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Comparative Cytogenetic Evaluation of 8-Hydroxyquinoline, Paradichlorobenzene, and Colchicine on Mitosis in Allium cepa Root Meristem Cells

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ABSTRACT

This study investigates the cytogenetic effects of three chemical mutagens—8-hydroxyquinoline (8-HQ), paradichlorobenzene (PDCB), and colchicine—on the mitotic behaviour of *Allium cepa* var. aggregatum root meristem cells. Root tips were treated with 0.004 N 8-HQ, 0.1% PDCB, their combinations, and a mixture of all three agents for five hours. Cytological preparations were made using acetoorcein staining and examined microscopically. The untreated control showed normal mitotic stages, while treated samples exhibited various abnormalities including c-metaphase, chromosomal stickiness, lagging and vagrant chromosomes, micronuclei, polyploidy, binucleated cells, giant cells, and multipolar anaphase. Notably, colchicine and PDCB prominently induced c-metaphase and polyploidy, whereas 8-HQ treatments were associated with chromosomal stickiness and anaphase bridges. Combined treatments significantly intensified the frequency and diversity of chromosomal aberrations, suggesting a synergistic mutagenic effect. These results confirm the dose- and time-dependent genotoxic potential of these agents and emphasize their applicability in cytogenetic studies and plant breeding programs targeting genetic improvement through polyploidy induction.

INTRODUCTION

Allium cepa L., commonly known as onion, is a biennial herbaceous plant belonging to the genus *Allium* within the family Liliaceae (alternatively classified under Alliaceae by some taxonomists). Native to central Asia, it is now widely cultivated across the globe as an essential food crop. The species is primarily grown for its underground bulb, which serves as a nutrient storage organ and is valued for its culinary versatility and medicinal properties. The plant produces a hollow, cylindrical scape that may reach a height of 75–180 cm, terminating in an umbel of small flowers. The bulb of *A. cepa* is composed of modified, concentric leaf bases and contains high moisture content—approximately 90% water—along with dietary fiber, essential vitamins (B1, B2, and C), selenium, and potassium. These constituents contribute to the onion's broad spectrum of health-promoting effects, including antidiabetic, anticancer, antioxidant, and cardioprotective properties. In addition, onion peels have shown therapeutic applications such as the prevention of hypertrophic scars due to their bioactive compounds. From a cytogenetic perspective, *Allium cepa* is a well-established model organism used in genetic and mutagenic studies. It possesses a diploid chromosome number of 2n = 16, with a distinct karyotype comprising six pairs of metacentric, one pair of submetacentric, and one pair of subtelocentric chromosomes (12m + 2sm + 2st). These relatively large chromosomes allow for easy visualization of mitotic and meiotic stages under a light microscope, making *A. cepa* an ideal subject for chromosomal analysis and the evaluation of genotoxic agents such as Paradichlorobenzene, 8-Hydroxy quinoline, Paradichlorobenzene and Colchicine.

8-Hydroxyquinoline (8HQ), also known as oxine, is a quinoline derivative originating from both natural plant sources and synthetic processes. It was first synthesized by Hugo Weidel and Albert Cobenzl in 1880 by decarboxylating oxycinchoninic acid, with further developments by chemists like Zdenko Skraup and Otto Fischer. Among seven isomeric monohydroxyquinolines, 8HQ stands out for its potent ability to chelate metal ions, particularly divalent metals. This strong metal-coordinating property has led to its widespread use in analytical chemistry, agriculture (as a fungicide), and various industrial applications including textiles and wood preservation. In aqueous solutions, 8-hydroxyquinoline has a pKa of approximately 9.9 and forms stable 8-

hydroxyquinolinato chelates upon deprotonation. It has also found applications in advanced materials such as organic light-emitting diodes (OLEDs), where substituted derivatives affect luminescence properties. Furthermore, it exhibits various biological activities, functioning as an antiseptic, disinfectant, and even showing potential as a transcription inhibitor and anti-cancer agent. Biologically, 8HQ plays a role in influencing metal homeostasis, which is critical for maintaining metabolic balance. Its bioactivity and therapeutic potential stem from its capacity to chelate metals, making it useful in managing conditions related to metal overload or deficiency. Notably, the compound is also released by the invasive plant *Centaurea diffusa*, demonstrating allelopathic effects on non-coevolved species. The relevance of 8-hydroxyquinoline in cytogenetics arises from its c-mitotic action—its ability to interfere with spindle fiber formation during mitosis. It disrupts normal chromosomal movements by arresting cells at metaphase, which is a key feature exploited in chromosome studies and polyploidy induction. However, in isolation, 8HQ may not always be effective in inducing polyploidy. Studies such as those by Prakhar and Swaminathan (1951) confirmed its c-mitotic properties, although no polyploid cells were observed in *Trigonella foenum-graecum* even after treatments up to 80 minutes. This limitation is likely due to insufficient exposure time. The mechanism behind 8HQ-induced polyploidy is believed to involve chemical imbalance at the cellular level, particularly affecting nucleic acid synthesis and spindle apparatus organization. The disruption causes the formation of multinucleate cells and irregular chromosomal separations, which, if followed by cell division, can yield polyploid progeny. Such somatic reduction and abnormal mitotic figures support the idea of 8HQ as an important tool in cytological research and plant breeding programs aimed at developing homozygous lines and improving genetic variability.

Paradichlorobenzene (1,4-dichlorobenzene, PDCB) is an aryl halide compound with the chemical formula $C_6H_4Cl_2$. It is a white crystalline solid with a distinctive odor and is primarily used as a fumigant insecticide, deodorizer, and disinfectant. Due to its high volatility and low water solubility, PDCB is commonly found in mothballs, urinal cakes, and air fresheners. It is also a precursor in the manufacture of polymers and other industrial chemicals. While PDCB is useful in pest control, prolonged exposure raises concerns due to its potential carcinogenicity and toxicological effects on humans and the environment. Recent studies have highlighted a novel use of PDCB in the field of plant biotechnology—its role in inducing polyploidy. Polyploidy refers to the condition of having more than two complete sets of chromosomes and is a key mechanism in plant breeding for enhancing desirable traits such as fruit size, disease resistance, and stress tolerance. Polyploidization can be achieved chemically by disrupting normal spindle fiber formation during cell division, preventing chromosome separation and resulting in chromosome doubling. Like traditional agents such as colchicine, PDCB has been found to interfere with mitotic processes, potentially by binding to microtubule proteins and inhibiting proper chromosome segregation. This disruption leads to the formation of polyploid cells. Chemically induced polyploidy can bring about significant morphological and physiological changes, including increased cell size, altered plant architecture, and enhanced biomass. In fruits and other horticultural crops, polyploidy can translate into commercially beneficial traits such as larger fruit size and improved shape—advantages that are difficult to consistently achieve through conventional cultural practices or hybridization at the diploid level.

Colchicine is an alkaloid that features a carbotricyclic structure, consisting of a 5,6,7,9-tetrahydrobenz [a]heptalene core with four methoxy groups at positions 1, 2, 3, and 10, an oxo group at position 9, and an acetamido group at position 7. It is derived from plants of the *Colchicum* genus. Colchicine acts as a microtubule-destabilizing agent and is a plant metabolite. It is categorized as a carboxylic acid, an alkaloid, an aromatic ether, and an acetamide. The natural product, N-(1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl)acetamide, is found in *Colchicum crocifolium*, *Colchicum doerfleri*, and other organisms. Colchicine is present in individuals only if they have used or taken the drug. It is a major alkaloid from *Colchicum autumnale* L. and other *Colchicum* species. Primarily, colchicine is used to treat gout and has also been used for familial Mediterranean fever (periodic disease). Although the precise mechanism of action is not fully understood, in gout patients, colchicine seems to interrupt the cycle of monosodium urate crystal deposition in joint tissues and the subsequent inflammatory response, thereby preventing acute attacks. It reduces leukocyte chemotaxis and phagocytosis and inhibits the formation and release of a chemotactic glycoprotein during urate crystal phagocytosis. Colchicine also inhibits urate crystal deposition, which is enhanced by low pH in tissues, likely by inhibiting glucose oxidation and subsequent lactic acid production in leukocytes. Colchicine does not possess analgesic or antihyperuricemic properties. Colchicine interferes with microtubule assembly in various cells, including leukocytes, by binding to and disrupting the polymerization of the tubulin subunit. While some studies suggest this action does not significantly contribute to colchicine's antigout effects, recent in vitro research indicates it might play a partial role.

Materials and Methods

The study was conducted at the Department of Botany, Maharani Cluster University, Bangalore. Root apical meristems of *Allium cepa* var aggregatum (onion) were used as plant models to determine cell cycle modulation and metaphase-arresting activities.

- Sample Collection and Preparation for treatment

Allium cepa var aggregatum was obtained from K R Market, Bengaluru. Onion bulbs of similar sizes that had wintered and budded were selected. The dried external leaves and roots were removed before the bulbs were planted in soil until the roots sprouted. Rapidly growing root tips of *Allium cepa* (1.0-2.0 cm in length) were immersed in vials containing 0.004 N ~~8—~~8-hydroxyquinoline and 0.1% paradichlorobenzene separately and both of these combined in a separate vial and– both of these combined with colchicine solution in a separate vial. The immersion time for each treatment was 5 hours. The stem disc was positioned to just touch the solutions, and the samples were protected from direct sunlight. The effect of these treatments was tested for 5 hours. 0.004 N 8 hydroxyquinoline, 0.1% paradichlorobenzene and 0.1% colchicine were used to study their effects on mitosis inhibition. *Allium cepa* flower buds were treated with 0.004 N 8 hydroxyquinoline for 5 hours to study its effects on meiosis inhibition.

- Chromosome Preparations

Root tips were harvested between 9 am and 12 pm and transferred into a beaker containing 1N hydrochloric acid, kept in a water bath for 6 minutes at 60°C. The cell walls were dissolved by acid hydrolysis. The hydrolyzed root tips were then transferred to a watch glass with 8-9 drops of acetoorcein per treatment and one drop of 1N HCl was added. The watch glass was warmed using a spirit lamp and left for 4-5 minutes.

- Root tips Preparation for Microscope

Approximately 1.5 mm of the root tip was cut off and placed in a drop of acetocarmine stain on a clean microscopic slide and gently tapped to create a squash. An additional –acetocarmine stain was added and left for 2-3 minutes. Coverslips were placed over the squash, and excess stain was removed using blotting paper. The slides were then observed under a light microscope at different magnifications (10x, 40x, 100x) to observe various stages of mitosis, at 100x, cedar wood oil was used.

Results

The meristematic regions of *Allium cepa* roots without any treatment (control) displayed a normal mitotic distribution. All four stages of cell division—prophase, metaphase, anaphase, and telophase were observed [plate 1, figs.a,b,c,d,e,f]. Most actively dividing normal cells were in prophase, few in metaphase, anaphase, and telophase stages of cell division. The Metaphase chromosomes were lined up at the equator and were evenly pulled toward the spindle poles for the cells at anaphase. No abnormal chromosomes were observed.

Allium cepa showed various mitotic abnormalities in the root meristem cells. The results revealed several chromosomal abnormalities like laggards, stickiness, vagrant chromosomes, binucleated cells, nuclear lesions, giant cells, and c-mitosis at different levels of treatment. Aberrations were more in root tips

treated with 8 hydroxyquinoline compared to paradichlorobenzene. Paradichlorobenzene showed more prophase arrest than metaphase. Paradichlorobenzene induced polyploidy in prophase. Same results were seen in the combined treatment of paradichlorobenzene and 8-hydroxyquinoline. A combination of 3 (Colchicine, paradichlorobenzene and 8 hydroxyquinoline) was more effective in inducing mutation. With an increase in time for treatment, more mutations were observed.

PLATE 1

Normal allium cepa

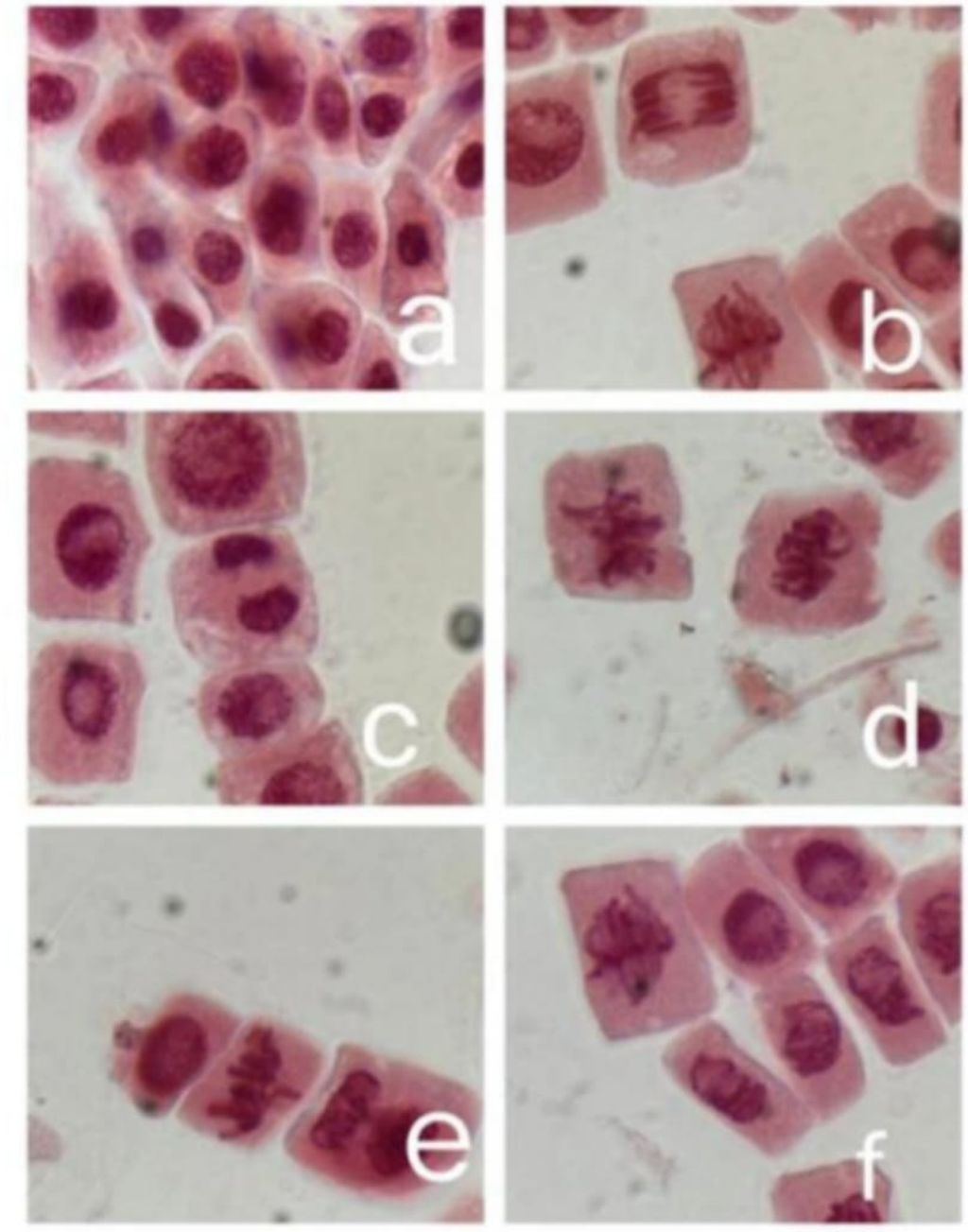

PLATE 1: *Allium cepa*; normal mitosis, a. Normal prophase, b. Normal metaphase and anaphase, c. Normal telophase, d. Normal metaphase, e. Normal anaphase, f. Normal telophase and metaphase

PLATE 2

Root tips of allium cepa
0.0048 hydroxyquinolone

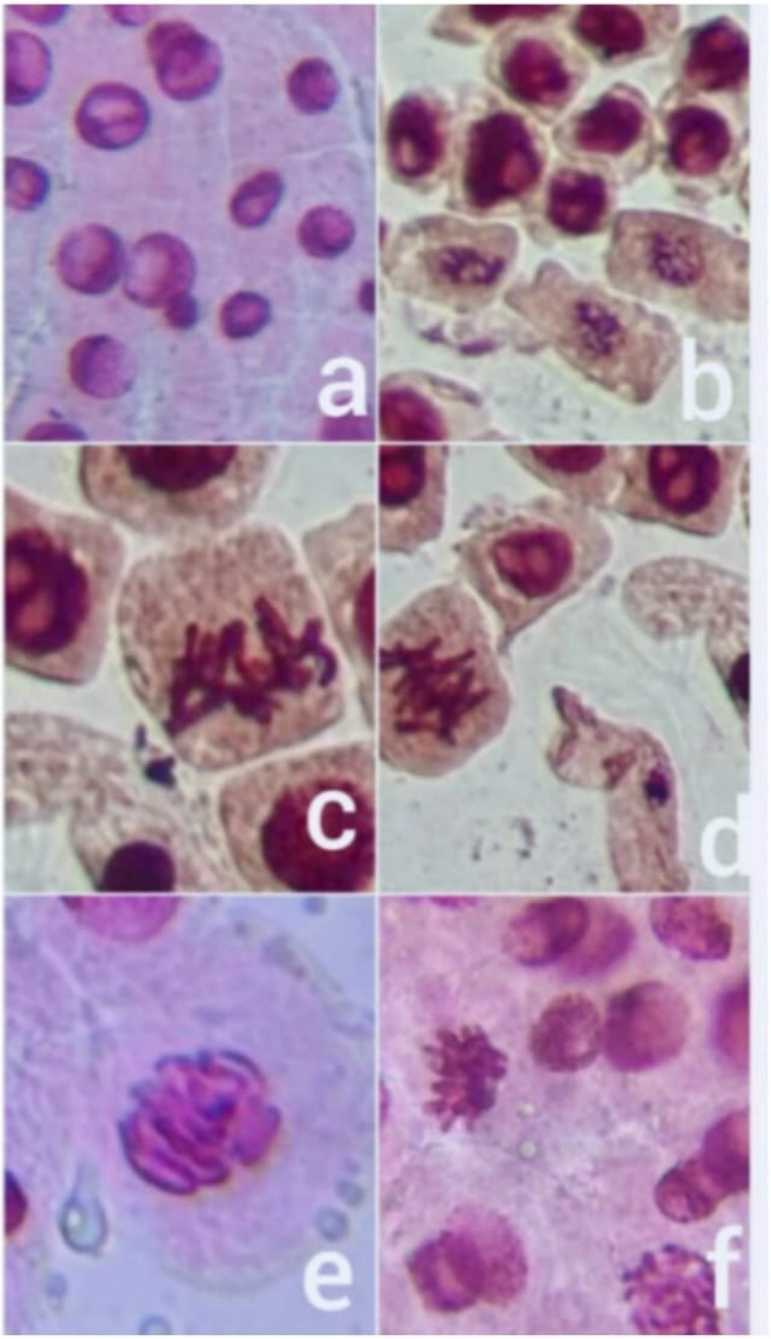

PLATE 2: *Allium cepa*; 0.004N 8 Hydroxy quinolone treated roots, a. Micronucleus, b. Fragmented chromatin, c. Sticky chromosomes, d. C-metaphase, e. Irregular metaphase, f. Nuclear lesions with C-metaphase

PLATE 3

**Root tips of allium cepa
0.004N 8 hydroxy
quinolone treated roots**

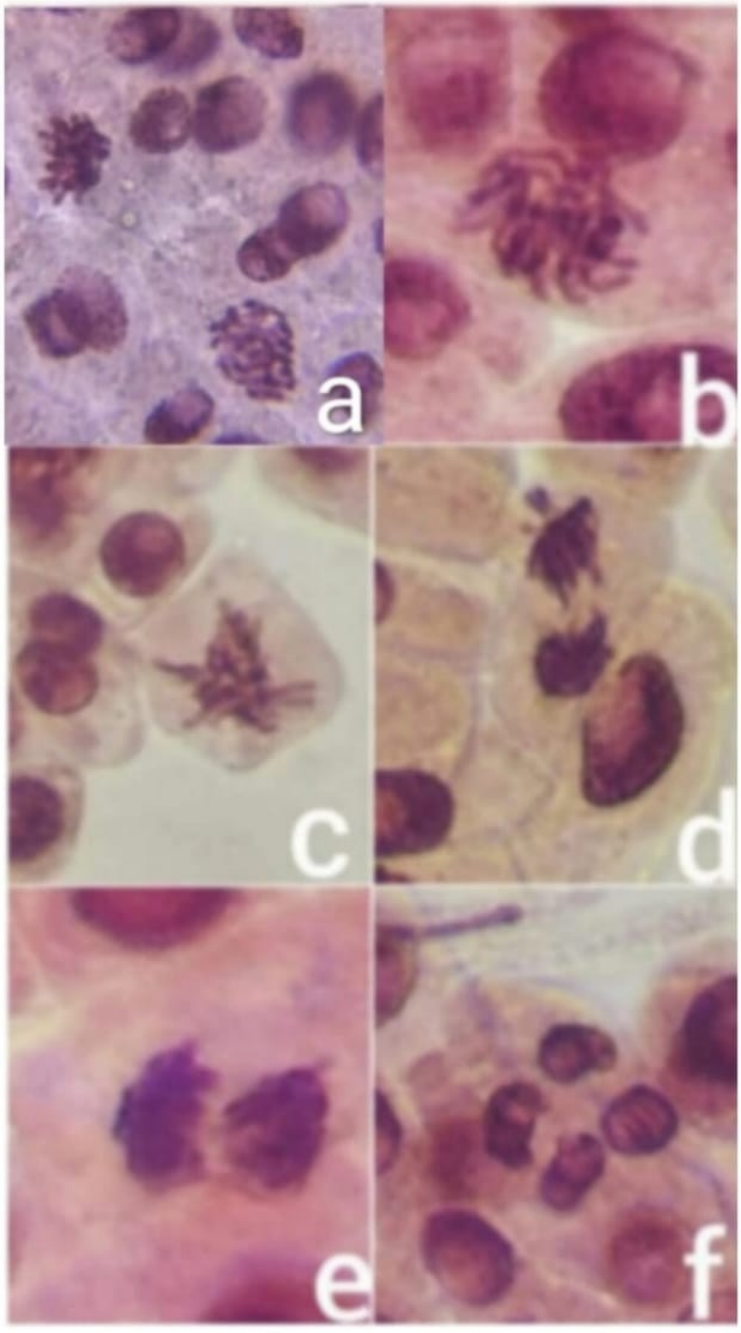

PLATE 3: *Allium cepa*; 0.004N 8 Hydroxy quinolone treated roots, a. Polyploid anaphase, b. Polyploid metaphase, c. Irregular metaphase, d. Vagrant and laggard, e. Irregular anaphase, f. Irregular anaphase

PLATE 4

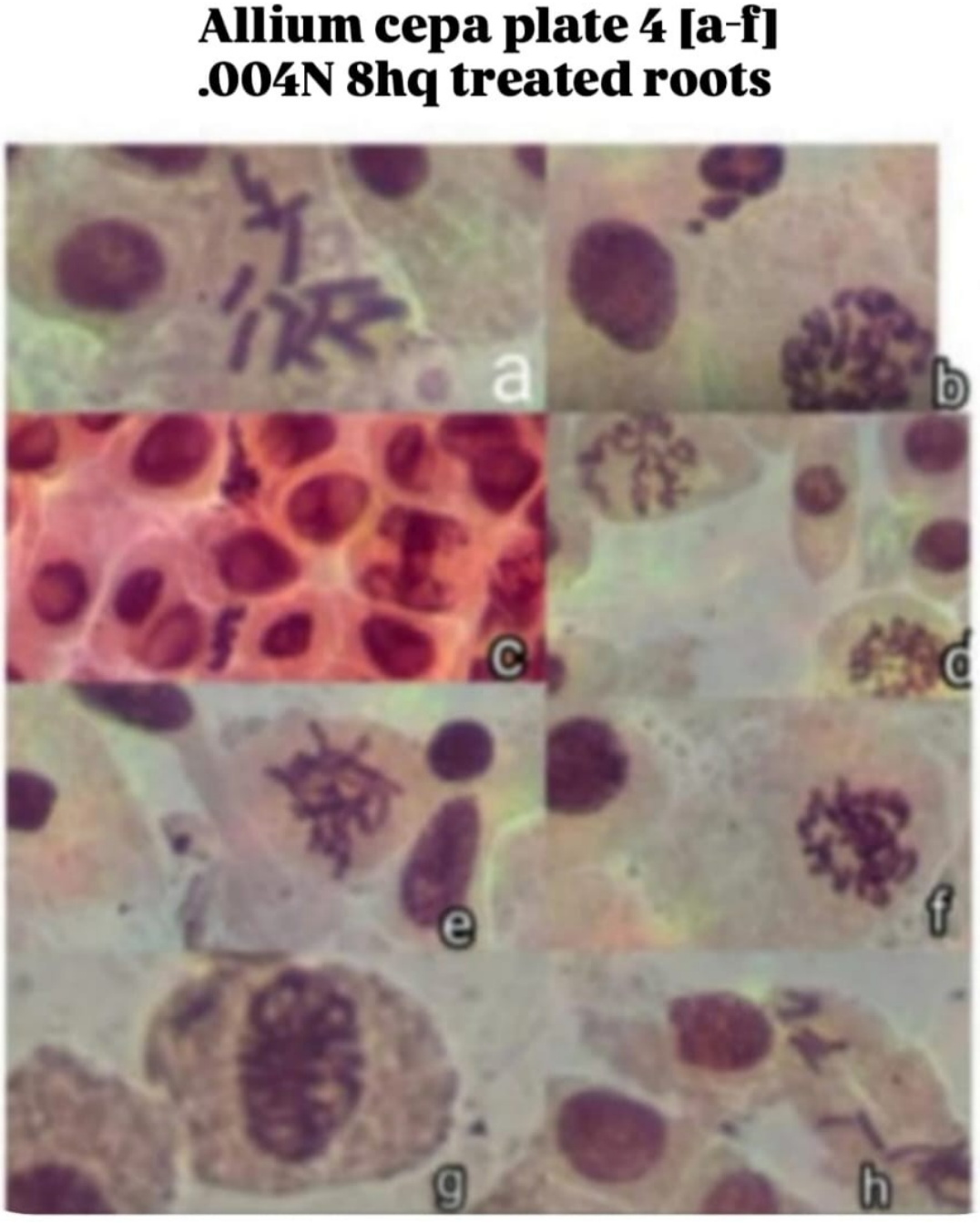

PLATE 4: *Allium cepa;* 0.004N 8 Hydroxy quinolone treated roots, a. Spindle distorted, b. Micronuclei, c. Irregular anaphase with irregular nucleus, d. Polyploid metaphase, e. Sticky chromosomes, f. Fragmented chromosomes, g. Polyploid metaphase, h. Irregular chromosomes

PLATE 5

Root tips of allium cepa 0.1 percent Paradichloro benzene

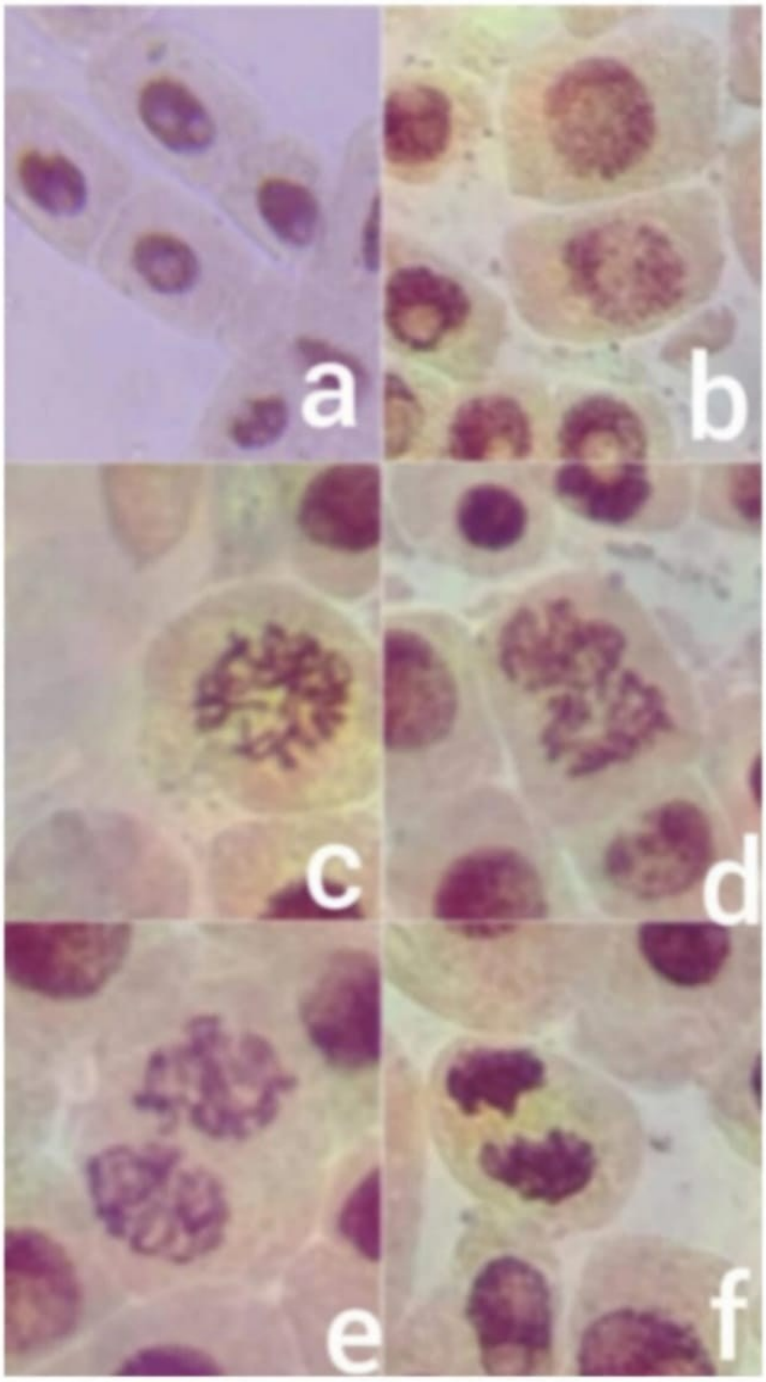

PLATE 5: *Allium cepa;* 0.004N 8 Hydroxy quinolone treated roots, a. Nuclear lesions, b. Polypliod prophase, c. Polypliod metaphase, d. Polypliod anaphase, e. Polypliod anaphase, f. Irregular anaphase

PLATE 6

Root tips of allium cepa 0.1 percent Paradichloro benzene

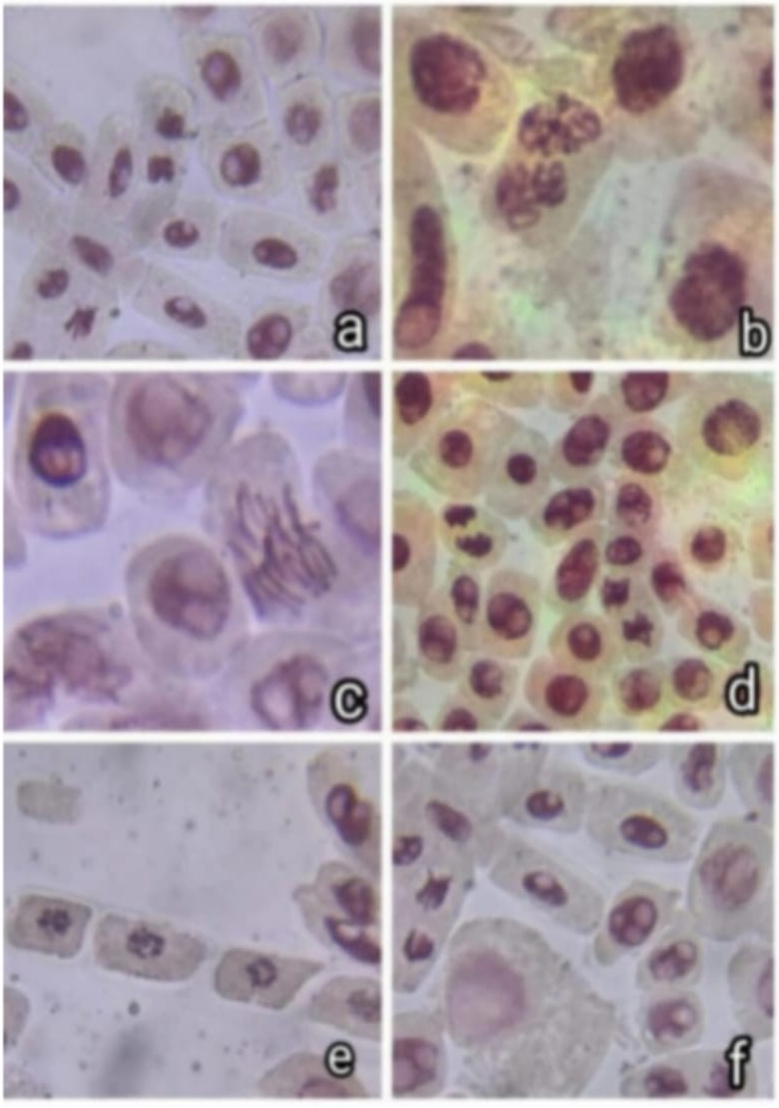

PLATE 6: *Allium cepa*; 0.1 percent Paradichloro benzene treated roots, a. Vagrant and nuclear lesions, b. Polyploid anaphase, c. Sticky chromosomes, d. Vagrant and laggard, e. Fragmented vhromosomes, f. Irregular anaphase with vagrant and nuclear lesions

PLATE 7

Root tips of allium cepa 0.004N hydroxy quinolone + 0.1 percent Paradichloro benzene

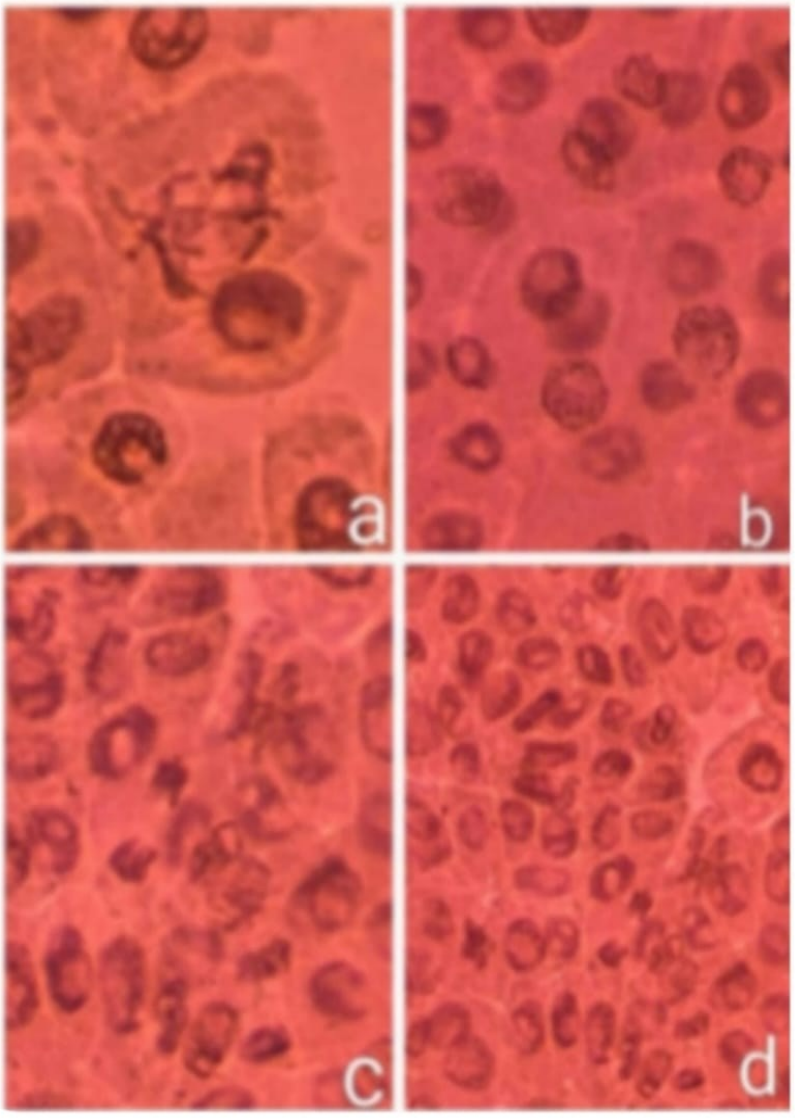

PLATE 7: *Allium cepa*; 0.004N 8 Hydroxy quinolone + 0.1 percent Paradichloro benzene treated roots, a. Irregular anaphase, b. Prophase with lesions, c. Vagrant and irregular anaphase, d. Nuclear lesions

PLATE 8

Root tips of allium cepa 0.004N hydroxy quinolone + 0.1 percent Paradichloro benzene

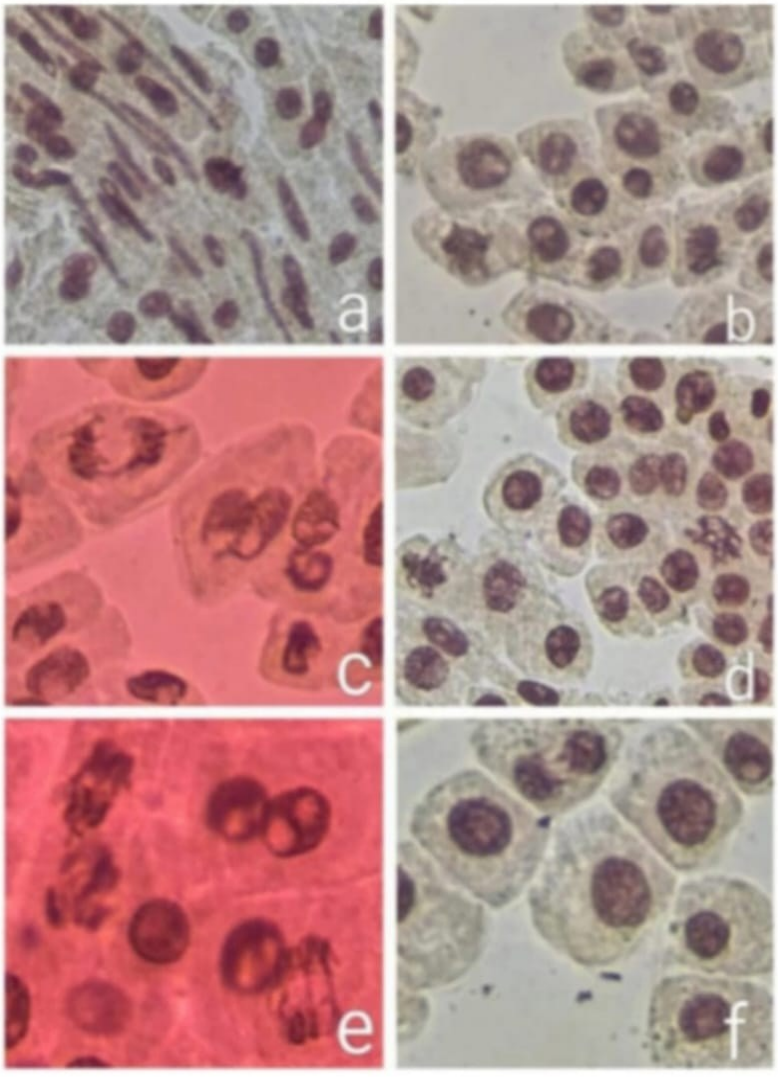

PLATE 8: *Allium cepa*; 0.004N 8 Hydroxy quinolone +0.1 percent Paradichloro benzene treated roots, a. Irregular cells with irregular nucleus, b. Sticky chromosomes, c. Vagrant and laggard d. Irregular anaphase, e. Laggard and vagrant, f. Laggard and vagrant

PLATE 9

Root tips of allium cepa 0.004N 8hydroxy quinolone + 0.1 percent Paradichloro benzene + 0.1 percent colchicine

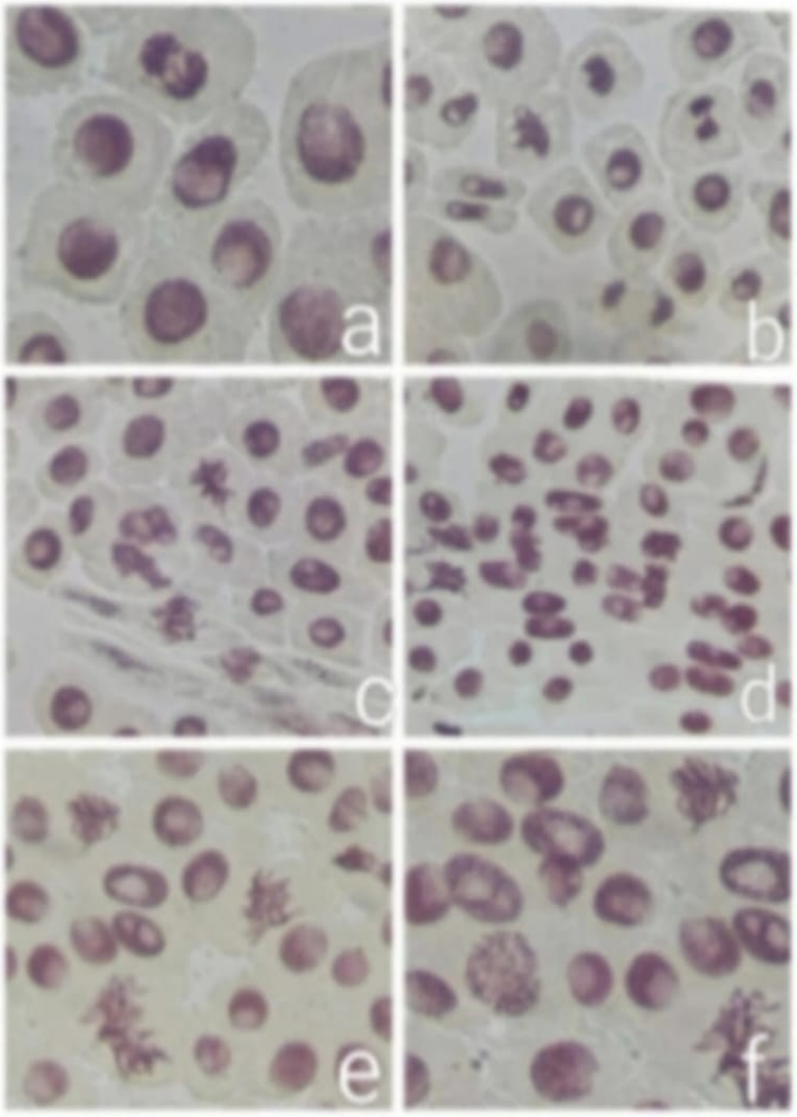

PLATE 9: *Allium cepa;* 0.004N 8 Hydroxy quinolone + 0.1 percent Paradichloro benzene + 0.1 percent cholchicine treated roots, a. Irregular anaphase, b. Nuclear lesions with sticky chromosomes, c. Sticky chromosomes, d. Irregular anaphase, e. Polyploid metaphase, f. Polyploid prophase

PLATE 10

Root tips of allium cepa 0.004N 8 hydroxy quinolone+0.1 percent Paradichloro benzene+0.1 percent colchicine

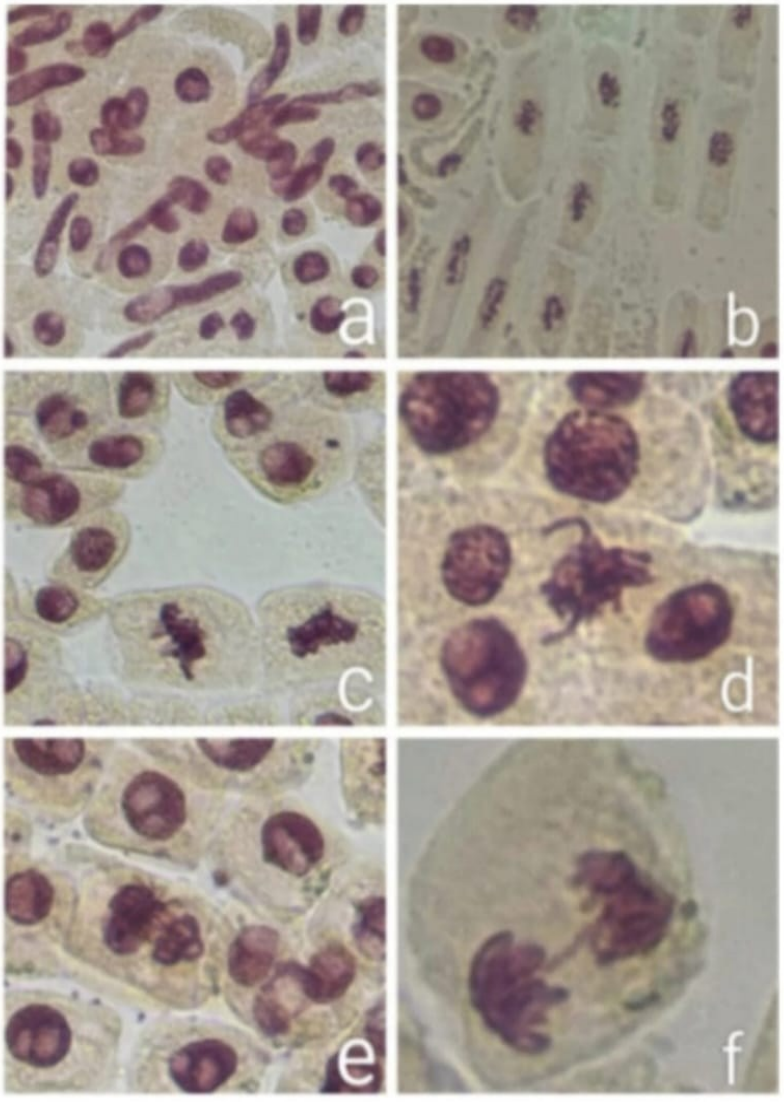

PLATE 10: *Allium cepa*; 0.004N 8 Hydroxy quinolone + 0.1 percent Paradichloro benzene + 0.1 percent cholchicine treated roots, a. Irregular cells with irregular nucleus, b. Fragmented chromatin, c. C- metaphase, d. Sticky chromosomes with abnormal orientation, e. Irregular anaphase, f. Laggard

PLATE 11

Root tips of allium cepa 0.004N 8 hydroxy quinolone+ 0.1percent Paradichloro benzene+0.1 percent colchicine

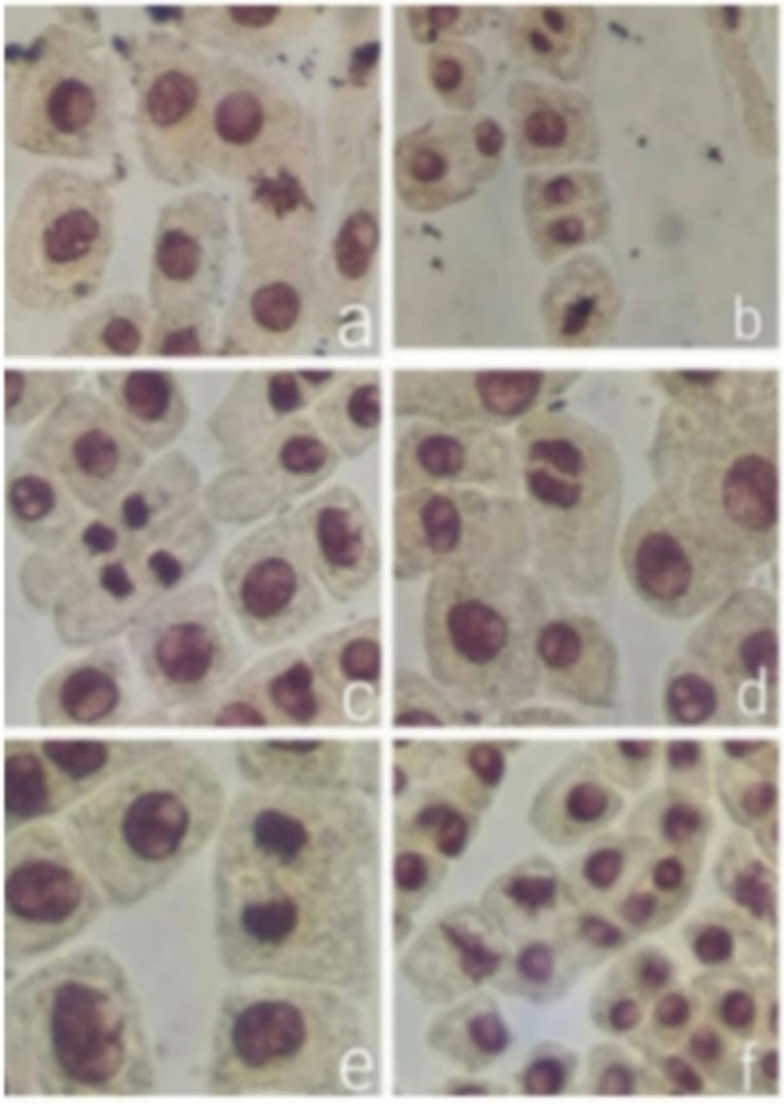

PLATE 11: *Allium cepa*; 0.004N 8 Hydroxy quinolone + 0.1 percent Paradichloro benzene + 0.1 percent colchicine treated roots, a. Sticky chromosomes, b. Nuclear lesions, c. Vagrant, d. Laggard, e. Laggard, f. Sticky chromosomes

DISCUSSION

The present investigation demonstrates the cytogenetic potential of colchicine, paradichlorobenzene (PDCB), and 8-hydroxyquinoline (8-HQ) in inducing mitotic abnormalities in *Allium cepa* root meristem. The untreated control cells exhibited a normal sequence of mitotic stages—prophase, metaphase, anaphase, and telophase—without any observable chromosomal aberrations, serving as a standard for comparison. This conforms with existing literature where untreated *Allium* cells show well-defined mitotic figures, indicative of genomic stability (Bonciu et al., 2018).

A notable cytological response in the treated samples was the induction of c-metaphase, particularly prominent in colchicine and 8-HQ treatments. C-metaphase is characterized by the condensation and random scattering of chromosomes due to inhibition of spindle fiber formation. This aberration is a hallmark of spindle poisons such as colchicine, which has been extensively studied for its ability to disrupt microtubule polymerization (Levan, 1938). The presence of c-metaphase in 8-HQ-treated samples corroborates findings by Fiskesjö (1985), who noted that 8-HQ similarly interferes with microtubule assembly, thereby halting metaphase progression. The increased frequency of c-metaphase with prolonged treatment durations confirms a time-dependent mode of action, as also suggested by Amer and Farah (1974) in their study on herbicide toxicity.

Another prevalent abnormality was chromosomal stickiness, primarily observed in cells exposed to 8-HQ and combination treatments. Chromosomal stickiness is considered an indicator of chromatin decondensation failure, resulting in entangled chromosomes that are unable to segregate properly (Yüzbaşioğlu et al., 2003). This can be attributed to toxic effects at the

molecular level, including inhibition of DNA repair enzymes or chromatin-binding proteins. Stickiness often led to the formation of anaphase bridges, another frequent abnormality seen in this study. Anaphase bridges arise due to dicentric chromosomes or incomplete separation of sister chromatids, indicative of clastogenic damage (Sudhakar et al., 2001). These aberrations disrupt the fidelity of chromosomal segregation, leading to further structural anomalies in daughter cells.

The observed presence of laggard and vagrant chromosomes in treated cells further supports the spindle-disruptive nature of the chemicals tested. Laggards are chromosomes that fail to migrate to either pole during anaphase, while vagrant chromosomes exhibit erratic movement and fail to align at the metaphase plate. Both types of chromosomal misbehavior contribute to the formation of micronuclei, which are extranuclear bodies containing chromosomal fragments or whole chromosomes excluded from the main nucleus (Majewska et al., 2003). In this study, micronucleus formation was notably higher in 8-HQ and combination treatments, suggesting a significant level of genomic instability. Micronuclei serve as reliable biomarkers of mutagenicity and are commonly used in environmental toxicity screening (Rank & Nielsen, 1994).

PDCB-treated samples showed pronounced signs of polyploidy, especially in prophase and metaphase stages. Polyploidy is a condition in which cells contain more than the diploid number of chromosomes, often resulting from mitotic slippage or cytokinesis failure induced by spindle poisons. Chauhan and Sundararaman (1990) reported similar findings in their study of substituted ureas, where polyploid cells were prevalent due to mitotic arrest. The formation of giant cells with multiple nuclei in PDCB- and colchicine-treated samples in the current study suggests that these chemicals not only inhibit spindle formation but also disrupt the regulatory checkpoints of the cell cycle. This is consistent with reports by Sharma and Sharma (1999), who emphasized the role of spindle dynamics in maintaining ploidy levels during cell division.

Multipolar anaphase, distorted metaphase alignment, and irregular anaphase movement were also recorded in treated cells, particularly under combined chemical exposures. These abnormalities arise when cells form more than two spindle poles, leading to chaotic chromosomal segregation and the potential for aneuploidy or genetic imbalance (Sharma & Sharma, 1999). The appearance of such multipolar divisions supports the assertion that the chemicals tested interfere not only with microtubule polymerization but also with spindle pole formation and orientation. The distortion of chromosomal alignment at metaphase and anaphase further reflects the cytotoxic influence of these agents on mitotic fidelity.

The formation of binucleated and multinucleated cells was another frequent observation across treatments, especially in cells exposed to 8-HQ and colchicine. These abnormalities arise when cytokinesis is either incomplete or entirely bypassed, often due to sustained metaphase arrest or missegregation of chromosomes (Amer & Farah, 1974). The resulting multinucleated cells tend to enter subsequent cycles with unbalanced chromosomal content, leading to either cell death or polyploid cell formation. These findings align with Abdel-Salam et al. (2012), who observed similar effects of pesticide exposure on *Vicia faba* root tip cells, linking multinucleation with impaired cytokinesis.

Nuclear lesions, indicative of DNA damage and chromatin fragmentation, were particularly evident during interphase in colchicine- and 8-HQ-treated cells. Such lesions suggest that the damage caused by chemical exposure extends beyond mitotic divisions and affects overall nuclear integrity. Abdel-Salam et al. (2012) described similar nuclear alterations in response to pesticide-induced genotoxicity, supporting the idea that mitotic poisons can induce both structural and functional damage to the genome. These lesions, coupled with increased chromosomal breaks and missegregation, point toward a high degree of cytogenetic stress in the exposed tissues.

Moreover, the correlation between treatment duration and the severity of mitotic abnormalities suggests a dose- and time-dependent genotoxic effect, consistent with the findings of Amer and Farah (1974). The highest frequency and diversity of chromosomal abnormalities occurred in cells subjected to combined treatments of colchicine, PDCB, and 8-HQ, indicating a synergistic effect of these agents. These results reinforce the cumulative cytogenetic burden posed by chemical combinations, which may be more harmful than single-agent exposure.

CONCLUSION

The present study was conducted in the Department of Botany, Maharani Cluster University, Bangalore, to assess the cytogenetic effects of chemical mutagens on *Allium cepa* root tips. The experiment aimed to evaluate the mitotic and meiotic abnormalities induced by 8-hydroxyquinoline (8-HQ), paradichlorobenzene (PDCB), and colchicine, both individually and in combination. Root tips of *Allium cepa* were treated with 0.004 N 8-HQ, 0.1% PDCB, their combination, and a mixture of all three chemicals for five hours. Treated samples were processed through acid hydrolysis, stained with acetocarmine, and squashed for microscopic observation. The results revealed a range of mitotic abnormalities including c-metaphase, chromosome stickiness, lagging and vagrant chromosomes, polyploidy, chromatid breaks, multipolar anaphase, binucleated cells, and giant cells. The frequency of abnormalities was significantly higher in combined treatments, indicating a synergistic mutagenic effect. C-metaphase and polyploid cells were particularly prominent in colchicine and PDCB treatments, whereas chromosome stickiness and laggards were frequently observed in 8-HQ and mixed treatments. With increased exposure time, the severity and variety of abnormalities also increased, highlighting the dose- and time-dependent nature of these mutagens. These findings collectively demonstrate that all three mutagens are effective in inducing chromosomal abnormalities, with the combination of colchicine, PDCB, and 8-HQ showing the strongest impact. The study highlights the potential of using these plant systems as reliable indicators for evaluating the genotoxic effects of chemical agents.

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