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Cytotoxic and Genotoxic Effect of Aqueous Leaf Extract of *Salvia officinalis* L. on *Allium cepa* Root Meristem Cells

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Abstract
The cytotoxic and genotoxic effects of aqueous extracts of *Salvia officinalis* L. (SAGE) on the root meristem cells of Allium cepa were determined using an Allium cepa cytogenetic assay. After four days, root growth inhibition was determined for different concentrations of extract (0–20%) using the cytogenetic assay to establish the EC50 value based on mean root lengths. Cytogenetic effects were evaluated by calculating mitotic indices and measuring the number of chromosomal aberrations. One of the primary objectives of this study was to show the dose-response relationship of the aqueous extract of *S. officinalis* L. with respect to inhibition of root growth. This relationship is represented in the results of the Allium cepa bioassay. Inhibition of root growth was shown to increase in a dose-dependent manner, with the EC50 identified as 20% aqueous extract *S. officinalis* L. The percentage of mitotic index was increased at various lower concentrations of the aqueous extract, but this increase represented metaphase arrest rather than mitotic stimulation. As the concentration of the aqueous extract increased, however, the percentage of mitotic index decreased, indicating that mitodepression was present for those concentrations. In addition, other chromosomal aberrations were observed further indicating the cytotoxic and genotoxic properties of the aqueous extract *S. officinalis* L.

Keywords: *Salvia Officinalis* L, *Allium Cepa* Assay, Cytotoxicity, Genotoxicity, Mitotic Index, Chromosomal Aberrations

1. Introduction

Salvia officinalis L. (Sage) is an important medicinal and aromatic plant belonging to the family Lamiaceae. It is native to the Mediterranean basin and is currently cultivated in many regions of the world with temperate climates, including Libya, where it is widely used in traditional medicine as well as in pharmaceutical and food industries (Lima *et al*., 2005) [18]. This importance is mainly attributed to its richness in bioactive compounds, particularly essential oils, phenolics, flavonoids, and terpenoids, which exhibit a broad spectrum of biological activities (Bozin *et al*., 2007; Durling *et al*., 2007) [7, 9].

Several studies have reported that essential oils in sage leaves account for approximately 1–2% of their dry weight and contain biologically active compounds such as thujone, carnosol, and rosmarinic acid. These compounds have been shown to possess strong antibacterial, antifungal, and antiviral activities (Schnitzler *et al*., 2008; Kamatou *et al*., 2008; Martins *et al*., 2016) [24, 15, 19]. Traditionally and in modern applications, *S. officinalis* has been used for the treatment of oral and gingival inflammations, gastrointestinal disorders, and for relieving muscle spasms and pain (Hajhashemi *et al*., 2010; Martins *et al*., 2016) [13, 19]. In addition, sage has been reported to reduce low-density lipoprotein (LDL) levels and increase high-density lipoprotein (HDL) levels, thereby exerting beneficial effects on cardiovascular health (Alarcón-Aguilar *et al*., 2001) [3].

At the Libyan level, most published studies on the genus *Salvia* have focused primarily on chemical and ethnomedicinal aspects, particularly essential oil composition and traditional uses (Agiel & Mericli, 2017) [2]. A Libyan study has also reported the chemical characterization of *S.officinalis* essential oil, highlighting variations in its composition depending on geographic origin and environmental conditions (Awen *et al.,* 2011) [5]. However, despite their importance in chemical characterization and pharmaceutical applications, these studies did not directly address the cytotoxic and genotoxic effects of aqueous sage extracts—the most commonly used form in daily traditional practices—at the level of cell division and chromosomal stability, thus revealing a clear research gap.

In summary, despite the widespread use of *S. officinalis* in the Libyan environment, particularly in its aqueous form, information regarding its cytotoxic and genotoxic effects remains limited and insufficient, especially with respect to its

influence on cell division and chromosomal stability. Most previous studies have focused on alcoholic extracts or essential oils, without an integrated approach linking root growth inhibition to cytological and genotoxic analyses within a comprehensive dose–response framework.

Accordingly, the present study aims to evaluate the cytotoxic and genotoxic effects of a sterilised aqueous extract of *S. officinalis* L. leaves on onion root tips (*Allium cepa assay*) by integrating assessments of root growth inhibition, mitotic index, and chromosomal aberrations across a graded series of concentrations, with determination of the half-maximal inhibitory concentration (EC50). This study seeks to provide a precise genotoxic evaluation of the biological safety limits of this widely used medicinal plant, contributing to biosafety assessment, sustainable agriculture, and mutation-based plant breeding programs.

2. Materials and Methods

Preparation of the Aqueous Extract of Salvia officinalis

Fresh leaves of *S. officinalis* L. were collected from local gardens in the city of Tripoli, and then were brought to the Genetics Laboratory, Faculty of Science; the leaves were thoroughly washed with distilled water to remove surface dust and contaminants, air-dried in the shade, and then ground into a fine powder using a household blender (Philips).

To prepare the aqueous extract, 20 g of leaf powder were mixed with 1000 mL of distilled water and incubated in an oven at 60 °C for 24 h. The mixture was then allowed to cool and filtered using a Büchner funnel to remove solid residues. The filtrate was subsequently sterilised in an autoclave at 121 °C for 30 min to eliminate any potential microbial contamination. Although autoclaving may reduce certain volatile constituents, the persistence of significant cytotoxic and genotoxic effects suggests that heat-stable, water-soluble bioactive compounds are primarily responsible for the observed responses (Bozin *et al*., 2007) [7]. Working concentrations (0%, 5%, 10%, 15%, 20%, and 25% v/v) were freshly prepared by diluting the stock extract immediately before use.

Experimental Design

Root Growth Inhibition Test (EC50 Determination)

The inhibitory effect of the aqueous extract on root growth was evaluated using the *Allium cepa* assay following the protocol described by (Rank, 2003) [21]. Uniform-sized onion bulbs were pre-soaked in distilled water for 24 h at room temperature to initiate root growth. Each bulb was then transferred to a container containing one of the extract concentrations. Four bulbs were used per concentration, and the exposure period lasted four days.

At the end of the exposure period, the lengths of 20 representative roots per concentration were measured, and the mean root length was used to estimate the concentration causing 50% inhibition of root growth (EC50).

Cytogenetic Assay (Allium cepa assay)

Fixation and Staining

Root tips were excised and fixed in Carnoy's fixative (ethanol: acetic acid, 3:1 v/v) for 24 h, then stored in 70% ethanol at 4 °C until further analysis. Prior to slide preparation, the root tips were hydrolyzed in 1N HCl at 60 °C for 7 min, rinsed with distilled water, and stained with 2% acetocarmine. The meristematic regions were gently squashed on clean microscope slides to obtain well-spread chromosomes, particularly at metaphase.

Microscopic Examination and Calculations

Prepared slides were examined under a light microscope. Dividing cells were counted and classified according to the mitotic stages: prophase, metaphase, anaphase, and telophase. Chromosomal aberrations observed during mitosis were recorded, including chromosomal bridges, laggard chromosomes, chromosome stickiness, vagrant chromosomes, and micronuclei. Classification and scoring were conducted in accordance with standard cytogenetic methodologies described by (Fiskesjö, 1985; Rank, 2003; Leme & Marin-Morales, 2009) [12, 21, 17].

Cytogenetic Indices

Mitotic Index (MI%)

The mitotic index, an indicator of mitotic activity in meristematic cells, was calculated using the following standard formula:

$$MI\ (\%) = (\text{Number of dividing cells} / \text{Total number of observed cells}) \times 100$$

where the number of dividing cells represents the total cells in prophase, metaphase, anaphase, and telophase.

Chromosomal Aberration Frequency (CA%)

The frequency of chromosomal aberrations (CA%) was calculated based only on dividing cells, following the standard *Allium cepa* assay protocol described by (Rank, 2003) [21]. It was calculated using the following equation:

$$CA\ (\%) = (\text{Number of aberrant dividing cells} / \text{Total number of dividing cells}) \times 100$$

Cells in interphase were excluded from this calculation, in accordance with internationally accepted cytogenetic methodologies.

Statistical Analysis

Root length data were expressed as mean ± standard deviation (Mean ± SD). Statistical differences among treatment groups were assessed using one-way analysis of variance (One-way ANOVA), followed by Tukey's post hoc test for multiple comparisons. A significance level of $p < 0.05$ was adopted. In addition, MI% and CA% were calculated for each concentration to provide a quantitative evaluation of the cytogenetic effects of the aqueous sage extract.

3. Results and Discussion

3.1 Effect of Salvia officinalis Aqueous Leaf Extract on Root Growth of Allium cepa

One-way analyses of variance (one-way ANOVA) revealed highly significant differences ($p < 0.001$) among the tested concentrations of *S. officinalis* L. aqueous leaf extract in their effects on the mean root length of onion (*Allium cepa*). This data further supports the sensitivity and suitability of the *Allium* assay as a bioindicator for detecting phytotoxic and cytotoxic impacts of plant extracts (Liman *et al*., 2018). Tukey's HSD post hoc test indicated that all applied concentrations (5–25%) caused a significant reduction in root growth when compared to the control treatment (0%),

consistent with a clear allelopathic potential of the extract.

The control group exhibited the highest mean root length (4.18 ± 0.66 cm), reflecting normal root growth in the absence of the extract. In contrast, treatments at 10%, 15%, and 25% produced marked inhibition, with root lengths decreasing to <1 cm. Complete cessation of root growth was also observed in some replicates at 10% and 25%, suggesting that these concentrations approached a near-maximal inhibitory threshold. The 5% concentration yielded a clear decline in root length while maintaining some residual growth capacity. Notably, the 20% treatment showed a relatively higher mean root length (2.05 ± 0.22 cm) than the 10%, 15%, and 25% concentrations, although it remained significantly lower than the control. This pattern indicates concentration-dependent variability in toxicity intensity and reflects differential sensitivity of meristematic tissues to extract constituents.

Table 1: Effect of different concentrations of *Salvia officinalis* L. aqueous leaf extract on mean root length of onion (*Allium cepa*) after 4 days of exposure (Mean ± SD). Different letters indicate significant differences among means according to Tukey's HSD test at α = 0.05

Concentration (%)	Mean Root Length (cm) ± SD	Statistical Group (Tukey HSD)
0 (Control)	4.18 ± 0.66	a
5	1.45 ± 0.19	c
10	0.48 ± 0.45	d
15	0.65 ± 0.28	d
20	2.05 ± 0.22	b
25	0.49 ± 0.09	d

As shown in Table 1, Tukey's grouping classified treatments into four distinct response groups: the control formed group (a) with the highest mean root length; the 20% treatment constituted group (b); the 5% treatment fell within group (c) with lower values; whereas 10%, 15%, and 25% clustered together in group (d), reflecting comparably severe inhibitory effects. This partition suggests that, beyond a certain toxicity threshold, numerically different concentrations may converge toward similar biological outcomes. Fig 1 illustrates the graphical representation of the effect of extract concentrations on mean root length, showing a transition from normal growth in the control to sharp inhibition at concentrations ≥10%. The relative position of the 20% treatment as an intermediate response point aligns with the values in Table 1 and underscores variation in toxicity severity with concentration.

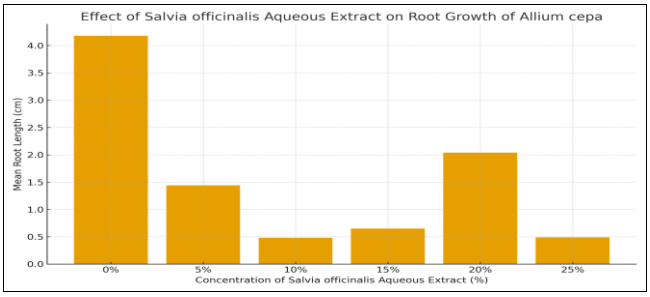

Fig 1: Effect of *Salvia officinalis* L. aqueous leaf extract on mean root length (cm) of onion (Allium cepa) after 4 days of exposure

3.2 Root Growth Inhibition and Determination of EC50

Based on the root growth inhibition assay, the mean root length at 20% concentration was approximately half the mean root length of the control, indicating that this concentration represents the half-maximal effective concentration (EC50) of the aqueous sage extract in this bioassay system. EC50 is a key endpoint in phytotoxic and ecotoxicological assessments, due to its significance in providing a practical benchmark for selecting effective concentrations before further cytological and genotoxic evaluations. This approach is consistent with Rank (2003) [21], who emphasised that a reduction in root growth is a sensitive and reliable criterion for identifying biologically effective concentrations in the *Allium* test before further cytogenetic characterisation.

The dose–response curve shown in Fig 2 demonstrates a decline in root growth (expressed as a percentage of the control) with increasing extract concentration. The curve indicates a clear intersection point at 50% of the control growth, supporting the EC50 estimate at approximately 20% and reinforcing the robustness of the statistical findings.

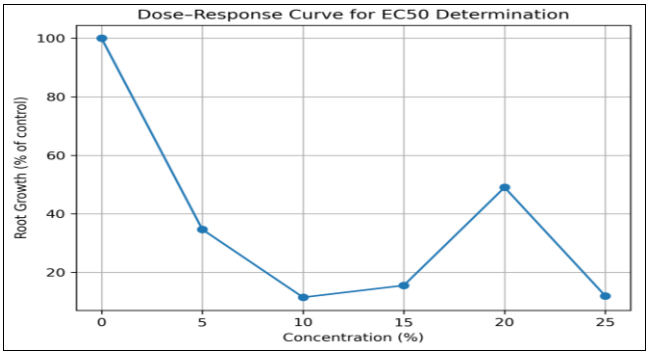

Fig 2: Dose–response curve for determination of the half maximal inhibitory concentration (EC50) of *Salvia officinalis* L. aqueous leaf extract on root growth of onion (*Allium cepa*) after 4 days of exposure, expressed as a percentage relative to the control

Collectively, these findings suggest that the aqueous sage extract exerts a pronounced allelopathic effect, indicated by a sharp decline in root growth as concentration increases, likely mediated through multiple interacting biological mechanisms. First, the extract may suppress cell division in the root apical meristem, leading to reduced growth rates and impaired mitotic progression, as reported for phenolic and terpenoid-rich extracts from medicinal and aromatic plants (Liman *et al*., 2018; Sadek, 2012 [23]; Kamatou *et al*., 2008 [15]). Second, phenolic compounds and terpenoids such as rosmarinic acid, thujone, and carnosol may interfere with enzymes and regulatory proteins that are involved in root cell elongation, thereby disrupting normal cell expansion and contributing to growth inhibition. Moreover, the extract may elevate oxidative stress by increasing reactive oxygen species production, damaging sensitive cellular components like DNA, which may explain the concordance between phytotoxic inhibition and genotoxic outcomes that were observed at higher concentrations (Liman *et al*., 2018; Sadek, 2012 [23]).

The comparatively less severe inhibition at 20% relative to the adjacent concentrations also suggests a possible hormetic response, characterised by a biphasic, non-linear pattern, in which moderate doses induce relatively reduced toxicity (or partial adaptation) before stronger inhibition re-emerges at higher doses. This phenomenon has been reported in other recent studies examining plant extract effects on germination and early seedling development (Ali

et al., 2022 [4]; Bonea, 2018; Ravlić *et al*., 2025 [22]). Thus, the allelopathic effect of sage in this system does not appear strictly linear, but reflects a complex interaction among extract concentration, cellular physiological mechanisms, and the adaptive capacity of meristematic cells under chemical stress.

These root growth and EC50 outcomes provide a critical basis for the selection of appropriate concentrations for subsequent cytological and genotoxic analyses, particularly those associated with strong inhibition and clear deviations in mitotic and chromosomal behaviour, to achieve a deeper understanding of cytotoxic and genotoxic effects of the aqueous sage extract in the *Allium cepa* model.

3.3 Mitotic Index and Cytotoxic Effects

Analysis of the mitotic index (MI%) in root meristem cells of *Allium cepa* treated with sterilised aqueous leaf extract of *S. officinalis* L. revealed a clear concentration-dependent response pattern (Table 2). The control group recorded an MI of 17.1%, which falls within the expected range of mitotic activity in untreated onion root meristems reported in *Allium cepa* assay literature.

At 5% concentration, the mitotic index increased markedly to 27.9%. This elevation should not be interpreted as a true stimulation of cell proliferation but instead reflects an apparent increase associated with the accumulation of cells in the metaphase stage. Similar patterns have been reported in other Allium cepa studies exposed to bioactive plant extracts and are commonly interpreted as indicative of mitotic disturbance rather than enhanced proliferative activity.

As the concentrations increased (10, 15, 20, and 25%), the mitotic index's decline was relative to the 5% peak, and ranged between 19.4% and 23.8%, accompanied by an increased proportion of interphase cells at the expense of dividing stages. This pattern suggests a mitodepressive effect and disruption of normal cell-cycle regulation. These are classical indicators of cytotoxicity in the *Allium cepa* assay upon exposure to agents capable of interfering with spindle assembly, chromatin condensation, or stage-to-stage progression within the cell cycle.

Fig 3 illustrates the overall changes in MI%, revealing a sharp increase at low concentrations (5%) followed by a gradual decline at higher concentrations. When interpreted alongside chromosomal aberration results, the apparent MI elevation at low concentration coincided with increased abnormalities, indicating that some cells were "apparently dividing" but in actuality were undergoing structural and functional impairment. These findings demonstrate that the aqueous sage extract affects not only the number of dividing cells but also the quality and stability of mitosis, potentially leading to mitotic failure, cell-cycle arrest, or cell death.

Overall, the mitotic index results indicate concentration-dependent cytotoxicity of the aqueous sage leaf extract and indicate that the extract disrupts cell-cycle regulation and mitotic stability. This outcome is consistent with root growth inhibition and chromosomal aberrations recorded in the present study, and aligns with published literature describing the cytogenotoxicity of medicinal plant extracts.

Table 2: Effect of *Salvia officinalis* L. aqueous leaf extract concentrations on mitotic index (MI%) in onion (*Allium cepa*) root meristem cells

Concentration (%)	Mitotic Index (MI%)
0 (Control)	17.1
5	27.9
10	19.4
15	23.8
20	21.7
25	21.7

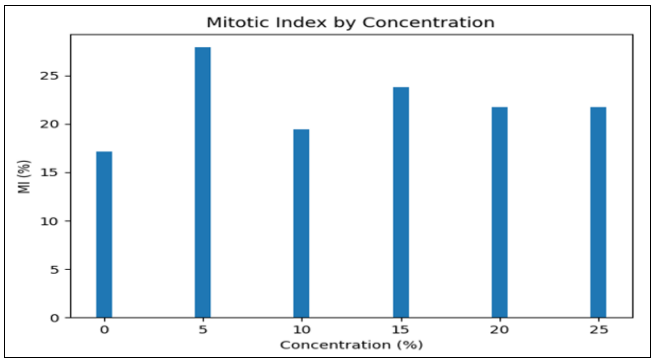

Fig 3: Mitotic index (MI%) in onion (*Allium cepa*) root meristem cells treated with increasing concentrations of *Salvia officinalis* L. aqueous leaf extract, showing a marked rise at 5% followed by a relative decline at higher concentrations

3.4 Chromosomal Aberrations and Genotoxic Effects

Cytogenetic analysis of *Allium cepa* meristematic root cells treated with *S. officinalis* L. aqueous extract revealed a clear spectrum of chromosomal aberrations varying in type and frequency with extract concentration. No aberrations were recorded in the control group, confirming that the effects observed were caused by the plant extract rather than experimental artefacts.

As shown in Table 3, chromosomal aberrations appeared at the 5% concentration extract, with a progressive increase in the number of aberrant dividing cells and a pronounced rise in total aberration percentage as concentration increased. The highest total aberration frequency was recorded at 20% (10.53%), which corresponded to the EC50 level for root growth inhibition, suggesting a critical concentration at which cytological and genetic effects become amplified.

Table 3: Frequency and distribution of chromosomal aberrations in onion (*Allium cepa*) root meristem cells treated with aqueous extract of *Salvia officinalis*

Concentration (%)	Sticky chromosomes	Chromosome bridges	Chromosomal fragments	C-metaphase	Disturbed metaphase	Total aberrant cells	Total aberrations (%)
0 (Control)	0	0	0	0	0	0	0.00
5	14	6	5	4	7	36	4.13
10	13	7	6	3	5	34	4.63
15	11	6	4	3	5	29	3.99
20	22	11	9	6	8	56	10.53
25	18	9	7	5	8	47	7.56

Note. Total aberrations (%) = (Number of aberrant dividing cells ÷ Number of dividing cells) × 100.

The observed aberrations were microscopically documented, as shown in Fig 4, and included chromosomal breakage, chromosome stickiness, disturbed metaphase, chromosomal fragments, C-metaphase, and chromosomal bridges. Collectively, these abnormalities indicate structural damage to the genetic material as well as pronounced disruption of spindle dynamics and chromatid segregation mechanisms.

Quantitative data in Table 3 show that chromosome stickiness was the most frequent aberration type across all tested concentrations, particularly at intermediate and high levels, suggesting impaired chromatin condensation and reduced chromosomal stability. Increased frequencies of chromosomal bridges and C-metaphase at higher concentrations further indicates spindle inhibition and microtubule disruption—mechanisms widely recognised as key indicators of genotoxicity in the *Allium cepa* assay.

Table 4 presents the proportional distribution (%) of abnormalities within the total aberrant cells at each concentration in order to clarify the relative contribution of each aberration type. Chromosome stickiness consistently represented the largest proportion, followed by bridges and fragments; whereas C-metaphase and disturbed metaphase occurred at comparatively lower proportions but increased with rising extract concentration. This pattern suggests that the genotoxic effect of the aqueous sage extract primarily targets chromatin organisation and spindle dynamics rather than inducing isolated random DNA breaks alone.

Table 4: Relative distribution (%) of chromosomal aberration types induced by *Salvia officinalis* L. aqueous leaf extract in onion (*Allium cepa*) root meristem cells (percent of total aberrant cells per concentration)

Concentration (%)	Sticky (%)	Bridges (%)	Fragments (%)	C-metaphase (%)	Disturbed metaphase (%)
5	38.9	16.7	13.9	11.1	19.4
10	38.2	20.6	17.6	8.8	14.8
15	37.9	20.7	13.8	10.3	17.3
20	39.3	19.6	16.1	10.7	14.3
25	38.3	19.1	14.9	10.6	17.0

Taken together, the concordance among (1) root growth inhibition and EC50 determination, (2) changes in mitotic index, and (3) the marked increase in chromosomal aberrations provides an integrated biological picture confirming that the aqueous sage extract exerts concentration-dependent cytotoxic and genotoxic effects. These findings are consistent with published evidence supporting the sensitivity of the *Allium cepa* assay in detecting cytogenotoxic impacts of plant extracts, particularly those rich in phenolics and terpenoids that may interfere with genomic integrity and mitotic regulation.

Given that the present study employed a pooled scoring approach (pooling method) for dividing cells, chromosomal aberrations were evaluated in a comparative descriptive–quantitative manner following standard *Allium cepa* protocols. This methodological approach helps strengthen the reliability of the reported patterns and enhances the interpretive value of the genotoxicity findings.

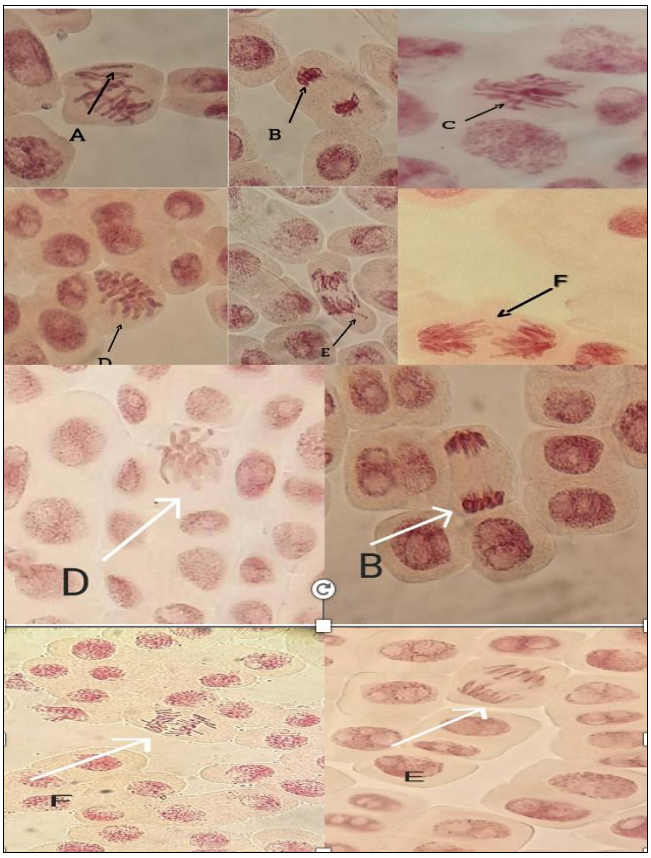

Fig 4: Types of chromosomal aberrations induced by *Salvia officinalis* L. aqueous extract in onion (*Allium cepa*) root meristem cells: (A) chromosomal breakage, (B) sticky chromosomes, (C) disturbed metaphase, (D) chromosomal fragments, (E) C-metaphase, and (F) chromosomal bridges

4. Conclusion

In this study, the sterilised aqueous extract of *S. officinalis* L. exhibited concentration-dependent cytotoxic and genotoxic effects on Allium cepa meristematic root cells. Root growth inhibition analysis identified 20% as the EC50 value, indicating a clear inhibitory threshold. Cytogenetic assessment revealed changes in the mitotic index (MI%) and the presence of various chromosomal aberrations, reflecting both mitodepressive and genotoxic effects at higher concentrations. The increase in MI% observed at low concentration was attributed to metaphase arrest rather than true mitotic stimulation. These findings confirm the sensitivity of the Allium cepa assay in evaluating the biological safety of medicinal plant extracts and further highlight the need for careful assessment of widely used herbal products.

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