

Cytotoxic Effects of Ethidium Bromide on Onion (*Allium cepa*) Root Meristem Cells

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Abstract

Ethidium bromide (EtBr) is a widely used nucleic acid-intercalating dye with well-documented mutagenic potential, yet its cytogenetic effects on plant model systems remain poorly characterised. This study evaluated the cytotoxic effects of EtBr on *Allium cepa* root meristem cells using the *Allium* bioassay. Uniformly graded onion bulbs were exposed for ten days to five graded concentrations of EtBr (2–10 μ L of stock solution per 100 mL distilled water) alongside an untreated control, after which root tips were fixed, hydrolysed and stained for microscopic examination. Germination rate was highest in control (4.67 ± 1.80) and progressively decline as EtBr increases with the least observed in 10 μ L (0.20 ± 1.80), and the total mean value of roots growth was highest in control (4.67 ± 1.80) while the least was observed in 10 μ L (0.20 ± 1.80). The control of the mitotic phase distribution had 14 dividing cells and normal cells in all the phases of 2.80 ± 0.83 , with zero abnormal cells, while the number of abnormal cells increases and normal cells declined as EtBr concentration increase, and at 10 μ L EtBr (0.00 ± 0.00), there was zero normal cells observed. The frequency of chromosomal aberrations (CAs) was more pronounced in 10 μ L EtBr (42), while in the control and 2 μ L EtBr had little to no chromosomal aberrations (2 and 6 respectively). EtBr produced a concentration-dependent decline in both root traits and mitotic phase distribution, indicating suppressed cell proliferation. Chromosomal aberrations, including fragmented chromosomes, multinucleated cells, sticky prophase and metaphase, bridges in anaphase, and abnormalities in metaphase during the various mitotic phases, rose significantly with increasing dosage of EtBr, and the highest concentration tested produced the most pronounced clastogenic damage. These findings confirmed that EtBr is cytotoxic to *A. cepa* root cells, underscore the environmental risk posed by the handling and release of EtBr as laboratory effluent, and support the continued use of the *Allium cepa* assay as a sensitive, low-cost bioindicator for chromosome-damaging agents.



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Introduction

Ethidium bromide (EtBr), a planar, tricyclic phenanthridinium dye, has been a mainstay of the molecular biology laboratory for decades, valued as a nucleic acid stain in agarose gel electrophoresis and other visualisation techniques (Borst, 2005). Its ability to intercalate between the base pairs of double-stranded DNA underpins both its usefulness as a fluorescent probe and its toxicological profile (Amirijavid & Mohammadi, 2015). Intercalation distorts DNA conformation, interferes with replication and transcription, and has been linked to mutagenic outcomes across diverse biological systems (Reema et al., 2024; Amirijavid & Mohammadi, 2015). Structural and biophysical studies have repeatedly confirmed EtBr's intercalative binding mode and sequence preferences, providing a mechanistic basis for its capacity to induce base-pair perturbations and frameshift events when retained in cellular DNA (Galindo-Murillo & Cheatham, 2021).

Despite this widespread laboratory use and longstanding concern about its mutagenic potential, the cytotoxic and genotoxic effects of EtBr remain incompletely characterised in non-mammalian systems and at the chromosomal level (Kuypers et al., 2013; Reema et al., 2024; Singh et al., 2024). Much of the historical toxicology has focused on prokaryotic mutagenicity assays or on mitochondrial DNA depletion in cultured mammalian cells, leaving ecotoxicological and clastogenic endpoints in plant systems comparatively neglected (Amirijavid & Mohammadi, 2024). At the same time, EtBr is known to persist in laboratory waste and environmental matrices, which has prompted growing interest in its degradation and environmental fate and, with it, a clearer need to understand its biological effects beyond standard cell culture (Singh et al., 2024).

The *Allium cepa* (onion) root meristem assay is a well-established, cost-effective bioindicator for cytotoxicity, genotoxicity and chromosomal aberration induced by chemical agents (Halida et al., 2013; Animasaun et al., 2024). Its large, easily scored chromosomes, rapid meristematic cell cycle, and demonstrated concordance with mammalian

test systems across many classes of genotoxins have kept the assay in continuous methodological development, with reviews progressively extending its endpoints to include mitotic index, chromosomal breaks and bridges, micronuclei frequency and comet-assay integration (Nicuță et al., 2025). These properties make it a suitable model for investigating the roles of EtBr, as both an intercalating agent and an environmental contaminant, and whether it produces measurable cytogenetic damage at the chromosomal level (Mohammed et al., 2023).

Intercalating dyes, including acridines and other planar molecules, have long been linked to frameshift and chromosomal effects. Nonetheless, only relatively few studies have quantified EtBr-induced chromosomal aberrations in plant meristem cells using scoring criteria across a concentration range relevant to laboratory effluent or accidental spillage (Abubakar et al., 2025). Much of the available literature predates contemporary genotoxicity-testing standards, clear dose-response documentation, adequate replication, and standardised scoring of mitotic phases and aberration subtypes, while environmental-degradation studies have concentrated on removal technologies rather than in vivo genotoxic outcomes in sentinel organisms (Abubakar et al., 2025). This is more than an academic gap: waste-management decisions, laboratory safety policy and environmental risk assessment all stand to benefit from quantitative, mechanistic data on EtBr's cytogenetic effects in a widely used plant bioassay (Singh et al., 2024).

Mechanistically, EtBr intercalation is expected to manifest as chromosomal bridges, breaks, lagging chromosomes and related structural anomalies during mitosis (Cariello et al., 1998), since intercalators can generate strand breaks indirectly or induce mispairing that promotes deletions and other structural aberrations (Singh et al., 2024). The *Allium* system allows such aberrations to be observed directly by microscopy, in intact, actively dividing tissue; combined with mitotic index measurement, it can distinguish cytostatic effects (reduced cell-cycle progression) from genuinely genotoxic or clastogenic damage (Animasaun et al.,

2024). Notably, EtBr also acts as a topoisomerase-I poison in some clinical anticancer drugs, an action that does not support the popular assumption that EtBr is a potent human mutagen, though it does confirm toxicity at high concentration (Gentry et al., 2011).

Clarifying EtBr's chromosomal effects in *Allium cepa* therefore is crucial for several practical reasons. Many laboratories worldwide continue to use EtBr for its sensitivity and low cost, keeping the risk of occupational exposure or environmental release non-negligible (Galindo-Murillo & Cheatham, 2021); its persistence in waste streams and partial resistance to conventional wastewater treatment reinforce the need to evaluate impacts on non-target organisms (Hartwig et al., 2020); and data from plant bioassays contribute to the weight-of-evidence approaches that inform regulatory guidance and the selection of safer alternatives, which must themselves be evaluated for comparative safety (Singh et al., 2024). While EtBr's DNA intercalation and mutagenic potential are well described at the molecular level, contemporary, rigorously controlled cytogenetic studies of chromosomal aberration in plant meristem cells remain scarce. The present study therefore used the *Allium cepa* assay, with its validated endpoints and translational relevance, to generate dose-responsive, microscopically resolvable evidence of EtBr-induced clastogenic or aneugenic activity, evaluating root growth, mitotic indices and chromosomal aberrations across a concentration range, and considering the implications for laboratory waste handling and the selection of safer nucleic-acid stains.

Materials and Methods

Plant Material

Onion (*Allium cepa*) bulbs were purchased from Unilorin campus market Ilorin Nigeria (latitude 8°30'N and longitude 4°33' E/ latitude 8.500° N and 4.550° E), and sun-dried for six days. Healthy bulbs of uniform weight and size were selected and cleaned before use.

Preparation and Treatment with Ethidium Bromide

Ethidium bromide (0.015 g) was dissolved in 2 mL of distilled water to prepare a stock solution of 7.5 mg/mL ethidium bromide (0.75% w/v). Aliquots of the stock (0, 2, 4, 6, 8 and 10 µL, corresponding to treatments T0–T10) were each dissolved in 100 mL of distilled water to give six working concentrations, with T0 (0 µL) serving as the control. The base of each onion bulb was suspended over a beaker containing one of the working solutions so that the root zone remained in continuous contact with the treatment, and the set-up was prepared in three replicates following the approach of Animasaun et al. (2024).

Growth: Treatment of Onion Root with Different concentrations of Ethidium Bromide.

Onion roots were grown in beaker-type containers, with three replicated bulbs per treatment containing different concentrations of ethidium bromide (0, 2, 4, 6, 8 and 10 µL). Root tips were harvested daily over the exposure period of nine days for cytological analysis. There was differentiation in root length for different concentrations of EtBr compared to the control, marked by reduced root length, abnormal root tips, and colour variation, which was in contrast with the control following the approach of Animasaun et al. (2024).

Root Tip Fixation, Hydrolysis and slide Preparation

Tender root tips (1-2 cm) were excised with sterile scissors between 08:00am and 10:00am, coinciding with peak meristematic activity, and collected into labelled specimen tubes. Roots were pre-treated with ice-cold water (24 hours) to arrest the cell cycle and improve chromosome resolution, and then rinsed twice in distilled water. Fixation followed in freshly prepared 1:3 glacial acetic acid for 30 minutes to halt cellular activity without introducing artefacts. After a further rinse, root tips were hydrolysed in 5N HCl at room temperature for 15 minutes to soften the tissue for squashing. The hydrolysed tips were transferred to a clean slide, and a 1–2 mm section of the tip was dissected, stained with aceto-orcein for 15 minutes, and

covered with a coverslip. Gentle squashing spread the tissue into a single-cell layer suitable for microscopy following the approach of Animasaun et al. (2024).

Cytotoxicity Determination

Chromosome morphology and behaviour were compared between EtBr-treated and control cells following the approach of Animasaun et al. (2024). A concentration was scored as cytotoxic where treated cells showed mitotic irregularities, a reduced mitotic index, chromosomal damage, or cell death.

Microscopic Examination of Cytogenetic Characteristics

Slides were examined under a light microscope, beginning with $\times 10$ magnification and progressing systematically to $\times 40$. Dividing and non-dividing cells were scored for mitotic irregularities, chromosomal damage and cell death, and micronuclei frequency was recorded across treatment groups. Photomicrographs were captured with a camera Lucida attachment.

Data Analysis

Data on root growth were presented in a graph using SPSS version 20 (IBM Corp., Armonk, NY, USA), and the mean germination rate and mitotic phase were manually calculated.

Results

Effect of Different Concentrations of Ethidium Bromide on Germination Rate of Onion bulbs.

The results of the germination rate of onion bulbs treated with different concentrations of Ethidium bromide over nine days period, based on root tip emergence, revealed that only the control produced root tips (3.00 ± 0.10) after 24 hours, which were harvested, while there were no observable root tips for all the onion bulbs treated with EtBr (2-10 μL), as presented in Table 1. Root tips emerged on day two solely for the bulbs treated with 2 μL (2.00 ± 0.20), while EtBr (4-10 μL) onion bulbs had no root tips. The control had the highest average root growth value (4.67 ± 1.80), followed by 2 μL (1.70 ± 1.80) and 4 μL (1.10 ± 1.80), and the lowest was 10 μL (0.20 ± 1.80), as presented in Table 1. The increase in EtBr concentrations delayed root tip

emergence and reduced the number of root tips, as EtBr concentrations (2 and 4 μL) germinated and produced roots more readily than those exposed to higher concentrations (6, 8 and 10 μL), while untreated controls grew fastest of all, confirming a clear inhibitory, dose-dependent effect of EtBr on root growth and germination.

The number of *Allium cepa* cells observed at each mitotic phase, and the corresponding proportion of normal and abnormal cells, across ethidium bromide concentrations as presented in Table 2, showed that lower concentrations of EtBr (2-4 μL) had a higher number of actively dividing cells in the various mitotic phases, and the number of cells undergoing division decreased as the concentrations of EtBr increased. The control had the highest number of dividing cells (14) and normal cells in all the phases (2.80 ± 0.83), with no abnormal cells recorded, while abnormal cells were recorded in EtBr (2-8 μL) treated bulbs and a complete absence of cellular activity in 10 μL of EtBr. The Increase in concentrations of EtBr resulted in greater mitotic inhibition. Thus, as the concentration increased, cellular division was increasingly inhibited. The total root growth across the treatments showed a decline in root growth as concentrations of EtBr increased (2-10 μL), and the control had the highest root growth, as presented in Figure 1.

The cytological examination of root-tip mitotic cells revealed chromosomal aberrations in all treated groups, as illustrated in figure 4. Cytological analysis of the root meristem of *A. cepa* treated with different concentrations of EtBr (2-10 μL) revealed varying degrees of chromosomal abnormalities in 6 μL and 8 μL EtBr concentrations (5.10 ± 0.44 and 5.10 ± 0.54 respectively), while there was no normal cellular activities observed for 10 μL (0.00 ± 0.00). The observed abnormal divisions and chromosomal aberrations are shown in figure 4. The chromosomal aberrations across the various mitotic phases revealed stickiness in prophase, abnormal metaphase, bridges and lagging in anaphase and telophase, fragmented chromosomes, and multinucleated chromosomes with condensation, all of which were notable across the various concentrations of EtBr (2-10 μL). Higher

concentrations of EtBr (8-10 μL) were characterised by marked separation of nuclear material across the poles that resulted in abnormal metaphase, anaphase and telophase bridges, indicating that higher concentrations of EtBr are toxic and result in cytotoxicity of onion cells. The chromosome damage was evident by comparison with the untreated control, and the reduction in mitotic activities with increasing EtBr concentration is consistent with cytotoxic and mutagenic activity.

Discussion

Effect of Different Concentrations of Ethidium Bromide on Germination Rate and Root of Onion bulbs.

Plant roots are significant in mineral absorption from the soil because of their direct contact with soil, which also exposes them to contaminants present within the soil.

Table 1: Effect of Different Concentrations of Ethidium Bromide on Germination Rate of Onion bulbs (number of root tips harvested per day, T₀–T₁₀).

Number of roots harvested/Day	Treatments (μl)					
	T ₀ :0	T ₂ :2	T ₄ :4	T ₆ :6	T ₈ :8	T ₁₀ :10
1	3.00±0.10 ^e	0.00±0.00 ^e	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^b
2	2.00±0.20 ^f	1.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^b
3	5.00±0.10 ^c	3.00±0.10 ^b	2.00±0.10 ^b	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^b
4	3.00±0.20 ^e	0.00±0.00 ^e	1.00±0.10 ^c	2.00±0.10 ^b	0.00±0.00 ^c	0.00±0.00 ^b
5	7.00±0.20 ^a	3.00±0.10 ^b	0.00±0.00 ^d	1.00±0.10 ^c	2.00±0.10 ^a	0.00±0.00 ^b
6	4.00±0.10 ^d	2.00±0.20 ^c	0.00±0.00 ^d	1.00±0.10 ^c	1.00±0.10 ^b	0.00±0.00 ^b
7	5.00±0.20 ^c	4.00±0.20 ^a	5.00±0.20 ^a	3.00±0.20 ^a	0.00±0.00 ^c	2.00±0.10 ^a
8	7.00±0.20 ^a	2.00±0.10 ^c	0.00±0.00 ^d	1.00±0.10 ^c	1.00±0.10 ^b	0.00±0.00 ^b
9	6.00±0.20 ^b	1.00±0.10 ^d	2.00±0.10 ^b	0.00±0.00 ^d	2.00±0.10 ^a	0.00±0.00 ^b
Total mean value	4.67±1.80 ^a	1.70±1.80 ^b	1.10±1.80 ^b	0.70±1.80 ^c	0.6±1.80 ^c	0.20±1.80 ^d
P-value	0.001	0.001	0.001	0.001	0.001	0.001

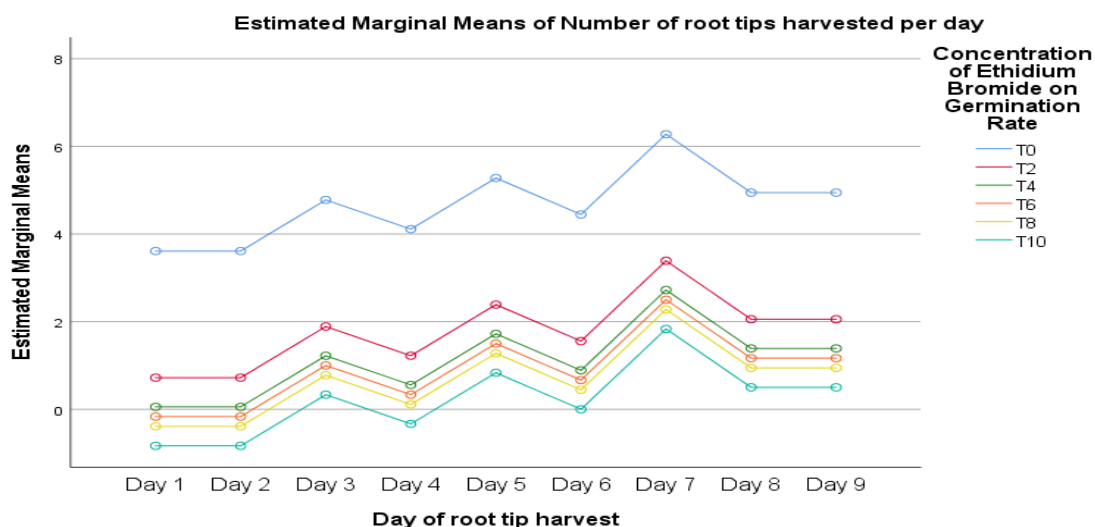
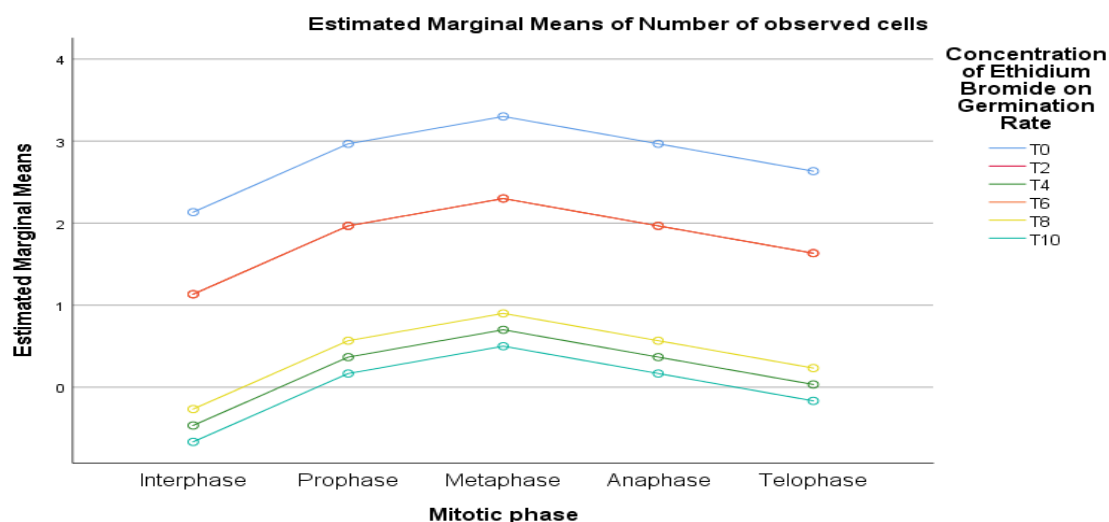
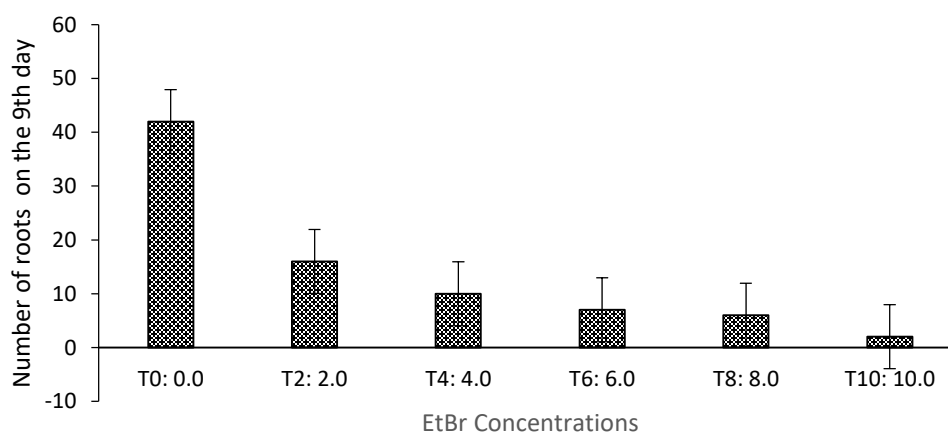


Figure 1: Relationship Between Ethidium Bromide Concentration and Germination Rate of Onion bulbs.

Table 2: Effect of Different Concentrations of Ethidium Bromide on Mitotic Stages and Cytological Abnormalities in Onion Root Tips Cells.

Treatments (μ l)	Number of observed cells in the mitotic phases					Normal cells	Abnormal cells
	I	P	M	A	T		
T ₀ :0	2.00 $\pm$ 0.20 ^a	4.00 $\pm$ 1.30 ^a	3.00 $\pm$ 1.10 ^a	2.00 $\pm$ 0.10 ^b	3.00 $\pm$ 1.00 ^a	2.80 $\pm$ 0.83 ^a	0.00 $\pm$ 0.00 ^c
T ₂ :2	0.00 $\pm$ 0.00 ^c	2.00 $\pm$ 1.10 ^b	3.00 $\pm$ 0.20 ^a	2.00 $\pm$ 1.00 ^b	2.00 $\pm$ 0.40 ^b	1.80 $\pm$ 1.09 ^b	0.10 $\pm$ 0.00 ^b
T ₄ :4	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^c	1.00 $\pm$ 1.10 ^c	0.00 $\pm$ 0.00 ^d	0.00 $\pm$ 0.00 ^d	1.80 $\pm$ 0.83 ^b	0.10 $\pm$ 0.00 ^b
T ₆ :6	1.00 $\pm$ 0.20 ^b	2.00 $\pm$ 1.20 ^b	2.00 $\pm$ 0.30 ^b	3.00 $\pm$ 1.10 ^a	1.00 $\pm$ 1.01 ^c	0.20 $\pm$ 0.44 ^c	5.10 $\pm$ 0.44 ^a
T ₈ :8	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^c	1.00 $\pm$ 0.20 ^c	1.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^d	0.40 $\pm$ 0.54 ^c	5.10 $\pm$ 0.54 ^a
T ₁₀ :10	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^d	0.00 $\pm$ 0.00 ^d	0.00 $\pm$ 0.00 ^d	0.00 $\pm$ 0.00 ^d	0.00 $\pm$ 0.00 ^c
TMV	0.50 $\pm$ 0.83 ^d	1.33 $\pm$ 1.63 ^c	1.67 $\pm$ 1.21 ^c	1.33 $\pm$ 1.21 ^c	1.00 $\pm$ 1.26 ^c	3.83 $\pm$ 5.307 ^a	2.00 $\pm$ 2.366 ^b
P-value	0.042	0.042	0.042	0.042	0.042	<0.001	<0.001

Key: TMV = Total mean value, I = interphase, P = prophase, M = metaphase, A = anaphase, T = telophase

**Figure 2: Relationship between Ethidium Bromide Concentration and Mitotic Phases, and Cytological Abnormalities in Onion Root Tips Cells.****Figure 3: Effect of Different Concentrations of Ethidium Bromide on Onion Root Growth on the Ninth Day of Treatment.**

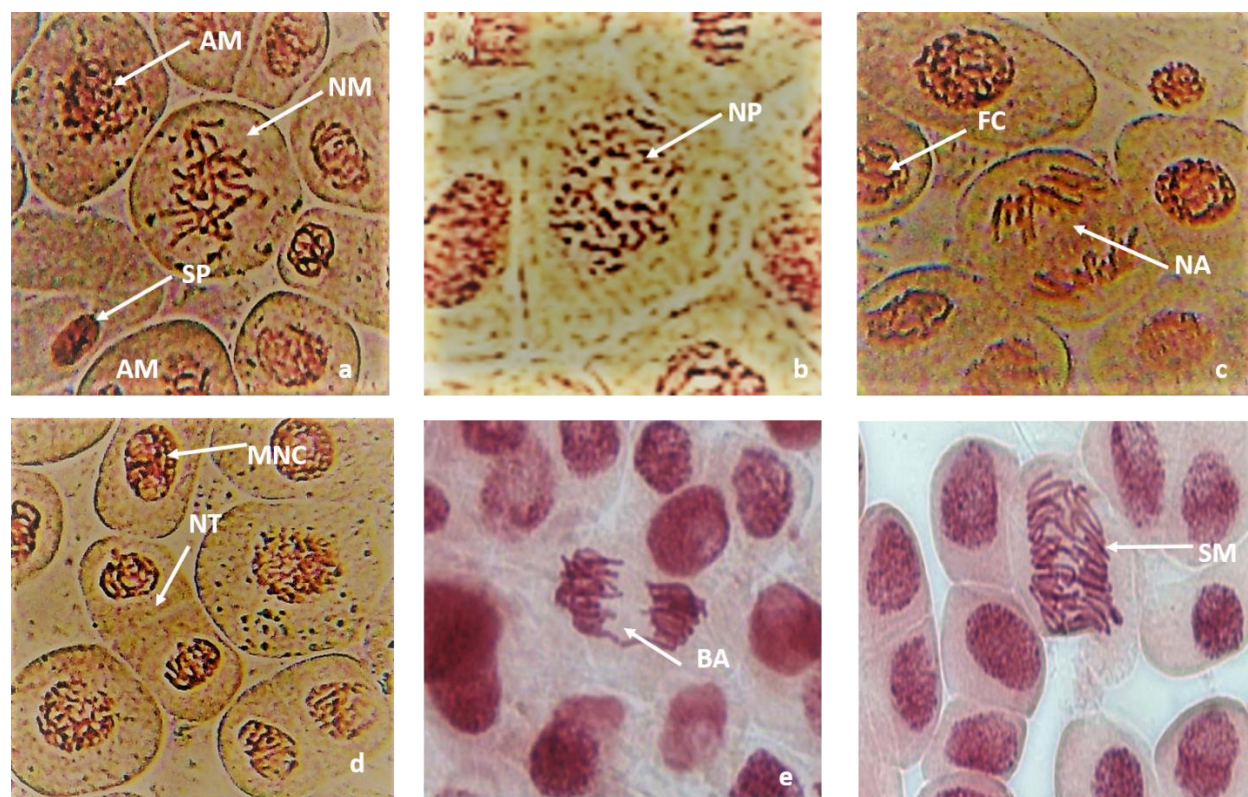


Figure 4: Chromosomes of an ethidium bromide-treated *Allium cepa* root cell observed at successive mitotic stages (a-f, above).

(a) AM: Abnormal metaphase; NM: Normal metaphase; SP: Sticky prophase; (b) NP: Normal prophase; (c) FC: Fragmented chromosome; NA: Normal anaphase; (d) MNC: multinucleated chromosomes; NT: Normal telophase; (e) BA: Bridged anaphase; (f) SM: Sticky metaphase.

Therefore, the cytotoxic evaluation of ethidium bromide (EtBr) on the germination, root development and chromosomal root formation of *Allium cepa* could provide insight into the effects of varying concentrations of EtBr on plants as a model of possible cytotoxicity in cells generally.

The increase in concentrations of ethidium bromide had a progressive negative effect on the germination rate, root growth and chromosomal behaviour of *A. cepa* exposed to EtBr (2-10 μL), while decreased EtBr concentrations produced a better germination rate. The control had the highest average number of root tips harvested per day within 1-9 days (4.67 ± 1.80), and the least was from EtBr treated with 10 μL (0.20 ± 1.80). This could be attributed to the cytotoxic effect of EtBr on the cells by reducing water influx and nutrient absorption, which negatively affects the growth and development of the root. Indicating that increasing

concentrations of EtBr interfere with the biocatalytic activities within the cell, disrupting the physiological processes that promote root formation and development. This is in tandem with Animasaun et al. (2024), who observed a decrease in root formation with increased concentrations of HgCl_2 (0.6–1.0%) and increased frequencies of multipolar cells, binucleated cells, and nuclear abnormalities in the treated bulbs. Similarly, Tavareker et al. (2025) reported that substances that inhibit *A. cepa* cell division and cause cell death can be categorised as cytotoxic through these parameters, while the genotoxic effects are evaluated by examining chromosomal aberrations, such as breaks, gaps, and micronuclei formation, which indicate DNA damage or chromosomal instability of *A. cepa*. This is in tandem with the observed effects of the tested concentrations of EtBr in this study.

Effect of Different Concentrations of Ethidium Bromide on Mitotic Stages and Cytological Abnormalities in Onion Root Tips Cells.

The EtBr concentrations reflect both disrupted metabolic activity and impaired nutrient uptake, as chromosomal disruption was evident even at the lowest concentration tested (2 μ L) and became more pronounced at higher concentrations at 6 μ L and 8 μ L EtBr (5.10 ± 0.44 and 5.10 ± 0.54), while there was no normal cellular activities observed for 10 μ L (0.00 ± 0.00), consistent with genetic alteration arising from mitotic interference. This is in alignment with the report of Animasaun et al. (2024) that the frequency of chromosomal aberrations differs notably with different concentrations of HgCl₂ (0.2-1.0%), and reflects in anaphase bridges characterised by incomplete segregation of nuclear material to the poles owing to persistent DNA entanglement between sister chromatids, stickiness, C-mitosis, chromosome fragmentation, and micronucleus formation. Onion root cells treated with higher concentrations (8 and 10 μ L) of EtBr had more aberrations, a pattern consistent with the findings of Tavarekere et al. (2025) and Animasaun et al. (2024) who reported that root cells subjected to elevated concentrations of HgCl₂ leads to an increased occurrence of chromosomal aberrations while low concentrations (0.2–0.4%) of HgCl₂ resulted in chromosome genotoxicity and damage in treated cells. This indicates that EtBr concentrations (2-10 μ L) possess cytotoxic effects on cells, causing chromosomes to become sticky clumps, fragmented and multinucleated, resulting in cell sterility.

Growth and survival tracked concentration closely revealed that roots exposed to 2, 4 and 6 μ L EtBr exhibited reduced growth alongside elevated chromosomal aberration, whereas 8 and 10 μ L were associated with outright plant death. The lower the EtBr concentration, the better *A. cepa* grew, while the untreated control developed faster and was the most consistent of all. This is linked to the planar aromatic nature of EtBr, which allows it to easily slide between DNA base pairs, intercalating into essential cellular processes, resulting in dysfunction, interruption of the movement of DNA materials that facilitates replication and

transcription and leading to frameshift mutations. This aligns with Animasaun et al. (2024) who reported that concentration of 0.4% (HgCl₂) resulted in the development of micronuclei in a considerable number of cells, with an even greater amount found in those exposed to 0.6% (HgCl₂), which demonstrates the negative effects of the chemical on the root growth of *A. cepa* due to its capacity to react with the sulfhydryl groups of tubulins to inhibits spindle function and results in various forms of chromosomal aberrations, and inducement of polyploidy and osmotic stress which prompts apoptosis and consequent DNA damage. Similarly, Bakare et al. (2021) reported that plant morphology ultimately reflects underlying metabolic activity, and phenotype is the visible output of a gene-controlled cascade of physiological and biochemical reactions. Therefore, the disruption of these processes by EtBr provides a plausible route to the cytotoxicity of *A. cepa* in this study. The disruption of spindle-fibre organisation and movement during cell division, which is ATP-dependent. Reduced ATP synthesis in EtBr-treated root-tip cells may therefore impair spindle assembly, in turn disrupting chromosome alignment at metaphase and poleward migration during anaphase. This aligns with Siddiqui & Hasan (2007), who linked defective spindle formation and chromosome segregation during mitosis to lagging chromosomes, chromosome stickiness and bridge formation. Konuk et al. (2007) reported that EtBr's genotoxicity has been documented for decades through chromosome stickiness that arises from improper folding of chromosomes into single chromatids, causing chromatin fibres to intermingle and chromosomes to adhere via sub-chromatid bridges, with the incidence of lagging chromosomes. Jamil et al. (2025) also reported that the concentration (EC₅₀) on the growth of *A. cepa* cells calculated for methylparaben and propylparaben was 75 ug/mL (2.70 ± 0.10 cm) and 25 ug/mL (2.70 ± 0.2 cm), respectively and the dose and time dependent decrease in mitotic index (MI), increase in chromosomal aberrations (CAs) and increase in DNA damage were observed by the exposure of *A. cepa* root tips to methylparaben and propylparaben upon 24 h and 48 h exposure periods is both

cytogenic and genotoxic. The concentrations and consistent exposure to EtBr have a damaging impact on cellular activities, DNA replication, and repair.

This study demonstrates a clear impairment to the root germination, growth and development of *A. cepa* by EtBr presence at varying degree of impairment from different concentrations and its subsequent mutagenic effect resulting in chromosomal aberrations of *A. cepa*, which result in stunted development and eventual death confirms the cytogenic effect of EtBr and the suitability of *A. cepa* as an excellent organism for cytotoxic screening for monitoring of environmental mutagens that is cost effective, and ethically seamless unlike human and animal cells with a substantial correlation in outcome.

This study provides information that suggests that careful handling and disposal of EtBr is important to avoid biological and environmental hazards.

Conclusion

Ethidium bromide concentrations as low as 2 µL impaired the cellular activity of onion root meristems and disrupted normal cell division. Toxicity increased with concentration (2-10 µL), evident in poorer germination, reduced root development, and irregular chromosomal form and function, including mitotic bridges, sticky and lagging chromosomes, fragmented chromosomes, and lobulated nuclei. These findings indicate that ethidium bromide interrupts normal cell development and can produce mutagenic outcomes in the chromosome complement as a result of its toxicity. Careful handling and proper disposal of this compound are therefore essential to limit its adverse effects.

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Authors Contribution

Study design: Buah, J. D., & Animasaun, D. A. Data Collection: Adejumo, G. A. & Buah, J.D. Analysis and result interpretation: Buah, J. D., & Animasaun,

D. A. Draft manuscript: Buah, J.D., Omotoso, A. A., & Animasaun, D. A.

Conflict of interest

There is no conflict of interest amongst the authors

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