

Research

Hazard assessment and cytogenotoxic effect of different concentrations of mercury chloride sterilant using the *Allium cepa* assay

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Received: 29 December 2023 / Accepted: 14 May 2024

Published online: 04 June 2024

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Abstract

The *Allium cepa* assay represents a crucial in vivo model for evaluating the cytotoxicity and genotoxicity of substances. This study investigated the cytogenotoxicity of mercury chloride (HgCl_2), a laboratory disinfectant and catalyst, using the *Allium cepa* assay. Mitotic slides were prepared from onion root tip cells grown on media supplemented with different concentrations of HgCl_2 (0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%). The slides were observed to evaluate cytogenotoxicity based on the assessment of the mitotic index, mitotic inhibition, clastogenic effect, and root length. The results showed that the concentrations used are harmful to the cell, leading to adverse impacts on the mitotic index, mitotic inhibition, root growth, and chromosome structure. Different chromosomal aberrations, such as bridge formation, fragmentation fragments, wandering chromosomes, stickiness, binucleate cells, and micronucleus formation, were detected depending on the concentration. Although lower concentrations (0.2–0.4%) had fewer effects on the cells, they still had a significant cytogenotoxic effect ($p < 0.05$) compared to that of the control (0.0%). The higher the concentration, the greater the effects on clastogenic changes. The abnormalities in both mitotic spread and root growth indicate that mercury chloride is cytotoxic even at low concentrations and can cause mito-depressive effects at higher concentrations. The results of this investigation can be used as a guide to guarantee sufficient safety precautions for people and organs during the regular use of HgCl_2 .

Keywords *Allium cepa* · Cytotoxicity assay · HgCl_2 · Mitotic index · Mitotic inhibition · Chromosome aberrations

1 Introduction

Cytotoxicity describes the harmful impact of chemical agents on living cells. An agent may also be genotoxic if it induces genetic changes within a cell, resulting in a mutation, a condition that can be fatal or extremely harmful to different systems. This alteration may produce permanent and heritable effects on DNA, impacting either the organism's somatic or germ cells,

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which can be passed down to future generations [1, 2]. Cytological examination of either mitotic or meiotic behaviour is a reliable way of measuring mutagenic potential [3]. Cytotoxicity testing holds great significance in the biological assessment of chemicals in cells. The extent of cytotoxicity and damage to cellular structure and physiology, such as disruption of the cell membrane, inhibition of protein synthesis, and irreversible binding to receptors, can be determined by conducting tests on chemicals, drugs, and extracts. Therefore, rapid, accurate, and cost-effective cytotoxicity assays that evaluate toxicity to whole organisms by examining their impact in vivo or in vitro are needed [4–7].

Assays can be invaluable in determining the effects of chemicals or biologics in living tissues. Cytotoxicity assays aim to affect various cellular mechanisms, such as cell membrane permeability, ATP production, enzyme activity, coenzyme production, and nucleotide uptake activity [3]. Onion (*Allium cepa*) is a suitable model for experimentally determining the in vivo cytotoxicity of chemicals and complex mixtures [1, 8–10]. The *A. cepa* assay is considered a cost-effective and easy-to-use screening method that surpasses other short-term screening methods requiring prior preparation of test samples and the inclusion of exogenous metabolic systems. This assay can evaluate the toxicity and genotoxicity of substances [2]. This includes assessing chromosomal irregularities and mitotic anomalies [8, 11–13]. The *Allium cepa* test is commonly used to evaluate the damage caused by mutagens to DNA. This test has also been effective at screening the cytotoxicity of chemical agents and studying the genotoxicity of environmental pollutants [5, 10, 14, 15]. The *Allium cepa* assay is significantly correlated with mammalian testing systems. Bakare et al. [12] used this assay to evaluate the cytogenotoxicity induced by e-waste leachate. Also, Kaymak and Goc-Rasgele [16] explored the genotoxicity of Raxil while Das et al. [17] investigated the cytogenetic effects of anticancer drugs Doxorubicin and Erlotinib using an *Allium cepa* assay.

Mercuric chloride (HgCl_2) is a chemical compound consisting of mercury and chlorine atoms. It is a laboratory reagent regularly used as a catalyst for converting acetylene to vinyl chloride, the precursor for polyvinyl chloride synthesis [18]. In addition, HgCl_2 functions as a depolarizer in batteries. Due to its effectiveness as a germicide, it is frequently used to sterilize the surfaces of explants and workspaces in plant tissue culture laboratories before culture inoculation. Moreover, it is employed in dental amalgam fillings [19] as well as in energy-efficient light bulbs [20]. During the Middle Ages, Arab physicians employed HgCl_2 to sterilize wounds. This practice persisted for several decades, even throughout the twentieth century, although it was ultimately deemed unsafe for modern medicinal use [21].

Although HgCl_2 is considered toxic and should not be handled carelessly, it is required for the daily operation of many laboratories and factories, and some individuals are exposed to this chemical daily during their work. The increasing concern about contamination from mercury compounds in laboratories and the environment is of global significance. HgCl_2 is a highly corrosive and toxic substance that can cause acute and cumulative poisoning [22]. Mercury poisoning can occur not only through ingestion but also through skin absorption into the bloodstream, resulting in detrimental effects on the kidneys and nervous system [23]. Exposure to HgCl_2 can lead to various symptoms, including skin rashes, vision impairment, memory loss, and mental disorders [22, 24].

Assays aimed at detecting genotoxic molecules entail analysing the levels of DNA damage in exposed cells. This damage can arise due to chromosomal breakage, encompassing both single and double-strand breaks, loss of excision repair, cross-linking, point mutations, and structural as well as numerical chromosomal aberrations [2, 7, 15]. In most scenarios, negative alterations in genetic material can give rise to a range of disorders. Numerous methods for conducting in vitro and in vivo toxicological tests have been developed to evaluate the genotoxicity of chemicals. Hence, this study aimed to assess the toxic impact of different concentrations of HgCl_2 on the root and mitotic characteristics to determine the occurrence of mitotic indices and assess chromosomal aberrations, nuclear abnormalities, and micronuclei at various mitotic stages using the *Allium cepa* model.

2 Materials and methods

2.1 Plant material preparation and treatments

Bulbs of onions (*Allium cepa*) were sourced commercially from a market in Ilorin, Nigeria. After being left to dry in the sun for five days and then cleaned, healthy bulbs of approximately equal sizes were selected for the experiment. Mercury chloride (HgCl_2) purchased from Sigma Aldrich (USA) was used at concentrations of 0.2, 0.4, 0.6, 0.8, and 1% w/v, which falls within the range commonly used as sterilant, to create a concentration gradient for the assessment of cytogenotoxicity in *Allium cepa*. Deionized water was used as the negative control. The bulbs were prepared by removing the dry, scaly brown covering leaves and the old roots attached to the basal stem, leaving the ring of the primordial roots intact. The toxicity of the HgCl_2 solution on root growth and in vivo induction of *Allium cepa*

chromosomal aberration was evaluated following the methods outlined by Khalil et al. [25]. The basal stem of the onion bulbs was suspended in 50 mL conical tubes for each of the tested concentrations of HgCl_2 , with five different bulbs as replicates for each mercury chloride concentration and the control. The sample was kept at a temperature of $27 \pm 1^\circ\text{C}$ in a dark laboratory cupboard. The treatment solutions were changed every other day, and onion bulbs were carefully examined daily to monitor root emergence. The root length of the bulbs was measured on the 3rd and 10 days, which aided in identifying the growth-inhibiting impact of HgCl_2 at various concentrations.

2.2 Root harvest and slide preparation

When the roots of *Allium cepa* had grown to a length of approximately 3 cm, the root tips were excised using sterilized scissors. The tips are then collected and labelled in specimen tubes according to the treatments. The pretreatment of roots and slide preparation followed [5], with a few modifications. The root tips were pretreated with Para-dichlorobenzene for four hours to arrest a large number of metaphase cells and to make the chromosome more condensed for easy counting. The treated root tips were then extracted from the solution with forceps, rinsed twice in double distilled deionized water, and subsequently transferred to a tube containing freshly prepared ethanol:glacial acetic acid fixatives (3:1, v/v) for 24 h. The root tips were rinsed with ice-cold distilled water after being removed from the fixative. Following this, the tips were hydrolysed in a watch glass with 5 N HCl for 10 min at room temperature, after which the HCl was discarded. Next, the tips were transferred to clean microscopic slides, and 1–2 mm of the growing tip was removed while the tips were retained and the other root parts discarded. Then, 2% (w/v) acetic orcein was added to cover the tip, which was left for 10 min before being covered with a coverslip. The root tips were then squashed in 45% acetic acid and stained through gentle tapping until a turbid suspension was formed, and the slide was covered with a cover slit in preparation for microscopic observation.

2.3 Scoring of slides and data analysis

Slides were prepared to observe the effects of the HgCl_2 concentrations at different stages of mitosis. The slides were observed using a light microscope, starting with the lowest objective lens ($\times 10$) and progressing steadily to higher objectives ($\times 40$). The mitotic index was calculated from the dividing and nondividing cells observed with the aid of a digital camera (AmScope Canon SLR/DSLR, Canon Electronics Inc. Japan), attached to the microscope's eyepiece. Chromosome forms, aberrations or anomalies seen were captured at each stage of mitosis. Approximately 600 cells were observed for each treatment in five replicates resulting in a total of 3000 cells per treatment. The following parameters were assessed: (a) the mitotic index (MI); (b) the frequency of cells displaying chromosome aberrations (CAs), which include bridges, fragments, sticky chromosomes, polar deviation, pulverization, and others; and (c) the frequency of micronucleus (MN) formation.

Cytotoxicity determination: a comparison was conducted to analyse the behaviour and morphology of chromosomes in cells exposed to HgCl_2 or without such exposure (control). For cells that display mitotic irregularities, decreased mitotic index, chromosome damage, or cell death, the HgCl_2 dosage was considered cytotoxic. Additionally, the presence of micronuclei (MNs) was examined among the treatment groups. The mitotic index (MI) was determined according to Eq. (1) [26]. Chromosomal aberrations (CAs) refer to alterations in either the structure or total number of chromosomes (karyotypes) following exposure to a chemical agent. Mitotic inhibition was measured using the method of Fiskejo [27] Eq. (2). The frequency of MNs was evaluated as % MNs in interphase cells per 1000 cells, as outlined by Pavlica et al. [28]. Representative micrographs demonstrated frequent abnormalities.

$$\text{Mitotic index} = \frac{\text{Dividing cells in all phases}}{\text{Total number of cells}} \times 100 \quad (1)$$

$$\text{Mitotic inhibition} = \frac{\text{mitotic index in the control} - \text{mitotic index of test concentration}}{\text{Mitotic index of the control}} \times 100 \quad (2)$$

2.4 Data analysis

The data collected on root parameters, mitotic indices and chromosome aberrations were subjected to the analysis of variance (ANOVA) using SPSS version 20 (IBM Corp. Armonk, NY, USA). The significance level between the treatment means was determined using Tukey's honest significant difference (HSD) test at a *p*-value of less than 0.05. The percentage of cells with chromosome aberrations was determined.

3 Results

The effects of different levels of HgCl_2 on root growth are illustrated in Fig. 1. An inhibitory impact on the growth of onion root was observed at all five doses. Onions treated with lower concentrations (0.2% and 0.4%) demonstrated delayed rooting, whereas higher concentrations (0.6%, 0.8%, 1%) exhibited significantly delayed emergence of roots. In the replicates where the onions underwent no HgCl_2 treatment (control setup), the root growth was optimal. Onions commenced sprouting on the third day and reached a length exceeding 1 cm. The delay in root emergence increased with increasing HgCl_2 concentration. Even at the 10 day mark, the average root lengths of the untreated control and those treated with 0.2 and 0.4% HgCl_2 were 9.28, 5.16, and 4.18 cm, respectively. However, higher dosages significantly inhibited root growth. The most detrimental effect was observed in samples treated with 1.0% HgCl_2 , in which the length of the roots was less than 2 cm at 10th days (Fig. 2).

Analysis of the cytological impacts of different concentrations of HgCl_2 revealed a direct relationship between the number of dividing cells, and the mitotic inhibition (Table 1). A lower concentration (0.2 and 0.4%) resulted in a greater number of actively dividing cells, while the number of cells undergoing division decreased as the concentration increased. Additionally, the untreated bulbs (control) contained the greatest quantity of dividing cells in various phases. Similarly, the percentage of mitotic index (%MI), which indicates the proportion of actively dividing cells in comparison to the total number of cells observed, decreased proportionally with concentration. In this study, the MI was used as a quantitative measurement of cell division rates, which decreased as the concentration of HgCl_2 increased. In contrast, the percentage of mitotic inhibition showed a concentration-dependent pattern. Increased concentrations of HgCl_2 resulted in greater mitotic inhibition. Thus, as the concentration increased, cellular division was increasingly inhibited.

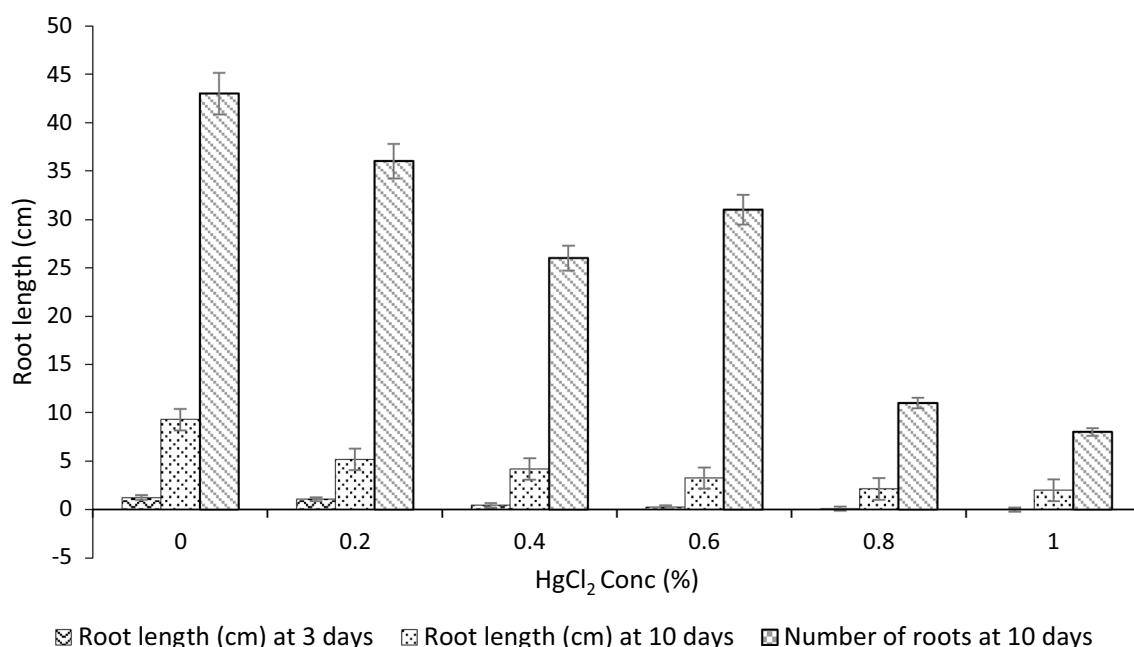
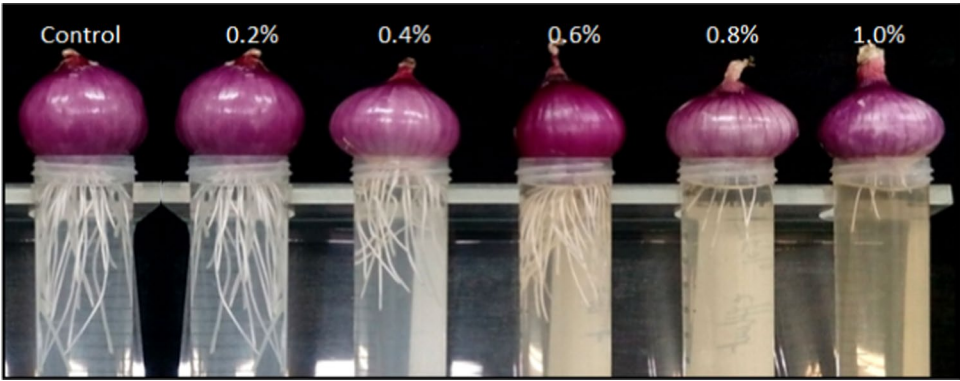


Fig. 1 Variations in root lengths of *Allium cepa* bulbs were observed under different concentrations of HgCl_2 . The mean value of five replicates $\pm$ SE is presented. The root number was determined by counting at least 0.5 cm long roots after ten days of treatment

Fig. 2 *Allium cepa* root formation in response to exposure to varying concentrations of HgCl_2 in 10 days after treatment



Cytological analysis revealed various types and frequencies of chromosomal abnormalities in the root meristem of *A. cepa* treated with varying concentrations of HgCl_2 , a surface sterilization agent commonly used in industry or laboratories. The observed normal division and chromosomal aberration data are shown in Figs. 3 and 4, while Table 2 presents the findings. The frequency of chromosomal aberrations, including anaphase bridge, stickiness, C-mitosis, chromosome fragmentation, and micronucleus formation, exhibited notable differences among the various concentrations of HgCl_2 . Anaphase bridges, characterized by incomplete segregation of nuclear material to the poles owing to persistent DNA entanglement between sister chromatids, were more evident in onion root cells treated with higher concentrations (0.6–1.0%) of HgCl_2 . The pattern is consistent for all the other identified abbreviations. Exposing root cells to elevated concentrations of HgCl_2 leads to an increased occurrence of chromosomal aberrations.

Low concentrations (0.2–0.4%) of HgCl_2 resulted in chromosome genotoxicity and damage in treated cells. Nevertheless, such concentrations are toxic to cells, causing chromatin to become sticky clumps, which may lead to sterility and chromosome fragmentation (Table 2). Moreover, a concentration of 0.4% resulted in the development of micronuclei in a considerable number of cells, with an even greater amount being found in those exposed to 0.6%. A small number of chromosomal aberrations, such as irregular prophase, anaphase lagging and pulverization, were observed in the root cells of onions that were not treated. Nevertheless, with higher concentrations of HgCl_2 , their frequency increased. Exposure to 0.6–1.0% HgCl_2 resulted in increased frequencies of multipolar cells, binucleated cells, and nuclear abnormalities in the treated bulbs. Essentially, the investigation revealed a substantial percentage of

Table 1 Cytological effects of exposure to varying concentrations of HgCl_2 on *A. cepa* mitotic cells

HgCl_2 concentrations (%)	Total number of cells counted	Dividing cells	Mitotic index (%)	Mitotic inhibition (%)
Control	2957.64 ± 15.09	413.35 ± 3.46	14.01 ± 2.11	0.00 ± 0.00
0.2	2972.81 ± 19.17	361.44 ± 17.61	12.16 ± 1.89	13.20 ± 1.16
0.4	2413.66 ± 26.57	277.36 ± 18.48	11.49 ± 1.64	17.99 ± 1.33
0.6	2433.33 ± 12.38	237.66 ± 6.69	9.77 ± 1.05	30.26 ± 4.15
0.8	2341.33 ± 25.59	117.41 ± 19.35	5.01 ± 0.45	64.24 ± 4.25
1.0	2129.66 ± 36.46	61.33 ± 25.40	2.89 ± 0.09	79.37 ± 7.01

The values designated by different superscript letters in each column are significantly different from each other at a level of $p < 0.05$

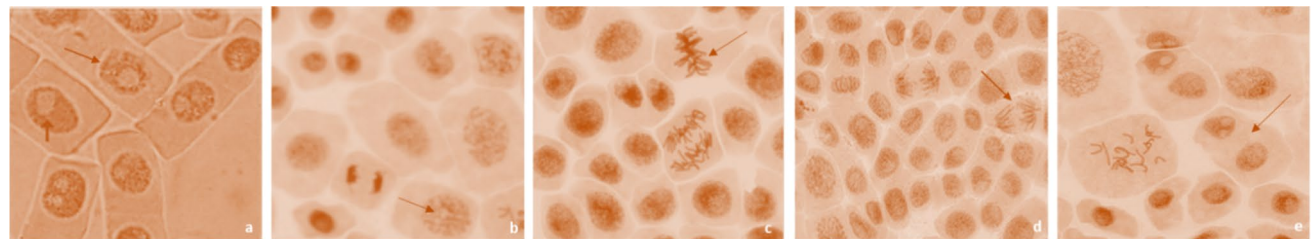


Fig. 3 Microscopic appearance of normal interphase and actively dividing cells in *Allium. cepa* root tip meristematic region **a** Interphase; **b** prophase; **c** metaphase; **d** anaphase; and **e** telophase

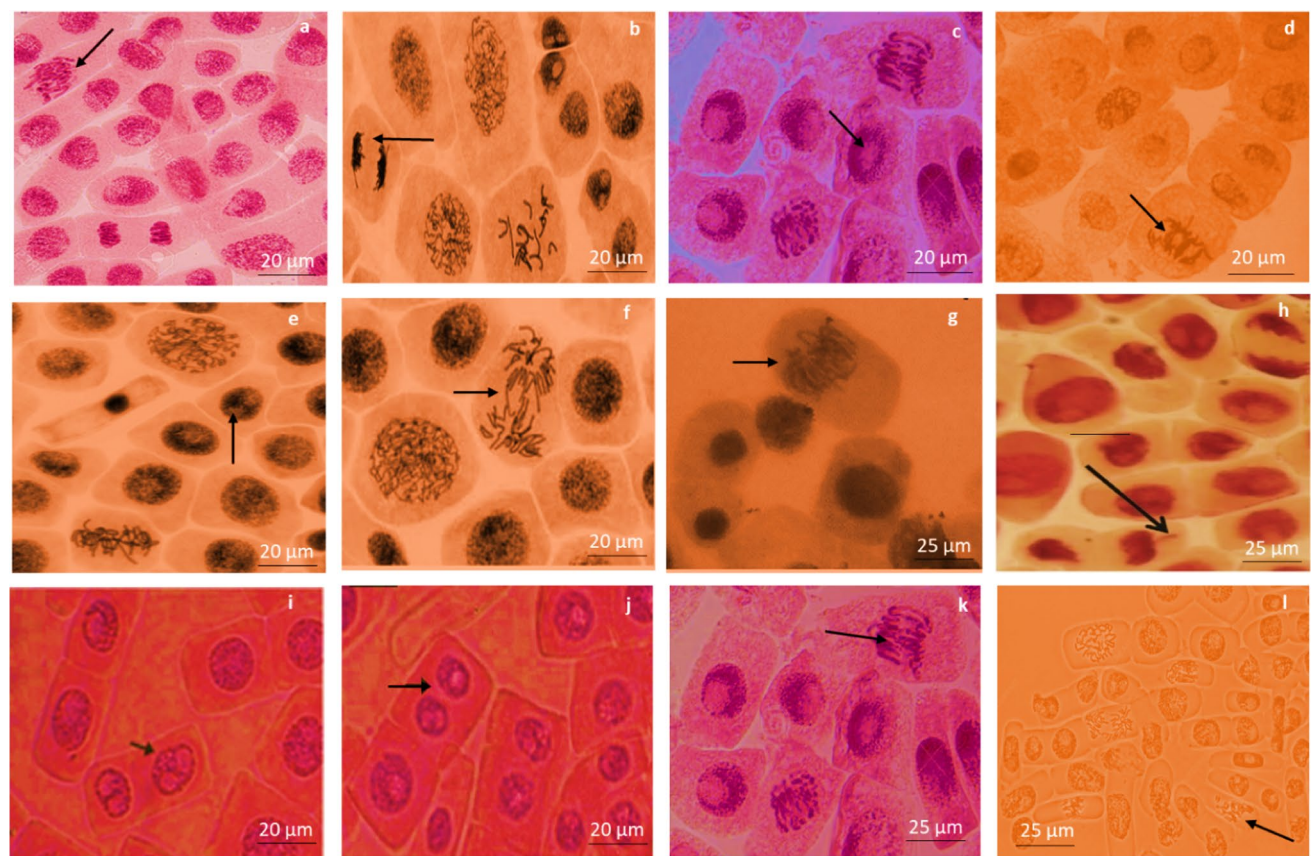


Fig. 4 Representative microscopic appearance of the nuclear abnormalities in the *Allium cepa* root tip cells exposed to different concentrations of HgCl₂ which is used as a sterilant in laboratories. **a:** Irregular metaphase; **b:** sticky anaphase; **c:** sticky chromosome at prophase; **d:** anaphase bridge; **e:** condensed nucleus prophase; **f:** polyploidy; **g:** sticky metaphase; **h:** telophase with vagrant chromosome; **i:** nuclear buds; **j:** binucleated cell; **k:** delayed anaphase; **l:** chromosome missegregation and fragmentation

Table 2 Chromosome aberration induced in *A. cepa* root tip cells in response to exposure to varying concentrations of HgCl₂

HgCl ₂ Conc. (%)	Total number of counted cells ^a	Anaphase bridge	Stickiness	Fragment	Micronucleus	Others ^b	% Chromosome aberration
0.0	2957.64a	1.00d	9.01e	0.00c	5.11de	4.20cd	0.65
0.2	2972.81a	11.13c	24.16cd	13.20b	6.24cd	2.91cd	1.94
0.4	2413.66a	8.33cd	21.49cde	7.99bc	14.25bc	3.83cd	2.32
0.6	2433.33a	31.56ab	19.77de	10.26b	11.25c	8.25bc	3.32
0.8	2341.33a	43.45a	45.01b	64.24a	23.25b	11.48b	8.00
1.0	2129.66a	33.42a	102.89a	79.37a	42.45a	36.15a	13.82

^aThe average total number of mitotic cells counted for each HgCl₂ treatment was determined in three replicates. ^bOther observed chromosomal aberrations include nuclear abnormalities, binucleated cells, irregular prophase, polyploidy, pulverization, anaphase lagging chromosome, multipolar cells, and polyploidy. The values designated by different superscript letters in each column are significantly different from each other at a level of *p* < 0.05

chromosome abnormalities, signifying cytogenotoxicity for HgCl₂ concentrations ranging from 0.6 to 1.0%. The order of aberrations triggered by interventions was 0.0 < 0.2% < 0.4% < 0.6% < 0.8% < 1.0%, indicating that high concentrations are associated with a greater risk of harm and genotoxicity.

4 Discussion

This study revealed chromosomal irregularities in mitotic cells of *Allium cepa* when exposed to different concentrations of HgCl_2 . Additionally, the results demonstrated the negative effects of the chemical on the root growth of *A. cepa*. The results revealed a range of chromosomal abnormalities at various stages of mitosis in onion roots exposed to the chemical, indicating that although this chemical can be used as a sterilizer or disinfectant alone or in combination with other chemicals as a catalyst, there are potential risks associated with its uses [18], due to its genotoxic and mutagenic properties [29, 30]. Plant root tips are usually the very part of the plant to come in contact with contaminants/pollutants in the soil or any growing medium. Hence the evaluation of the HgCl_2 effects on the root formation of *A. cepa* could provide insight into differential concentration effects and growth inhibition of the chemical on plants, as a model of possible cytotoxicity in cells generally. The observed decrease in the root formation with increased concentrations of HgCl_2 showed the chemical may have reduced pressure-induced water flux and nutrient conductivity thereby impacting negatively the root development. The tested concentrations in the current study might have affected the enzymatic activities of the pathways and the physiological processes that promote root formation and growth [31–33]. It is also evident from the current data that even low concentrations of HgCl_2 (0.2%) can induce arrays of mitotic disruptions, which can result in large-scale genetic alteration and mutation, and the chances of this increase with higher concentrations.

The genotoxicity of mercury primarily results from its capacity to react with the sulfhydryl groups of tubulins. This process inhibits spindle function and results in various forms of chromosomal aberrations, and this chemical can also induce polyploidy [29, 34]. Moreover, mercury triggers free radical production by generating osmotic stress [35, 36], which prompts apoptosis and consequent DNA damage [32]. Thus, the potential of HgCl_2 to cause disruptions in the sequential and directional process of mitotic cell division/cycle progression suggests cyto and genotoxicity. These effects can be inferred from the mitotic indices of the plants, as well as the various clastogenic and aneugenic events observed in the roots exposed to HgCl_2 . According to Schurz [37], mercury may hinder cellular antioxidant mechanisms, leading to cell degeneration and, ultimately, necrosis. Boujbiha et al. [38] confirmed that HgCl_2 is the most harmful mercury compound due to its strong affinity for proteins. They found that cells exposed to any amount of HgCl_2 experienced detrimental consequences, indicating that the chemical is hazardous to living tissues. In this study, even at a low concentration of 0.2% w/v, considerable chromosome damage was detected, implying that prolonged exposure to the substance might be hazardous, given its potential for penetrating living tissues and causing adverse effects on the genetic system [23, 39]. However, despite the potential risks associated with HgCl_2 , various laboratories, hospitals, and industries continue to use this chemical for different purposes [19, 20, 40]. This poses a threat to personnel who may be exposed to the chemical's harmful genotoxic effects.

In a study conducted by Hwang et al. [41], the toxic effects of HgCl_2 on keratinocytes and the subsequent impact on the expression of certain genes were demonstrated. The authors observed cell death and disruption of the cell membrane structure caused by the harmful effects of HgCl_2 . Several decades ago, Sander et al. [42] reported the occurrence of chromosomal damage during the mitotic process in barley exposed to mercury chloride. The results from the present study were consistent with earlier research. HgCl_2 toxicity was found to cause several chromosomal abnormalities, including fragmentation, lagging, stickiness, bridge formation, polyploidy, binucleation, and nuclear budding. These abnormalities could lead to DNA damage, alterations in the genetic system, and mutations or disorders [7]. As demonstrated in the present study, high levels of HgCl_2 hinder cellular division, leading to significant occurrences of diverse clastogenic changes and reducing the mitotic index. These findings support previous reports [43, 44] that suggest a correlation between high accumulation of heavy metals in plant roots and chromosomal irregularities, such as a decreased mitotic index, increased frequency of chromosome lagging, bridge formations, and nuclear lesions. These aberrations can interfere with cell division and result in disorders and even the death of cells. The occurrence of anaphase bridges, a decreased mitotic index, chromosome lag, and other chromosomal aberrations observed in this study is contingent on concentration. These findings are consistent with [45], which demonstrated that the genotoxicity induced by *Euphorbia hirta* extract also hinges on the concentration.

Chromosome bridging is attributed mainly to chromosome stickiness, which leads to delayed separation and unsuccessful detachment of these adhesive chromosomes. Internally within or between chromatids, chromosomal structures become bridged through a combination of homologous and nonhomologous chromosome exchanges, usually due to dicentric formation or defective replication processes. These events can result in disorders [46]. Chromosomes can become adhesive in two ways: through contraction or condensation, DNA depolymerization and partial dissolution of nucleoproteins. This study demonstrated a high rate of stickiness, suggesting the harmful impact of HgCl_2 on nuclear

materials, which may cause irreversible damage and cell death. Additionally, the occurrence of unaligned chromosomes suggested impaired organization and function of the spindle apparatus caused by HgCl_2 . The different degrees of C-mitosis detected in onion root cells suggest the presence of harmful substances in the growth media [47]. The *Allium cepa* test demonstrated a substantial correlation with potential impacts in mammals [10, 13], and because the assay can be employed without ethical concerns, it functions as a valuable model for assessing cytotoxicity. The *Allium cepa* test has been utilized to evaluate the possible chemical impacts of chromosomal aberrations and cytotoxicity [12, 44]. In these previous studies, the authors reported considerable chromosome distortion and cytotoxicity when high levels of chemicals were applied.

The results of this study may hold weight for tissue culturists, microbial laboratory technologists, patients, and industrial workers who are at risk of HgCl_2 toxicity due to their occupation as the information regarding the cytotoxicity of HgCl_2 obtained in this study could serve as a vital reference for ensuring adequate safety measures for those frequently working with this compound. Such measures are crucial given the documented potential harmful effects [48, 49].

5 Conclusion

The present study explored the cytotoxic impact of different HgCl_2 concentrations on onion root tips. The results showed that concentrations as low as 0.2% of the chemical can be detrimental and toxic to cells as it inhibits root formation and development, negatively impacts the cell division process, and causes chromosomal abnormalities, including lagging chromosomes, chromosome bridges in various stages of cell division, vagrants, sticky chromosomes, binucleated cells, chromosome fragmentation, and loculated nuclei. The majority of the induced abnormalities exhibited concentration dependence. HgCl_2 caused significant clastogenic changes in onion root tip cells, which suggested that it interferes with cell growth and the cell cycle, possibly affecting the genetic system and behaviour of the cells. The data gathered on HgCl_2 toxicity in this study can serve as a point of reference to ensure the implementation of appropriate safety measures by those who frequently use HgCl_2 to prevent its harmful effects.

Author contributions DAA, PAA, SI and RK designed the study. All the authors carried out the cytogenetic studies. DAA and PAA analysed the data. DAA, SAO KAA and RK interpreted the results and validated the correctness of the information. All the authors prepared and vetted the final manuscript.

Funding No funding was received for this study.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study protocol complies with relevant institutional, national, and international guidelines and legislation.

Competing interests The authors declare no potential competing interests.

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References

1. Sainis I, Banti CN, Owczarzak AM, Kyros L, et al. New antibacterial, non-genotoxic materials, derived from the functionalization of the anti-thyroid drug methimazole with silver ions. *J Inorg Biochem.* 2016;160:114–24.

2. Banti CN and Hadjikakou SK 2019. Evaluation of genotoxicity by micronucleus assay in vitro and by *Allium cepa* test in vivo. *Bio-protocol*. 9(14) e3311. <https://doi.org/10.21769/BioProtoc.3311>
3. Aslantürk OS. In vitro cytotoxicity and cell viability assays: principles, advantages, and disadvantages. In: Larramendy ML, Soloneski S, editors. *Genotoxicity—a predictable risk to our actual world* (1st edn). London: IntechOpen Ltd; 2018. p. 122.
4. Ishiyama M, Tominaga H, Shiga M, Sasamoto K, Okhura Y, Ueno KA. Combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. *BioloPharm Bull*. 1996;19(11):1518–20.
5. Asita AO, Matebesi LP. Genotoxicity of hormoban and seven other pesticides to onion root tip meristematic cells. *Afri J Biotechnol*. 2010;9(27):4225–32.
6. Sahu SC, Njoroge J, Bryce SM, Yourick JJ, Sprando RL. Comparative genotoxicity of nanosilver in human liver *HepG2* and colon *Caco2* cells evaluated by a flow cytometric in vitro micronucleus assay. *J Appl Toxicol*. 2014;34:1226–34.
7. Chrysouli MP, Banti CN, Kourkoumelis N, Panayiotou N, et al. Chloro (triphenylphosphine) gold (I) a forefront reagent in gold chemistry as apoptotic agent for cancer cells. *J Inorg Biochem*. 2018;179:107–20.
8. Lerda D, Biagi BM, Pelliccioni P, Litterio N. *Allium cepa* as a biomonitor of ochratoxin a toxicity and genotoxicity. *Plant Biol*. 2010;12(4):685–8.
9. Tedesco BS, Laughinghouse HD IV. Bioindicator of genotoxicity: the *Allium cepa* test. INTECH. 2012. <https://doi.org/10.5772/31371>.
10. Milonis I, Banti CN, Sainis I, Raptopoulou CP, et al. Silver ciprofloxacin (CIPAG): a successful combination of chemically modified antibiotic in inorganic-organic hybrid. *J Biol Inorg Chem*. 2018;23(5):705–23.
11. Leme DM, Marin-Morales MA. *Allium cepa* in environmental monitoring: a review on its application. *Mutat Res*. 2009;682:71–81.
12. Bakare AA, Adeyemi AO, Adeyemi A, Alabi OA, Osibanjo O. Cytogenotoxic effects of electronic waste leachate in *Allium cepa*. *Caryologia*. 2012;65(2):94–100. <https://doi.org/10.1080/00087114.2012.709786>.
13. Khalil AM, Salman WK, Al-Qaoud KM. Preliminary evaluation of acute cytogenotoxicity of a novel phenylboronic acid derivative; 2-(bromoacetamido) phenylboronic acid using the *Allium cepa* chromosome aberrations assay. *Caryologia*. 2016;70(1):34–41.
14. Fernandes TCC, Mazzeo DEC, Marin-Morales MA. Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide. *Pestic Biochem Physiol*. 2007;88:252–9. <https://doi.org/10.1016/j.pestbp.2006.12.003>.
15. Kassa BA. Cytotoxicity and genotoxicity evaluation of municipal wastewater discharged into the head of blue Nile river using the *Allium Cepa* test. *Sci Afri*. 2021;13(2021): e00911.
16. Kaymak F, Goc-Rasgele P. Genotoxic effects of raxil on root tips and anthers of *Allium cepa* L. *Caryologia*. 2009;62(1):1–9.
17. Das D, Mitra PK, Gupta S. Evaluation of cytotoxicity induced by the anti-cancerous drugs doxorubicin and erlotinib in *allium cepa* assay for eco-safety monitoring. *Cytologia*. 2021;86(3):19–199.
18. Simon M, Jönk P, Wühl-Couturier G, Halbach S. Mercury, mercury alloys, and mercury compounds. In: Simon M, Jönk P, Wühl-Couturier G, Halbach S, editors. *Ullmann's encyclopedia of industrial chemistry*. 6th ed. New York: Wiley-interscience; 2006. p. 15264–95.
19. Bengtsson UG, Hylander LD. Increased mercury emissions from modern dental amalgams. *Biometals*. 2017;30:277–83.
20. Bjorklund G, Dadar M, Mutter J, Aaseth J. The toxicology of mercury: current research and emerging trends. *Environ Res*. 2017;159:545–54.
21. Maillard AP, Fraise PA, Lambert J. Principles and practice of disinfection, preservation and sterilization. Hoboken Oxford: John Wiley & Sons; 2007. p. 4.
22. US Environmental Protection Agency, 2010. <http://www.epa.gov/hg/effects.htm> (accessed on 10th August 2023).
23. Oriquat GA, Saleem TH, Naik RR, Moussa SZ, Al-Gindy RM. A sub-chronic toxicity study of mercuric chloride in the rat. *Jordan J Biol Sci*. 2012;5(2):141–6.
24. Health Canada 2008 Human health risk assessment of mercury in fish and health benefits of fish consumption. Available online, http://www.hc-sc.gc.ca/fn-an/pubs/mercur/merc_fish_poisson-eng.php (accessed on 5th September 2023).
25. Khalil A, Maslat A, Hafez A, Mizyed S, Ashram M. Genotoxicity of different tert-butyl calyx [4] crowns. *Zeitschrift für Naturforschung C*. 2009;63:167–75.
26. Sehgal R, Roy S, Kumar VL. Evaluation of cytotoxic potential of latex of *Calotropis procera* and podophyllotoxin in *Allium cepa* root model. *Biocell*. 2006;30(1):9–13.
27. Fiskesjö G. *Allium* test I: a 2–3 day plant test for toxicity assessment by measuring the mean root growth of onions (*Allium cepa* L.). *Environ Toxicol Water Qual*. 1993;8(4):461–70.
28. Pavlica M, Besendorfer V, Roša J, Papeš D. The cytotoxic effect of wastewater from the phosphoric gypsum depot on common oak (*Quercus robur* L.) and shallot (*Allium cepa* var. *ascalonicum*). *Chemosphere*. 2000;41(10):1519–27.
29. Silva-Pereira LC, Cardoso PCS, Leite DS, Bahia MO, et al. Cytotoxicity and genotoxicity of low doses of mercury chloride and methylmercury chloride on human lymphocytes in vitro. *Braz J Med Biol Res*. 2005;38:901–7.
30. Shalan MG. Amelioration of mercuric chloride-induced physiologic and histopathologic alterations in rats using vitamin E and zinc chloride supplement. *Heliyon*. 2022;8(12): e12036.
31. Mondal NK, Das C, Datta JK. Effect of mercury on seedling growth, nodulation and ultrastructural deformation of *Vigna radiata* (L) Wilczek. *Environ Monit Assess*. 2015;187(5):241. <https://doi.org/10.1007/s10661-015-4484-8>.
32. Hossain KFB, Rahman MM, Sikder MT, Hosokawa T, et al. Selenium modulates inorganic mercury induced cytotoxicity and intrinsic apoptosis in PCl_2 cells. *Ecotoxicol Environ Saf*. 2021;207:1–10.
33. Kalinhoff C, Calderón NT. Mercury phytotoxicity and tolerance in three wild plants during germination and seedling development. *Plants (Basel)*. 2022;11(15):2046. <https://doi.org/10.3390/plants11152046>.
34. De Flora S, Benniceli C, Bagnasco M. Genotoxicity of mercury compounds a review. *Mut Res*. 1994;317:57–79.
35. Ehrenstein C, Shu P, Wickenheiser EB, Hirner AV, Dolfen M, Emons H, Obe G. Methyl mercury uptake and associations with the induction of chromosomal aberrations in Chinese hamster (CHO) cells. *Chem-Biol Interact*. 2002;141:259–74.
36. Durak D, Kalender S, Uzun FG, Demir F, Kalender Y. Mercury chloride-induced oxidative stress in human erythrocytes and the effect of vitamins C and E in vitro. *Afr J Biotechnol*. 2010;9(4):488–95.
37. Schurz F, Sabater-Vilar M, Fink-Gremmels J. Mutagenicity of mercury chloride and mechanisms of cellular defence: the role of metal-binding proteins. *Mutagenesis*. 2000;15:525–30.

38. Boujbiha MA, Hamden K, Guermazi F, Bouslama A, et al. Testicular toxicity in mercuric chloride treated rats: association with oxidative stress. *Reprod Toxicol*. 2009;28:81–9.
39. Su L, Wang M, Yin ST, Wang HL, et al. The interaction of selenium and mercury in the accumulations and oxidative stress of rat tissues. *Ecotoxicol Environ Saf*. 2008;70(3):483–9.
40. Dadar M, Adel M, Saravi HN, Dadar MA. Comparative study of trace metals in male and female *Caspian kutum* (*Rutilus frisii kutum*) from the southern basin of Caspian sea. *Environ Sci Pollut Control Ser*. 2016;23:24540–6.
41. Hwang T, Chen H, Changchien T, Wang C, Wu C. The cytotoxicity of mercury chloride to the keratinocytes is associated with metallothionein expression. *Biomed Rep*. 2013;1:379–82.
42. Sander C, Nillan RA, Kleinhof SA, Big BK. Mutagenic and chromosome breaking effects of mercury chloride in barley and human leucocyte. *Mut Res*. 1978;50:67–76.
43. Nefic H, Musanovic J, Metovic A, Kurteshi K. Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by alprazolam. *Med Arch*. 2013;67(6):388.
44. Sabeen M, Mahmood Q, Ahmad Bhatti Z, Faridullah Irshad M, et al. *Allium cepa* assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. *Saudi J Biolo Sci*. 2020;27(5):1368–74. <https://doi.org/10.1016/j.sjbs.2019.12.011>.
45. Ping YK, Darah I, Yusuf UK, Yeng C, Sasidharan S. Genotoxicity of *Euphorbia hirta*: an *Allium cepa* assay. *Molecules*. 2012;17(7):7782–91. <https://doi.org/10.3390/molecules17077782>.
46. Feretti D, Zerbini I, Zani C, Ceretti E, Moretti M, Monarca S. *Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of extracts from pesticide-treated vegetables and grapes. *Food Addit Contam*. 2007;24:561–72.
47. Bonciu E, Firbas P, Fontanetti CS, Wusheng J, et al. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia*. 2018;71:191–209.
48. Saljooghi AS, Delavar Mendi F. The effect of mercury in iron metabolism in rats. *J Clin Toxicol*. 2013;53:1–5. <https://doi.org/10.4172/2161-0495.53-006>.
49. Mohamed NA, Ali AM, Bakhoun SA, Abdel-Kader HH, Ahmed MA. Monitoring of oxidative stress biomarkers and toxicity of lead and mercury in catfish of Lake Mariout, Egypt: the role of Meso 2,3-Dimercaptosuccinic acid (DMSA) *Egyp. J Aqu Bio Fish*. 2019;23(2):165–82.

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