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# *Allium cepa* used as a dosimetry system in nuclear and radiological emergencies

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**Abstract** Effective provisions of preparedness and response are necessary to protect human life, health, property, and the environment in any nuclear and radiological emergency. Recently, the International Commission on Radiological Protection recognized the need to provide more quantitative guidance on environmental radiation protection to integrate these analyses. A required assessment is a correlation between dose and its effects in non-human biota. Plants are highly sensitive environmental monitors for the assessment of potentially genotoxic agents and avoid the controversial use of animal models. The *Allium* test is commonly used to assess genotoxicity for a wide variety of chemical and physical factors, as it allows for estimates of possible DNA damage in eukaryotes in general, including humans. In this work, onion (*Allium cepa*) seedlings were exposed to 20–200 mGy of $\alpha$-radiation. We studied the possibility of using cytogenetic analyses of irradiated onion cells to determine the biological dose. It was observed that the increase in the frequencies of chromosomal aberrations, mitotic abnormalities, and micronuclei occurred proportional to the radiation dose, but a reduction in cytological damage was observed from 100 mGy, suggesting the onset of cytotoxic activity. Our research shows the potential of *Allium cepa* as a sensitive support system for dosimetry, detection, and screening of cellular effects produced by low doses of environmental radiation.

## 1 Introduction

The increasing dissemination of applications of ionizing radiation sources in different areas of knowledge has increased the possibility of related accidents. Although these are rare events, mainly because the applications of nuclear technology are strictly controlled, it is important to note that no country is exempt from radiation accidents.

Nuclear and radiological emergencies are typically associated with unintended and unexpected events involving a source of ionizing radiation. Currently, these events also include

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possible criminal or terrorist actions combining chemical, biological, radiological, nuclear, and/or explosive agents. The common element among all these scenarios is the deployment or dispersal of radioactive material in the target areas. Therefore, they pose great risks to human and environmental health.

Health physics has been a fundamental area for the recognition, assessment, and control of risks to human and environmental health, allowing safe applications of ionizing radiations. However, it is rather consensual in the scientific community the need to better understand the biophysics involved in processes of damage induction in cases of sudden exposure to low and moderate doses. Dose increments produced by sudden exposures can initiate responses capable of altering the DNA of somatic cells, being one of the key events in the process of carcinogenesis [1, 2].

A fundamental requirement for developing effective strategies to control radiation levels or to respond promptly to possible nuclear and radiological emergencies is to establish a scale of risks associated with different levels and types of exposure, relating them to the induced biological effects.

The connection between the frequency of radiation-induced responses in cells, called endpoints, and the dose, is known as cytogenetic dosimetry [3]. With chromosomal aberrations (CAs) and micronuclei (MNs) formation as endpoints, the cytogenetic dosimetry using animal models has been well established and widely applied [3–10]. For humans, these endpoints have been used to monitor the dose/effect relationship in radiotherapy [4] or to determine the appearance of genomic instability in occupationally exposed individuals [5, 6], or victims of nuclear and radiological accidents [7, 8].

Recently, the International Commission on Radiological Protection (ICRP) has recognized the need to provide more quantitative advice on environmental protection as well. A required assessment is a correlation between dose and its effects in non-human biota [11, 12].

As a non-human biota option, higher plants are a viable alternative to support and complement environmental dosimetry. Cytogenetic analyses can be performed on organisms of all taxonomic categories, and higher plants are a system of choice because, in addition to providing a first screening for environmental genotoxicity, they avoid the use of animal models for testing [13]. The health of organisms in radioactively contaminated sites as those in Chernobyl or Fukushima Dai-ichi nuclear power plants is under intense interest, especially due to the controversial results showing resistance of several flora and fauna communities, while significant effects of chronic irradiation at relatively very low doses are also reported [14].

Onion (*Allium cepa*), a diploid species with a low chromosome number ($2n = 16$), is the current well-established standard in vivo model for assessing a wide variety of genotoxic chemicals and environmental contaminants [13, 15–16]. Besides, *Allium cepa* has also been used to analyze the effects of high radiation doses, especially from low linear energy transfer (LET) radiations. The onion roots while cultivated were exposed to different levels of radiation and proved to be robust in vivo model for determining cytotoxic and genotoxic activity [17–20]. DNA damage is expressed as CAs and disturbances in the mitotic cycle or presence of MNs in interphasic cells, which can be seen in cells of the first mitotic cycle after irradiation. By differentiating the effects caused by different dose levels or types of radiation, it would be possible to use *Allium cepa* as a cytogenetic dosimeter both to monitor the environment radiation level and to investigate the dose received by people in environments where a radiological emergency has occurred.

In this work, we focused on assessing the effects of $\alpha$-particles emitted by a $^{241}$Am source. $\alpha$-particles are a type of ionizing radiation with a short range in condensed matter, thus with no meaning in case of external irradiation, but this type of radiation is extremely harmful

when it is inhaled, ingested, or absorbed. It has been shown that very low doses of high LET $\alpha$-radiation initiate deleterious genetic consequences, which can result in several cellular and tissue disorders, including cancer [21]. Due to its high radiotoxicity, alpha radiation can induce harmful effects both on human and non-human biota.

The determination of low-dose effects is of high importance for the protection of the human population and the environment. However, the relationships between low doses and biological responses have yet to be analyzed. The objective of this study was to evaluate the effects of low doses of alpha radiation on the DNA of the plants using meristematic root cells of *Allium cepa* and to verify the possibility of using these effects to estimate the dose in case of sudden exposures.

## 2 Materials and methods

### 2.1 Germination of *Allium cepa* seeds

Approximately twenty onion (*Allium cepa*) seeds of the Brazilian brand ISLA™, variety Baia Periform, free of pesticides, were deposited on the bottom of Petri dishes previously coated with a double layer of germination filter paper. Subsequently, 4.0 mL of distilled water was added. Then, the Petri dishes were capped and sealed with Parafilm M® plastic. Preliminary germination, maintained at a temperature of 25 ± 1 °C, without exposure to any radiation source, except the background radiation levels, was performed until the roots grew approximately 5 mm in length.

### 2.2 Irradiation of *Allium cepa* seedlings

After *Allium cepa* roots grew, seven groups of samples were defined, randomly transferring four roots to new Petri dishes. The roots of six groups were individually irradiated with a point source of $^{241}$Am (Phywe System Co. Ltd, Germany), with a nominal activity of 74 kBq (2 μCi) as of August 11, 2003, and mean energy alpha particle emission of 5.48 MeV [22]. The dose rate was calculated by Monte Carlo simulation (MNCP6, ver. 1.0) using the standard soft tissue of the ICRU 44 [23]. Cylinders 5 mm long and 400 μm thick were used to simulate the roots of *Allium cepa*. The dose rate of $\alpha$-particles was 7.92 mGy/min at 1 cm from the source. The error (1 s.d.) of the Monte Carlo simulation is less than 1%. Additionally, an unirradiated control group was set up to determine the baseline frequencies of chromosomal abnormalities, micronuclei, and mitotic rate. The mean absorbed doses evaluated were 0, 20, 40, 60, 80, 100, and 200 mGy. The experiment was performed at a controlled temperature of 25 ± 1 °C, and the root collections were carried out 20 h after the irradiations, allowing for the conclusion of one mitotic cycle of *Allium cepa* [24].

### 2.3 Preparation of the slides of *Allium cepa*

After root collection, Carnoy fixation in a 3:1 solution of ethanol–acetic acid was performed for 24 h at room temperature (25 ± 1 °C). The roots were then stored in a fresh Carnoy solution at 4 °C for further analysis.

Microscopy slides were prepared by hydrolyzing the roots of *Allium cepa* for five minutes in a 1 N HCl solution kept at 42 °C, and, shortly thereafter, rinsing them with distilled water for a few minutes [25]. To increase the coloration quality/contrast and improve cell spread, the root tips of *Allium cepa* were stained with 2% acetic orcein (Sigma-Aldrich) for 30 min,

and then one drop of 45% acetic acid was added to remove possible coloration excesses. Shortly thereafter, the root tips were separated with a scalpel blade, carefully squashed with a glass stick on the microscopic slide, and covered with coverslips. Finally, the slides were assembled by applying colorless enamel on the edges of the coverslips.

### 2.4 Evaluation of mitotic index, chromosomal aberrations, and micronuclei in meristematic cells of *Allium cepa*

The slides were examined on an Olympus BX51 optical microscope at 1000 × magnification. In total, 8000 cells of two independents experiments were analyzed for each dose point, including the unirradiated control group. Cytotoxicity was assessed based on the mitotic index (MI), calculated as the ratio of the number of cells in the division to the number of cells analyzed. A total of 1000 cells of each slide were scored for MI and expressed as the percentage of the number of cells examined undergoing mitosis. Genotoxicity was assessed based on the frequency of chromosomal aberrations (CAs) and the frequency of cells with micronuclei (MNs). The micronuclei did not exceed one-third of the size of the nucleus. For the analysis of CAs, 100 dividing cells per slide were examined (except for the 200 mGy dose, which had an abrupt reduction in MI), while for the evaluation of MNs, 1000 interphasic cells were examined per slide. The frequencies of ACs and MNs were expressed as the number of aberrant cells per 100 cells and as the number of MNs per 1000 cells, respectively. To avoid bias in scoring, all measurements were performed by the same person using coded slides. As an inclusion criterion for the analysis, we considered only cells with intact cytoplasmic membranes. The data were expressed as the mean ± standard deviation (SD) of the means for MI and frequencies of aberrant cells and cells with micronuclei.

### 2.5 Statistical analysis

An analysis of variance of the MI, CAs, and MNs means (ANOVA one-way) followed by a Tukey test ($p < 0.05$) was performed to verify whether differences between the sample had a statistical significance [26]. Data analysis was performed using the software PAST 4.03 [27].

## 3 Results

Data on the mitotic index of *Allium cepa* seedlings exposed to alpha radiation from the source of $^{241}$Am are shown in Table 1. Based on statistical analysis using one-way ANOVA followed by the Tukey test ($p < 0.05$), a significant increase ($p < 0.01$) of the mitotic index was observed for the dose of 80 mGy when compared to the mitotic index of the unirradiated control group, and of the assays exposed to the doses of 20, 40, 60, 100, and 200 mGy. In contrast, the assay exposed to 200 mGy had the lowest mitotic index compared to the other assays, with a statistically significant cellular inhibition ($p < 0.01$) compared to the unirradiated control group.

The mitotic and nuclear abnormalities (MNAs) and CAs in meristematic root cells of *Allium cepa* induced after 20 h of exposure to alpha radiation were bridges, fragments, lagging, stickiness, abnormal kinetics, and nuclear buds (Table 2). Statistical analyses of CAs and MNAs were performed only with data from the unirradiated control group and assays exposed to doses from 20 to 100 mGy. For this dosing interval, except for 100 mGy, an increase with the dose of the average number of aberrant cells was observed with statistically

**Table 1** Mitotic index (MI) of meristematic root cells of *Allium cepa* exposed to different alpha radiation doses from a $^{241}$Am source (mean ± SD)

| Dose (mGy) | Number of dividing cells | MI (%) | Phase index | | | |
|---|---|---|---|---|---|---|
| | | | *P* | *M* | *A* | *T* |
| Unirradiated control (0) | 79 ± 21 | 7.9 ± 2.1 | 12 ± 3 | 23 ± 3 | 24 ± 4 | 20 ± 7 |
| 20 | 29 ± 8 | 2.9 ± 0.8 | 4 ± 5 | 8 ± 2 | 8 ± 4 | 9 ± 4 |
| 40 | 49 ± 24 | 4.9 ± 2.4 | 23 ± 7 | 8 ± 2 | 12 ± 3 | 6 ± 2 |
| 60 | 42 ± 13 | 4.2 ± 1.3 | 6 ± 4 | 10 ± 2 | 12 ± 6 | 14 ± 4 |
| 80 | 149 ± 32 | 14.9 ± 3.2[a, b, c, d] | 49 ± 25 | 31 ± 6 | 36 ± 6 | 33 ± 7 |
| 100 | 44 ± 9 | 4.4 ± 0.9 [e] | 3 ± 1 | 9 ± 2 | 13 ± 5 | 15 ± 3 |
| 200 | 15 ± 11 | 1.5 ± 1.1 [a, e] | 0 ± 0 | 5 ± 2 | 3 ± 1 | 7 ± 2 |

*P* prophase, *M* metaphase; *A* anaphase, *T* telophase
ANOVA with Tukey test: [a]Statistically significant at $p < 0.01$ when compared with unirradiated control; [b]Statistically significant at $p < 0.01$, when compared to the 20 mGy irradiated assay; [c]Statistically significant at $p < 0.01$, when compared to the 40 mGy irradiated assay; [d]Statistically significant at $p < 0.01$, when compared to the 60 mGy irradiated assay; [e]Statistically significant at $p < 0.01$, when compared to the 80 mGy irradiated assay

**Table 2** Chromosomal aberrations and nuclear abnormalities (mean ± SD) in the meristematic cells of *Allium cepa*

| Dose (mGy) | Chromosomal aberrations (CAs) and nuclear abnormalities (NAs) per 100 cells | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bridges | Fragments | Lagging | Stickiness | Abnormal kinetics | Nuclear buds | Total number of aberrant cells |
| Unirradiated control (0) | 1.5 ± 0.5 | 1.4 ± 0.5 | 1.2 ± 1.4 | 1.1 ± 1.6 | 1.5 ± 2.1 | 1.6 ± 1.4 | 8.3 ± 1.7 |
| 20 | 4.8 ± 0.9 | 5.0 ± 0.6 | 4.4 ± 2.7 | 4.1 ± 1.9 | 4.9 ± 2.4 | 3.8 ± 3.2 | 27.0 ± 4.2 [a] |
| 40 | 6.4 ± 2.9 | 5.4 ± 1.7 | 6.2 ± 1.9 | 5.8 ± 2.0 | 5.5 ± 4.5 | 6.3 ± 3.4 | 35.5 ± 3.1 [a] |
| 60 | 8.0 ± 3.5 | 7.9 ± 3.0 | 8.2 ± 3.4 | 6.3 ± 2.7 | 8.4 ± 3.5 | 7.6 ± 3.2 | 46.4 ± 6.0[a, b] |
| 80 | 12.5 ± 1.9 | 12.3 ± 1.8 | 10.4 ± 2.8 | 15.7 ± 4.3 | 12.6 ± 5.0 | 16.1 ± 3.4 | 80 ± 12[a, b, c, d] |
| 100 | 8.9 ± 2.7 | 9.1 ± 2.6 | 8.4 ± 3.4 | 9.1 ± 1.2 | 9.3 ± 3.6 | 10.5 ± 3.4 | 55.3 ± 8.0[a, b, c, e] |
| 200 | 0.4 ± 0.7 | 0.3 ± 0.7 | 0.3 ± 0.5 | 0.4 ± 0.5 | 0.0 ± 0.0 | 0.1 ± 0.3 | 1.6 ± 0.8 |

ANOVA with Tukey test: [a]Statistically significant at $p < 0.05$, when compared with unirradiated control; [b]Statistically significant at $p < 0.05$, when compared to the 20 mGy irradiated assay; [c]Statistically significant at $p < 0.05$, when compared to the 40 mGy irradiated assay; [d]Statistically significant at $p < 0.05$, when compared to the 60 mGy irradiated assay; [e]Statistically significant at $p < 0.05$, when compared to the 80 mGy irradiated assay

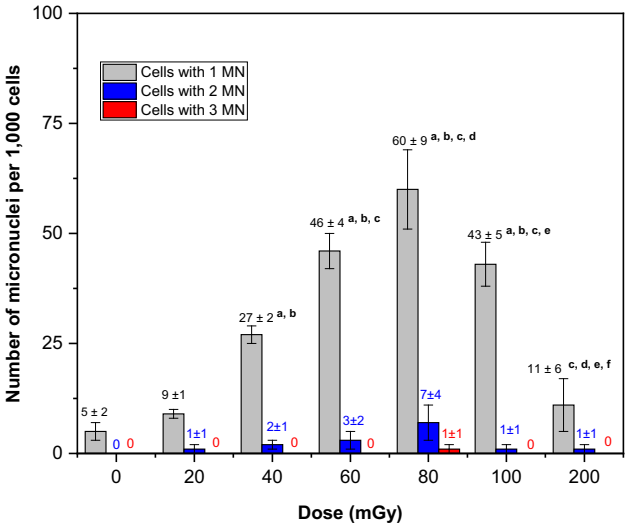


**Fig. 1** Distribution of micronuclei in meristematic root cells of *Allium cepa*. ANOVA with Tukey test: **a** Statistically significant at $p < 0.05$ when compared with unirradiated control; **b** Statistically significant at $p < 0.05$, when compared to the 20 mGy irradiated assay; **c** Statistically significant at $p < 0.05$, when compared to the 40 mGy irradiated assay; **d** Statistically significant at $p < 0.05$, when compared to the 60 mGy irradiated assay; **e** Statistically significant at $p < 0.05$, when compared to the 80 mGy irradiated assay; **f** Statistically significant at $p < 0.05$ when compared to the 100 mGy irradiated assay

significant differences ($p < 0.05$) compared to the unirradiated control group. We excluded from the analysis the data from the 200 mGy assay due to the low number of dividing cells induced by this dose.

The micronucleus data are shown in Fig. 1. In addition to the expected observation of MNs in interphasic cells of *Allium cepa*, MNs were also found in different phases of the cell cycle. Between 0 and 80 mGy, the increase in the average number of cells with MNs was proportional to the increase in the radiation dose. For this dosing interval, results were statistically significant ($p < 0.05$) compared to the unirradiated control group, except for the 20 mGy dose. However, for doses of 100 and 200 mGy, a reduction of about 28% and 82%, respectively, was observed in the average number of cells with MNs compared to the 80 mGy assay.

## 4 Discussion

The onion (*Allium cepa*) is an in vivo plant model widely used for the evaluation of cytotoxic and/or genotoxic activity of various physical and chemical agents, as it allows for estimates of possible DNA damage in eukaryotes in general, including humans [13, 16]. Its chemical composition is essentially aqueous, which makes the vegetable biota *Allium cepa* an equivalent tissue in vivo suitable for dosimetry. Indeed, previous works have indicated the *Allium cepa* as a potential in vivo model for dosimetry, including for screening nuclear and radiological emergencies. However, these analyses were done only in high doses [18–20].

In this work, cells of *Allium cepa* seedlings were evaluated after 20 h of exposure to low doses of alpha radiation to verify whether these exposures were capable of producing

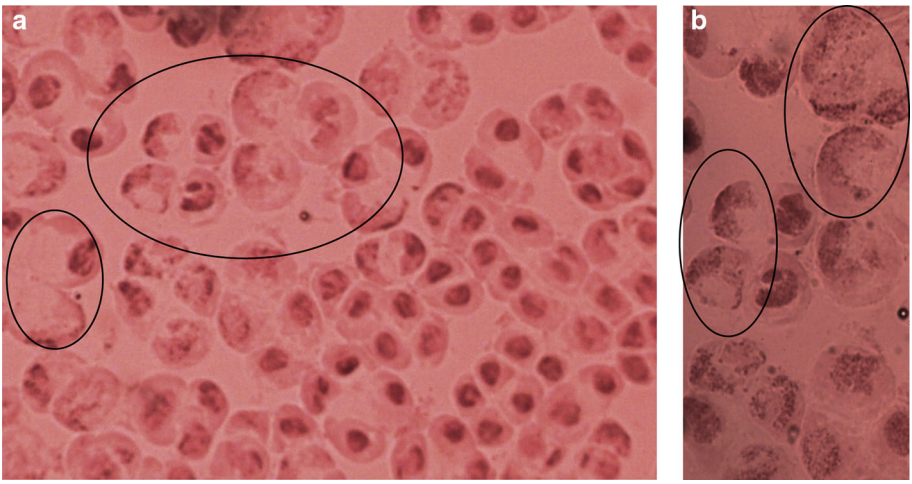


**Fig. 2** Cells of *Allium cepa* seedling exposed to 200 mGy of alpha radiation, indicating a possible effect of chromatin fragmentation. **a** 400 × magnification; **b** 1000 × magnification

detectable changes, i.e., simulating a possible emergency involving low doses in the environment. Thanks to its large chromosomes, effects are easily observable with an optical microscope. Mitotic and nuclear abnormalities, chromosomal aberrations, and the presence of MNs in the cell cycle of *Allium cepa* seedlings were observed as endpoints that indicate genotoxicity. The mitotic index was evaluated as a cytotoxicity meter.

An apparent reduction in the mitotic index was observed in the assays exposed to 20–60 mGy when compared to the unirradiated control group, but it did not show statistical significance. In contrast, the 80 mGy dose strongly stimulated the mitotic activity of the *Allium cepa* root cells, which when compared to the unirradiated control group and the assays exposed to the doses of 20, 40, and 60 mGy showed a statistically significant difference ($p < 0.01$). When MI is significantly higher than the control level, it can be harmful to the cells, leading to a disorder's cell proliferation and even to malignant transformations [13]. However, there is no consensus on the increase in MI to be considered a beneficial or harmful effect. In this study, the highest frequencies of cytogenetic damage were found for the assay that had the highest mitotic activity, that is, 80 mGy. But, we do not yet have an explanation for the MI increase observed in the 80 mGy assay.

In a previous study with human lymphoblast cells, it was reported that doses of $\alpha$-particles greater than 100 mGy could cause cell cycle arrest, delayed nuclear division, and reduced MN formation [28]. Indeed, a similar behavior was also observed with our samples of *Allium cepa* cells irradiated with $\alpha$-particles. The assay exposed to 100 mGy showed a statistically significant reduction in the mitotic index ($p < 0.01$) when compared to the 80 mGy assay (Table 1). However, this MI value is very close to the MI of the assays exposed to 40 and 60 mGy. This reduction in MI was most evident when the samples of *Allium cepa* were exposed to a dose of 200 mGy, which in addition to present the lowest mitotic activity of all the assays, including the control group, also showed an aspect of loss of cell integrity (Fig. 2). This effect suggests the onset of a cellular restriction checkpoint. DNA damage is the main indication for a cell to restrict and not enter the mitotic phase.

The observation of CAs occurred mainly at the anaphase. However, between 20 and 100 mGy, CAs were observed in all phases of the mitotic cycle of *Allium cepa* (Fig. 3). Mitotic

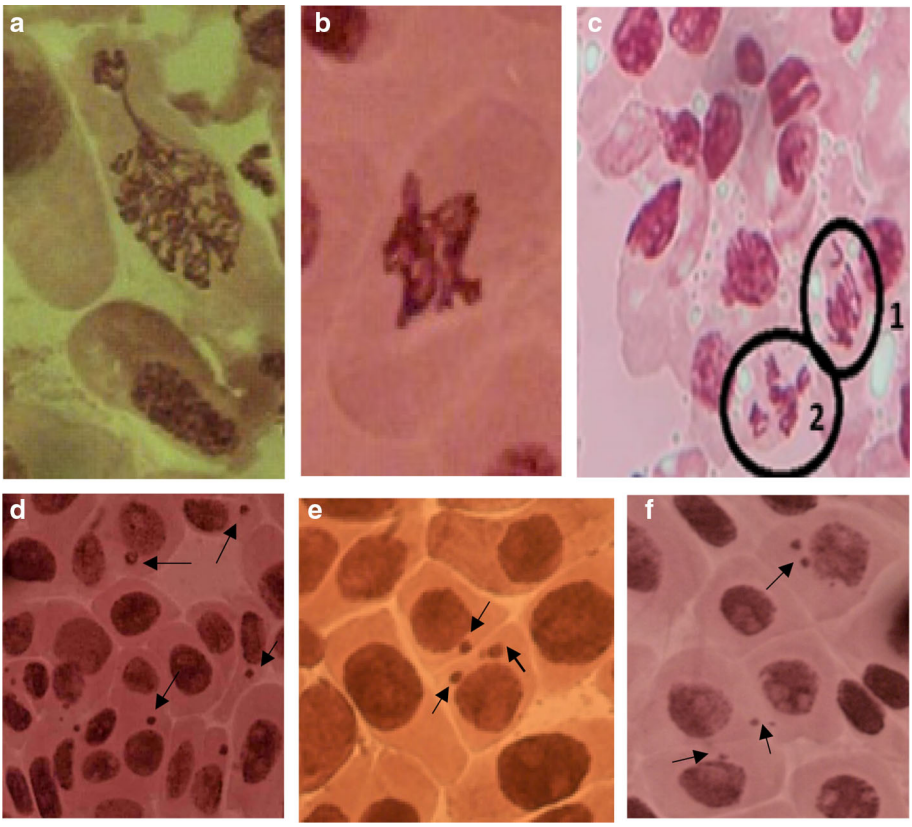


**Fig. 3** Disturbing on mitotic chromosome distribution and presence of micronuclei in *Allium cepa* seedling cells irradiated with 20–100 mGy of $\alpha$-particles from a $^{241}$Am source. **a** disturbed prophase; **b** sticky chromosome; **c** 1—lagging chromosome at metaphase, 2—multipolar metaphase (**d–f**) Micronuclei. (1000 × magnification)

abnormalities are typically associated with spindle disorders [29, 30]. The data on abnormal kinetics showed in Table 2 indicate that even the smallest dose evaluated of 20 mGy was able to impair the formation of spindle microtubules, the structure responsible for properly promoting cell division. The appearance of bridges, fragments, and lagging chromosomes was proportional to the dose increase, except for 100 mGy (Fig. 4). These aberrations are supposed to turn into micronuclei later in the cell cycle—if the cell survives the damage. These data are supported by the MN frequency analyses, which were proportional from 20 to 80 mGy, but which started to decrease from 100 mGy.

It is known that DNA damage induced by the $\alpha$-particles is difficult to be repaired because it leads to severe cellular damage and weakens the cell defense ability. We believe that the cellular restriction checkpoint processes are intensified starting from 100 mGy. This hypothesis becomes stronger when the data from the assay that was exposed to a dose of 200 mGy are observed. The abrupt reduction in the mitotic index, CAs, and MNs of *Allium cepa* samples exposed to 200 mGy indicates evidence of cytotoxic activity. This, in turn, is reinforced by observing the loss of cell integrity of the samples in this assay (Fig. 2).

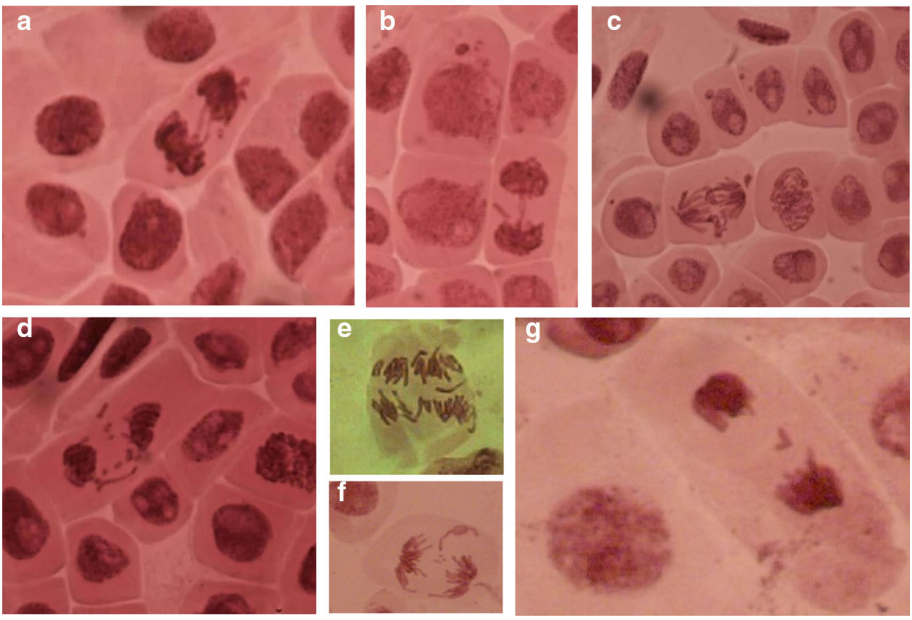


**Fig. 4** Chromosome aberrations in cells of *Allium cepa* seedling irradiated with 20–100 mGy of $\alpha$-particles from a $^{241}$Am source. **a**–**c** anaphase bridges and micronuclei; **d** fragments; **e** multipolar anaphase with lagging chromosome; **f** lagging chromosome and fragments; **g** lagging chromosome. (1000 × magnification)

## 5 Conclusions

The results of the present study indicate the utility of *Allium cepa* root meristem cells as a potential model to detect the genotoxic effects produced by low doses of alpha radiation. In this work, the combined analysis of the frequencies of MI, CAs, and MNs showed to be able to distinguish the effects produced by alpha irradiation in the *Allium cepa.* The frequencies of CAs, and MNs increased proportionally to the radiation dose up to 80 mGy. However, a reduction in cytological damage was observed from 100 mGy, suggesting the onset of cytotoxic activity. Our research shows the potential of *Allium cepa* as a sensitive support system for dosimetry, detection, and screening of cellular effects produced by low doses of environmental radiation. Yet, to establish cytogenetic dose–response curves, complementary analyses are necessary to determine DNA damage/repair rates. Future studies adding other types of ionizing radiation and exposure levels are needed to better understand the effects of low and intermediate doses on *Allium cepa* cells.

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**Declarations**

**Conflict of interest** The authors report no conflicts of interest.

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