 and 1.0 mGy.

Keywords

Leukocytes, Gamma Radiation, Cell Damage, DNA, Hsp70, p53

1. Introduction

Human beings are exposed to ionizing radiation (IR) through clinical therapeutic procedures or radiological accidents. Long-term effects from radiation exposure include health problems ranging from fibrosis to cancer [1].

Gamma rays are a type of electromagnetic radiation [2], which consists of high-energy photons, which cause diffuse damage at the cellular level throughout the body [3]. It has been observed that low levels of RI cause a stochastic health risk, the effect of which is proportional to the dose, and therefore has no

threshold. This means that this effect is likely to occur at very low doses with the likelihood of genetic damage and induction of cancer. High doses produce deterministic effects, acute tissue damage of which is certain to occur when there is a particularly large dose in a short period of time [4] [5] [6] [7].

The consequences of IR on organisms are classified as physical, chemical and biological effects [8]. After the physical effects, chemical effects are triggered in which damaged atoms and molecules interact with each other, increasing the damage to biological tissue. The damages produced to molecules and biological structures try to be repaired, and in the case of not being able to do it in time, or if the repair is defective, they will lead to cell death or the appearance of biological modifications such as genetic mutations [9].

After exposure of a cell to radiation, there is a latency period before any observable response, this could be decades for low doses of ionizing radiation (LDIR), but only minutes or hours for high doses [4] [10].

Cells that divide more are highly radiosensitive compared to those that divide less. This is because, during mitosis, the chromosomes are condensed and paired, there is more DNA present in one area at this point, so it is theorized that this is the most radiosensitive time. This has been observed in cancer cells whose mitotic rates are high, making them more radiosensitive than normal cells [11].

Leukocytes, due to their high division rate, are one of the most sensitive cells that show a high response to LDIR. They originate in the bone marrow, circulate through the bloodstream, and help fight pathogenic infections as well as tumor cells [12] [13].

People are often exposed to LDIR, for example, natural background radiation and artificial sources, especially those used in the medical diagnostic process, which can be repeated frequently [14].

The interaction of IR in the cell can be divided into two ways: direct or indirect interaction [15] [16]. In direct, the macromolecules of the cell (protein or DNA) are hit by IR, which kills the cell or mutates the DNA [17]. One of the main causes of cell death is the break of the double strand of DNA (DSB, Double strand DNA breaks). If not repaired or incorrectly repaired, DSBs can cause chromosomal aberrations, which can lead to human diseases, including cancer [18]. Because of this, identifying when DSBs appear in human leukocytes *in vitro* could be used as a biological indicator to estimate whether a person has been exposed to RI [19].

Indirectly, it occurs when the radiation energy interacts with water instead of macromolecules [20]. Given that in most living organisms 80% of their weight is made up of water, the probability of an event taking place on this molecule is very high. If after the interaction with radiation the water molecule is excited, it can happen that it dissociates generating free radicals (hydroxyls and protons) and consequently compounds such as highly oxidizing hydrogen peroxide can be formed [21] [22] [23]. When cell damage after irradiation is not corrected,

the cell often leads to cell death called apoptosis, which is a series of events that leads to nuclear and cell fragmentation [24].

When a cell is subjected to stress, either by physical or chemical agents, the expression of a family of proteins known as heat shock proteins (Hsp) has been observed. Heat shock and other forms of stress such as inflammation, infections, environmental pollutants, ultraviolet radiation, low-frequency radiation, and low-intensity gamma radiation fields, among other factors, can induce Hsp expression in all cells and tissues [25]. These proteins are found in cells and show a high degree of evolutionary conservation. This means that Hsp are necessary for successful survival in harsh environmental conditions [26]. In 1962, Ritossa discovered this group of proteins, which are expressed in all cells and organisms, from prokaryotes to humans [27].

Most Hsp function as molecular chaperones, having the ability to bind other proteins and mediate their folding, transport, and interactions with other proteins. They also participate in cell physiology such as in the transport of transmembrane proteins and in enabling the assembly and folding of polypeptides [28].

Hsp are overexpressed in a wide range of malignancies, contributing to tumor growth, differentiation, invasion, and metastasis. Its overexpression in tumor cells has also been shown to play an important role in tumorigenesis by inhibiting apoptosis and senescence [29]. Therefore, the study of these proteins is important when cells are exposed to IR.

Within the Hsp is the Hsp70 protein, it is expressed at low levels in healthy cells and without stress. Under different stresses, its expression increases [30]. The Hsp70 in the cell, help to refold the denatured proteins. Importantly, Hsp70 is frequently upregulated in disease states, including cancer [31]. Hsp70 interferes at several points in apoptotic signaling, including cytochrome C release, caspase activation, accumulation of misfolded proteins, generation of reactive oxygen species, and DNA fragmentation. Furthermore, Hsp70 inhibition increases the sensitivity of cells to apoptosis. Thus, Hsp70 directly or indirectly modulates the intrinsic and extrinsic apoptotic pathways [32]. Since Hsp70 is expressed in response to a wide variety of physiological and environmental stressors, its expression can be used as a bioindicator of cell damage.

It has also been observed that during stressful situations due to physical or chemical agents or diseases, a protein encoded by the p53 tumor suppressor gene is overexpre

ssed; it is a phosphoprotein that is located in the cell nucleus [33]. The participation of p53 in the intrinsic pathway of apoptosis, which is induced by radiation or agents that damage DNA, has been described. The p53 protein is normally present in very small amounts, but when cells are exposed to genotoxic stimuli, p53 levels rise rapidly and initiate a cell death program mediated by regulation of bax and fas transcription [34]. After irradiation, activation of p53 promotes cell survival through growth arrest and DNA damage repair.

However, depending on the DNA damage and the cell type, p53 can also acti-

vate pathways for the removal of damaged cells by apoptosis or senescence [35]. In the event of non-repairable DNA damage, p53 induces the action of pro-apoptotic proteins such as BAX or PUMA that inhibit anti-apoptotic proteins, activating caspase 9 that triggers apoptosis. Not all cell types are prone to apoptosis, as the balance between pro- and anti-apoptotic proteins may not be disrupted by p53 activation [36]. The diversity of the response to radiation in vivo is at least partially attributable to the status of p53. In certain cells, such as blood cells, p53 activation by radiation triggers the intrinsic pathway of apoptosis [37], so the expression of this protein can also be used as a bioindicator of cell damage.

The effect of IR on human cells, especially on leukocytes, has aroused great interest, since a decrease in hematological parameters has been observed in various clinical studies, mainly on occupationally exposed personnel [38] [39]. It is important to investigate the effect that IR has on leukocyte cells, since we know that direct or indirect energy deposits cause damage to vital macromolecules for cell survival. Thus, it is remarkable to analyze cell viability and damage to macromolecules, as well as the expression of proteins involved in maintaining cell homeostasis in human leukocytes when exposed to low-dose gamma radiation fields. For this reason, the objective of this work was to evaluate cell viability, DNA damage, and changes in the expression of Hsp70 and p53 proteins in human leukocytes exposed to low-dose gamma radiation fields.

2. Materials and Methods

Study model and sample collection: 3 healthy human donors aged 20 - 24 years were selected, with no history of radiotherapy, or consumption of alcohol, tobacco and drugs, previously evaluated with a general health study, an informed consent was given to each donor about this study. 20 mL of peripheral venous blood (antebrachial venipuncture) was obtained with heparin.

Obtaining leukocytes: Human leukocytes were obtained from peripheral blood samples [13] by the Ficoll Histopaque® technique (SIGMA-ALDRICH® 10771 protocol). Later, RPMI® culture medium was added to the cell package. After this, 6 aliquots of 500 µL of leukocytes were made in RPMI medium in eppendorf tubes to expose them to the radioactive source of 137Cs emitting gamma radiation (**Figure 1**).

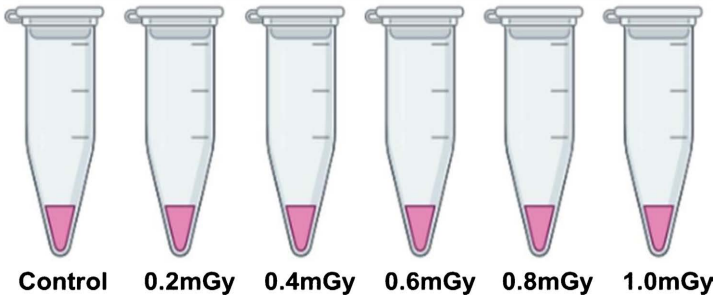

Figure 1. Experimental setup of leukocytes for further analysis.

Exposure dose calculation: Using a gamma radiation emitting ^{137}Cs source, the dose and irradiation time (t_i) necessary to expose the samples at each exposure dose ($D_{applied}$) was calculated using the Following Equation (1):

$$t_i = D_{applied} \Big/ D_o e^{-\lambda td} \quad (1)$$

In this equation D_o is the dose rate that the source had when it was manufactured in mGy/sec, λ is the decay constant of ^{137}Cs (8.43321/day) and td is the time elapsed since the source was manufactured until the day the samples were irradiated.

Exposure of leukocytes to gamma rays: The samples were exposed with a ^{137}Cs source that emits gamma rays of 0.662 MeV, packed in an Eppendorf-type polypropylene tube and in a lead container used as shielding. The radioactive source was placed in the central part of the experimental arrangement, around it, and the experimental samples were placed at a distance of 5 cm. Everything was deposited on a cooler that contained hot water bags to maintain the temperature at 37˚C (Figure 2). After irradiation, the leukocytes were placed in an incubator for 40 minutes. Subsequently, viability, DNA integrity, and Hsp70 and p53 expression were evaluated.

During exposure to the ^{137}Cs source, the absorbed doses of the samples were calculated as shown in Table 1, the exposure process was carried out under the regulatory measures of radiological protection for sealed sources.

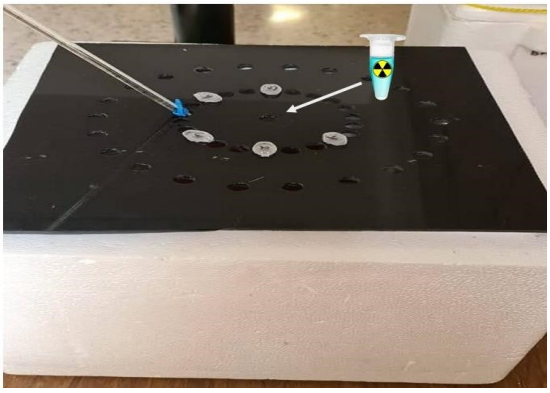

Figure 2. Experimental setup used for irradiation of leukocytes with gamma radiation from a ^{137}Cs source.

Table 1. Actual absorbed dose in each of the samples as a function of exposure time.

Sample	Irradiation time (sec)	Dose (mGy)
1	0	0.00 ± 0.00
2	55	0.24 ± 0.01
3	95	0.40 ± 0.02
4	138	0.60 ± 0.03
5	186	0.80 ± 0.04
6	235	1.02 ± 0.05

Determination of the Absorbed Dose. The absorbed dose was calculated using Harshaw type 100 thermoluminescent dosimeters (Lithium fluoride, Li natural), 3.2 × 3.2 × 0.89 mm tape type. TLD 100 is widely used for X-ray and gamma ray dosimetry from 10 pGy to 10 Gy. Before use, the TLDs were heated at 400˚C for 1 hour to erase them. During their exposure, groups of 4 TLDs were placed in eppendorf tubes, the TLD-100 groups were exposed at times of 0, 55, 95, 135, 186 and 235 seconds, at a distance of 10 cm between the ^{137}Cs source and the samples. A group of TLDs-100 was used to measure the background radiation. This same procedure was repeated where the dose was measured with a RaySafe ThinX RAD model solid state monitor that is activated when samples are exposed to the ^{137}Cs source. The same number of times used to expose the TLDs was used with the RaySafe equipment.

Calculation of the Absorbed Dose. During exposure to gamma rays, TLDs were used to evaluate the absorbed dose, whose response was measured with the Harshaw 3500 reader, heating them in a nitrogen atmosphere from 50˚C to 300˚C with a gradient of 10˚C/second. The responses from each set of exposed TLDs were averaged and corrected for the average response of the TLDs used to measure background. These values were correlated with the RaySafe monitor readings and a correlation (r^2 = 0.9987) was obtained between the response of the TLDs in nanoCoulombs and the number of times the cells were exposed to the ^{137}Cs source. The absorbed dose was based on the times shown in **Table 1**.

Evaluation of cell viability: After exposure of leukocytes to gamma rays, cell viability was evaluated. A 10 µL suspension of leukocytes was mixed with 10 µL of 0.4% trypan blue solution and observed under the microscope using a Neubauer chamber. Subsequently, a separate count of blue (dead) leukocytes and birefringent or white (alive) leukocytes that were observed in each of the quadrants was performed. Making use of the calculations proposed in the SOPs protocol (2013) [40], we continued to calculate the percentage of living cells. This was done for the control and the different samples exposed to gamma rays.

Evaluation of DNA damage (comet assay): Albuminized slides were used, which allowed effective adherence between the slide and the first layer of agarose, based on the protocol of Singh *et al.*, (1998) [41] for alkaline medium.

20 µl of leukocytes were taken and placed in an eppendorf tube containing 60 µl of 0.5% low melting point (LMP) agarose. Next, this mixture was plated on slides with 0.8% normal melting point (NMP) agarose, to then be covered with a third layer of LMP agarose.

The slides were kept at 4˚C for 24 hours immersed in a lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% sodium sarcosinate and 100 mM Tris at pH = 10), in order to provoke the rupture of the membranes.

The slides were placed in a horizontal electrophoresis chamber with alkaline buffer (10 N NaOH and 200 mM EDTA, pH = 10) for 30 min to allow decompaction of the DNA and exposure of damaged weak alkali sites. Subsequently, electrophoresis was performed for 30 minutes at 25 V and 300 mA. Once the

electrophoresis was finished, the slides were washed with a neutralization buffer solution (0.4 M Tris, pH = 7.5) to eliminate excess alkali and remove detergents. For staining, 50 µl of ethidium bromide prepared at 10% with bidistilled water were used.

Finally, they were examined under a fluorescence microscope (Carl Zeiss Axiover 25 Inverted) with a magnification of 200×, using a 590 nm filter.

Visual analysis: 100 random cells were observed, quantifying 100 comets in the gel. Each comet was classified according to the category or degree of corresponding DNA damage between 0 and 4. (**Figure 3**) The magnitude of DNA damage was expressed in arbitrary units (AU), with possible values in a range of 0 - 400, according to the following Equation (2):

$$UA = 0 \times TCG0 + 1 \times TCG1 + 2 \times TCG2 + 3 \times TCG3 + 4 \times TCG4 \quad (2)$$

TCG0 = Total grade 0 cells (undamaged cells). TCG1 = Total number of grade 1 cells (minimum frequency of DNA lesions). TCG2 = Total cells grade 2 (moderately low damage from DNA lesions). TCG3 = Total cells grade 3 (moderately HIGH damage from DNA lesions). TCG4 = Total cells grade 4 (totally damaged cells, in apoptosis) [42].

Identification of Hsp70 and p53 proteins

Cell lysis: To release the proteins from the leukocyte cells, 500 µL of lysis buffer (PBS 150 mM NaCl, 2 mM KCl, KH_2PO_4 and Na_2HPO_4) with protease inhibitor (Protease Inhibitor Cocktail, 11697498001, Roche Diagnostics) were added to the tubes to then centrifuge them at 14,000 rpm for 10 minutes, and collect the supernatant to recover the total soluble protein.

Dot Blot: On a gridded nitrocellulose membrane, 20 µL of the supernatant containing the total soluble protein were deposited. Subsequently, the membrane was dried at room temperature and blocked with phosphate saline solution (PBS-milk 3%) for 24 h at 4˚C.

After the non-specific sites on the nitrocellulose membranes were blocked, the primary antibody Hsp70 (SC-24 Santa Cruz Biotechnology®, USA.) or p53 (SC-126 Santa Cruz Biotechnology®, USA.) was added (Dilution 1:1000) for one hour at room temperature and shaking (25 rpm), followed by 5 washings with

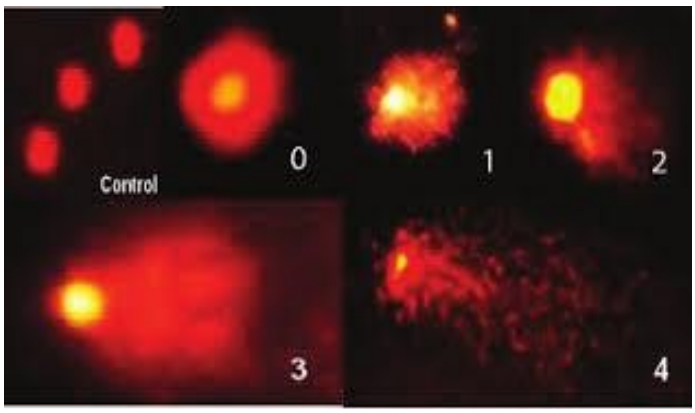

Figure 3. Qualitative morphological classification of comet length states [43].

PBS solution and PBS-Tween alternately (5 min and with constant shaking at 70 rpm), then the peroxidized secondary antibody was added (anti-mouse IgG) for one hour and with agitation (25 rpm), finally 6 washes with PBS and PBS-Tween were done alternately. Afterwards, the membranes were immersed in an ECL solution (GERPN2232 - ECL™ Prime Western Blotting System) to reveal the proteins, finally they were placed in the Bio-Rad © brand photodocumenter (Image Lab Bio-Rad ® Laboratories), to capture and analyze images, not exceeding 30 min of revealed.

Statistical Analysis: All experiments were carried out in triplicate. Statistical analyzes were performed using Microsoft Excel® and GraphPad Prism 8 programs. Cell viability of each sample was presented as a percentage. DNA damage of each cell was quantified by calculating arbitrary units, and finally the internal volume of protein expression was analyzed using Image Lab software version 2.0.1 build 18 (Copyright © 2009 Bio-Rad® Laboratories). Differences between experimental groups were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test with a 95% confidence level. Graphs comparing differences between experimental groups were plotted using GraphPad Prism 8 and expressed as mean ± standard deviation (SD). A value of $p \leq 0.05$ was considered statistically significant.

3. Results

Cell viability assessment

In leukocytes exposed to different doses of gamma radiation (0.2, 0.4, 0.6, 0.8, 1.0 mGy), a decrease in the average percentage of live cells was observed, which went from 98.7% ± 1.3% to 50.3% ± 10.7% with respect to the control ($p \leq 0.05$). In general, it was observed that as the radiation dose increases, the percentage of living cells decreases, Figure 4.

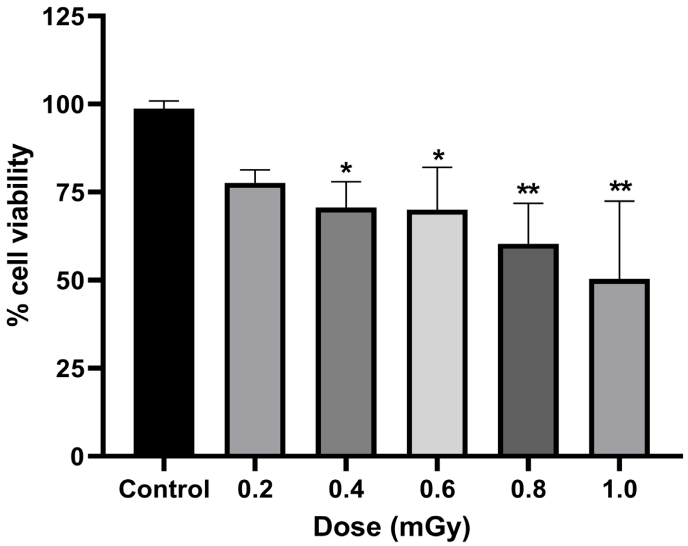

Figure 4. Mean values of the viability percentages (±SD) of human leukocytes exposed to different doses of gamma radiation. (Control, 0.2, 0.4, 0.6, 0.8 and 1.0 mGy) ($p \leq 0.05$).

DNA Damage (Comet Assay)

The comet assay showed that the doses of gamma radiation used induced DNA damage in leukocytes. The negative control presented only class 0 and 1 comets, while the exposure group showed class 0 - 4 comets, Figure 5 ($p \leq 0.05$), increasing the degree of damage as the exposure dose increases.

The results indicate that gamma radiation produced DNA damage from the first dose (AU 0.1 mGy = 105.66 ± 2.6). In the negative control, there was a mean arbitrary unit value of AU 0 mGy = 5 ± 0.5, and it was observed that as the radiation dose increases, the number of arbitrary units also increases ($p \leq 0.05$) (Figure 6).

Identification of Hsp70 and p53 proteins

When leukocytes were gamma-irradiated, basal expression of Hsp70 and p53 was observed in the control, followed by increased expression in the irradiated

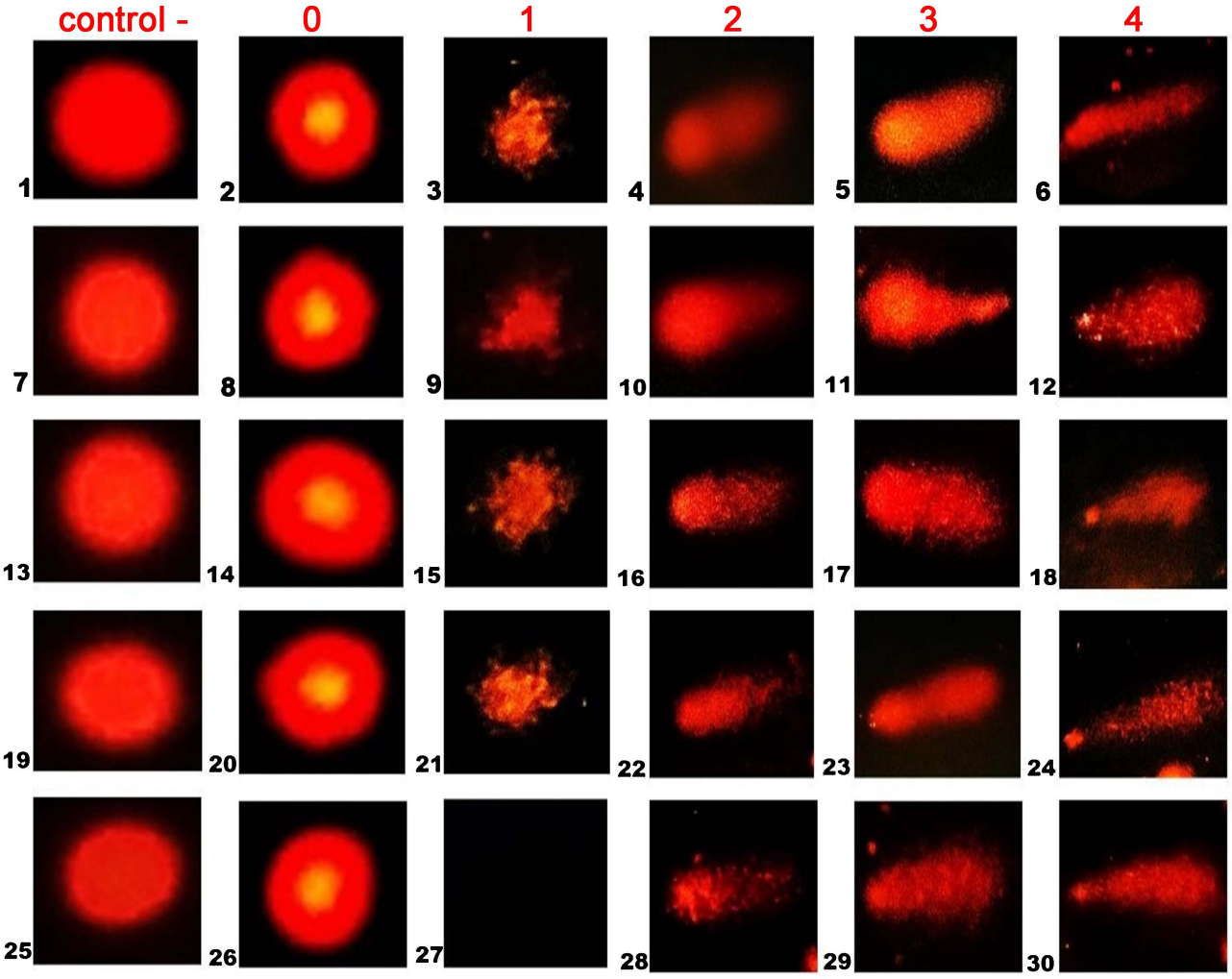

Figure 5. Comet assay of human leukocytes exposed to different doses of gamma radiation ($p \leq 0.05$). 1 - 6 cells exposed to 0.2 mGy gamma radiation, damage level 0. 7 - 12. Cells exposed to 0.4 mGy gamma radiation, damage level 0, 1, 2, 3, 4 from left to right. Image 13 - 18. Cells exposed to 0.6 mGy gamma radiation, damage level 0, 1, 2, 3, 4 from left to right. Image 19 - 24. Cells exposed to 0.8 mGy gamma radiation, damage level 0, 1, 2, 3, 4 from left to right. Image 25 - 30. Cells exposed to 1.0 mGy gamma radiation, damage level 1, 2, 3, 4 from left to right.

cells. The overexpression of the Hsp70 protein can be observed from the first dose (0.2 mGy). Regarding p53, an overexpression begins up to a dose of 0.4 mGy, both proteins increase as the exposure dose increases ($p \leq 0.05$), Figure 7.

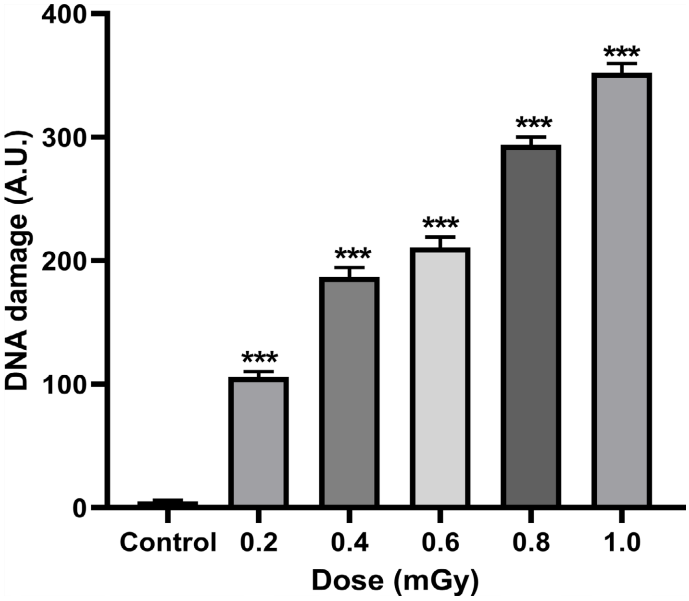

Figure 6. DNA damage shown in arbitrary units of human leukocytes exposed to different doses of gamma radiation (Zero (control), 0.2, 0.4, 0.6, 0.8 and 1.0 mGy) ($p \leq 0.05$).

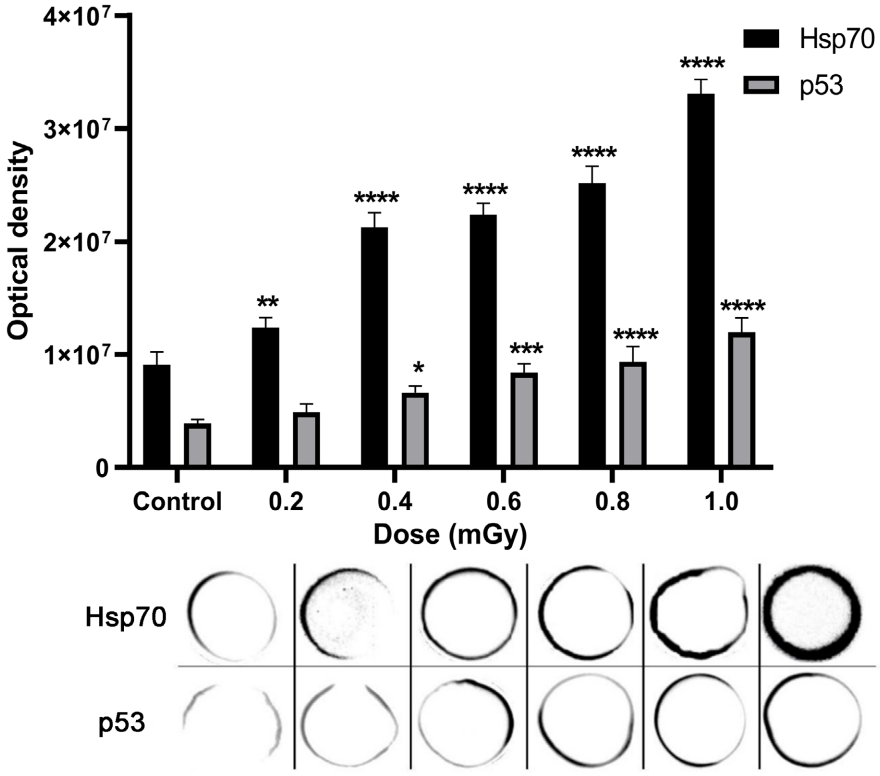

Figure 7. Expression of the Hsp70 and p53 proteins of human leukocytes exposed to different doses of gamma radiation (Zero (C), 0.2, 0.4, 0.6, 0.8 and 1.0 mGy) ($p \leq 0.05$) and Dot-blot technique.

4. Discussion

Various studies have contributed to demonstrating the effects of IR on cells of the human body. It is of great interest to study these effects, especially in leukocytes, which are the most affected when humans are exposed to RI. This was verified by Davudian Talab *et al.* in 2018 [12] where he observed a decrease in hematological parameters in occupationally exposed personnel.

The objective of this study was to evaluate cell viability, DNA damage, and expression of Hsp70 and p53 proteins in human leukocytes exposed *in vitro* to low-dose gamma radiation fields from a ^{137}Cs source.

Gamma radiation produces a decrease in cell viability. It was found that as the exposure dose increases, viability decreases. The exposure dose of 1 mGy was the one that presented the lowest percentage of live cells (50.3% ± 10.7%) compared to the control (98.7% ± 1.3%) ($p \leq 0.05$). This is attributed to the fact that cells, as a consequence of radiation exposure, suffer damage that alters their homeostasis, which leads to death. This phenomenon coincides with what was reported by Changizi *et al.* in 2017 [44] where exposure to 2 Gy gamma radiation significantly reduced the viability of lymphocytes compared to a control group. Similar effects on viability drop were observed in different study models, such as those reported by Almeida *et al.* in 2004 [45], where gamma radiation damage occurred in the DNA of Escherichia coli strains.

Because of this, the question arises, is the drop in cell viability due to DNA damage? Thus, in the present study, DNA damage was analyzed in leukocytes exposed to low doses of gamma radiation. For this analysis, the comet assay was used, since various articles have appeared in the literature indicating the suitability of the comet assay in studies on genotoxicity and biomonitoring to quantify DNA damage in cells exposed to low doses (0 - 5 cGy) of gamma radiation, in vivo and *in vitro* in a wide variety of experimental systems, including humans exposed to various stressors [41] [46] [47] [48] [49] [50].

Through the comet assay, it was found that as the exposure dose increases, DNA damage increases significantly ($p \leq 0.05$). Using this technique, it was possible to show that from the first dose of exposure (0.2 ± 0.01 mGy) there were alterations in the DNA molecule. The final dose of 1 mGy presented the highest grade 4 DNA damage and a high number of AUs (352.3 ± 4.3). It has been shown that small doses of RI can damage DNA, producing a variety of lesions, such as single-strand breaks, alkali-labile sites, and double-strand breaks [51]. This damage is considered to be the most important initial step in the development of cancer and genetic diseases after IR exposure [52].

These results agree with the investigations of Kuefner *et al.* in 2015 [53] where an increase in DNA double-strand breaks was observed in lymphocytes exposed to IR for 1 hour; Likewise, there are similar results with different experimental models, such as the evaluation of DNA damage induced by gamma radiation in Aedes aegypti reported by Shetty *et al.* in 2017 [54]; Similarly, Chaubey *et al.* in 2001 [55] observed the effect of gamma radiation on mouse peripheral blood

leukocytes, finding a significant linear increase in DNA damage.

Likewise, it was important to demonstrate the degree of stress imposed by IR on leukocytes. For this, the expression of the Hsp70 protein was determined. The results showed the presence of the Hsp70 protein in all the samples analyzed. This correlates with other authors who have shown that Hsp are constitutively and ubiquitously expressed in eukaryotic cells, where they participate in the maintenance of cell function [56] [57].

In the present study, when observing the behavior of the Hsp70 protein due to exposure to gamma radiation, an increase was observed as the exposure dose increased, with the last dose (1.0 ± 0.05) presenting a quantitatively more significant increase ($p \leq 0.05$). Hsp70 overexpression is considered as a bioindicator of cellular stress by gamma IR as reported by Mayer in 2013 [58]. Compared to other stress proteins, Hsp70 synthesis is faster and accumulates to higher levels in cells after exposure to environmental stress [59].

These data are consistent in different gamma-irradiated experimental models, such as mouse C3H 10T1/2 cells and NIH3T3 cells that showed Hsp70 overexpression after irradiation [60]. Something similar occurs in salivary gland cells of one of the most radio-tolerant organisms, Chironomus ramosus [61]. Other studies by Nishad and Ghosh In 2018 [62] showed slight expression of Hsp90, Hsp70, and GRP78 proteins in human peripheral blood mononuclear cells by low fields of natural ionizing radiation off the coast of Kerala, India, (in vivo and *in vitro*), using ^{60}Co. Likewise, Nogami *et al.* in 1994 [63] showed the expression of Hsp70 and Hsp72 in T cells of mice irradiated at low doses.

It should be noted that an important factor in the expression of heat stress proteins is the ability to generate an adaptation or radio resistance response, as demonstrated by Sadekova *et al.* in 1997 [64], where they showed the expression of proteins from the Hsp70 family, such as PBP74/mortalin/Grp75 by low fields of ionizing radiation indicating an adaptive response to this stressor, or in other terms generating a phenomenon of radio resistance in HT29 cells (human colon adenocarcinoma), or in mouse splenocytes expressing Hsp70 by radiation showing adaptive responses [65].

Previously, significant damage to DNA by IR was demonstrated in leukocytes, in this regard, a protein that is in charge of faithfully maintaining the integrity of DNA is the p53 protein, which, when damage occurs, is expressed for its correction. p53 is a tumor suppressor protein. Functionally, p53 is a transcription factor, particularly for genes that control cell cycle progression or initiate apoptosis [66]. The behavior of the expression of p53 was similar to that of Hsp70, since it was overexpressed as the radiation dose increased ($p \leq 0.05$). Radiation has been shown to activate signals in irradiated cells that cause cytogenetic damage, decreased survival, increased apoptosis, and biochemical changes in neighboring non-irradiated cells [67], as described by He *et al.* in 2020 [68], showing that the apoptosis signaling pathway and ATM-p53 are some of the main events that cells present to the human body by radiotherapy.

The analysis of Hsp70 indicates a degree of stress imposed by IR to which leukocytes are exposed. Likewise, the p53 protein is expressed by the damage that occurs to DNA by this IR, and that its expression tries to correct the damage to DNA, or failing that, if the damage is greater as shown by the results of the comet analysis, p53 is capable of inducing cell death through the intrinsic pathway of apoptosis.

Lastly, it is important to highlight that, in terms of dosimetry, specific units and quantities are used with the purpose of establishing a use and exposure limit to IR at work, where 100 mSv is established every 5 years and no more than 50 mSv per year [69]. For the general public, a lower exposure limit is established and it is recommended to avoid exposure to artificial radiation sources with equivalent doses greater than 5 mSv (0.5 rem) per year [69] [70]. Likewise, it must always be considered that during exposure to low doses of ionizing radiation, cells have DNA damage correction mechanisms, but over time, radiation doses are cumulative and their damaging effects can occur in the long term.

5. Conclusion

Low dose gamma radiation fields when interacting with human leukocytes induce cell damage. The main damage was to the DNA molecule with the subsequent drop in cell viability. The level of damage is proportional to the dose received. The cells under these low-dose radiation fields express bioindicator molecules that alert us to this damage, such as the Hsp70 and p53 proteins.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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