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Original Article

# Cytotoxicity Assessment of *Coffea arabica* Bean Extract Using *Allium cepa* Root Tip Assay

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**Abstract**

Coffee is one of the most widely consumed beverages worldwide; however, excessive caffeine intake has been associated with adverse health effects, including headaches, insomnia, anxiety, and increased heart rate. Therefore, investigation is needed into the potential long-term impacts of the caffeine consumed in coffee. This study evaluated the concentration-dependent and time-dependent cytotoxic and genotoxic effects of *Coffea arabica* (*C. arabica*) bean extract on *Allium cepa* (*A. cepa*) root meristem cells. Five concentrations of *C. arabica* bean extract were tested: 0% (zero), 25% (low), 50% (moderate), 75% (high), and 100% (extremely high), each with exposures of 24 and 48 hours. Cytotoxicity was evaluated based on the mitotic index (MI) and chromosomal aberrations. Based on the results, all the tested concentrations significantly reduced the MI compared to the control, indicating inhibition of cell division. In particular, the 48-hour treatments with 50%, 75%, and 100% (v/v), resulted in a reduction in the MI compared to the positive control. In addition, all treatments induced various chromosomal abnormalities at both exposure times, including anaphase bridges, telophase bridges, chromosome fragmentation, c-mitosis, chromosome stickiness, and binucleated cells. Among these, the binucleated cells were the most frequently observed abnormality, comparable to those observed in the positive control group. In conclusion, *C. arabica* bean extract demonstrated cytotoxic and genotoxic effects in *A. cepa* root meristem cells in a concentration-dependent and time-dependent manner. This study highlighted the utility of the *A. cepa* as a reliable bioassay for evaluating the potential toxicity of natural compounds.

***Keywords****: Allium cepa; Chromosomal Aberration; Coffee Extract; Mitotic Index; Root Tip*

## Introduction

Coffee is one of the most popular drinks in the world because of its unique taste and smell. It is also one of the most traded goods in the world. Large-scale production takes place in about 60 tropical and subtropical countries (Esquivel & Jiménez, 2012). More than 80% of the global population consumes coffee regularly, highlighting its major role in daily life (Samoggia & Rezzaghi, 2021). Among its bioactive components, caffeine is the most well-known; however, its health effects and potential adverse outcomes remain incompletely understood. *Coffea arabica* (*C. arabica*) is of the two major commercially cultivated species and contains approximately 1–2% less caffeine than *C. canephora* (robusta coffee) on a dry-weight basis (Shinde & Shinde, 2017). Generally, arabica coffee is preferred for its superior aroma and quality (Febrianto & Zhu 2023). Despite its popularity, excessive caffeine consumption has been associated with adverse health effects, including hypertension, cardiac

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complications, restlessness, muscle tremors, insomnia, and palpitations (Shinde & Shinde, 2017). In extreme cases, high doses of caffeine (150–200 mg/kg body weight) may be lethal (Mrvos *et al.*, 1989). Other studies have demonstrated that coffee extracts can exert biological effects at the cellular level. For example, robusta coffee extracts have been shown to reduce cell viability, alter cell cycle progression, and induce apoptosis in human prostate carcinoma (DU-145) cells (Bauer *et al.*, 2018). In contrast, arabica coffee extracts at a concentration of 100 mg/mL did not exhibit cytotoxicity toward breast cancer (MCF-7) cells or normal human peripheral blood mononuclear cells after 24 and 48 hours of exposure (Hutachok *et al.,* 2021). Despite extensive research, findings regarding caffeine's biological effects remain inconsistent and its safety levels need to be considered (Doepker *et al.*, 2016). Considering the widespread consumption of arabica coffee drinks, it is essential to understand the toxicity action involved. In addition, it is important to investigate the potential toxicological effects of arabica coffee extracts based on a stock concentration of 200 mg/mL, which is comparable to a single shot of espresso based on the extraction process baristas use.

Caffeine is a naturally occurring, bitter alkaloid belonging to the methylxanthine group (Anastassakis, 2022). It is present in various plant species, including *C. arabica* and *Camellia sinensis* [L.] Kuntze, where it functions as a natural defence compound against herbivores and insects (Singh *et al.*, 2001; Ashihara, 2006). Pharmacologically, caffeine acts as a mild central nervous system stimulant, a myocardial stimulant, and a peripheral vasoconstrictor (Riedel *et al.*, 2014). The present study aimed to evaluate the cytotoxic effects of *C. arabica* bean extract using the *Allium cepa* (*A. cepa*) assay. *A. cepa* is a well-established model organism due to its easily accessible root meristem, low chromosome number (2n = 16), and large chromosome size during the mitotic cycle (Chandraker *et al.*, 2014). In addition, the *A. cepa* assay is recognized widely as a reliable and sensitive system for assessing the cytotoxic and genotoxic effects of various environmental pollutants, chemicals, and natural compounds in both plant and animal systems (Bonciu *et al.*, 2022). Notably, this assay is free from the ethical concerns associated with animal testing and had a strong correlation with other established biological test systems (da Silva *et al.*, 2026).

## Materials and Methods

### *Preparation for aceto-orcein dye*

A sample (1 g) of orcein dye powder was combined with 45 mL of acetic acid and 55 mL of distilled water. Then, the orcein solution was boiled until the powder had dissolved completely (Bench Chem Technical Support Team, 2025). Afterward, the solution was passed through Whatman filter paper and stored in a dark bottle for use as a 1% w/v aceto-orcein dye solution in chromosome staining.

### *Preparation of arabica coffee extract*

Roasted beans of coffee (*C. arabica*) were purchased from a coffee shop belonging to The Coffee Factory at Wang Nam Khaio, Nakhon Ratchasima, Thailand. A sample (10 g) of arabica bean powder was boiled in 100 mL of distilled water until about one-half of the solution had evaporated (Madić *et al.*, 2019). Then, the resulting coffee extract was passed through Whatman filter paper and used as a stock solution with a concentration of 200 mg/mL. This boiling-evaporation method was used to simulate traditional coffee preparation while ensuring effective extraction of any water-soluble bioactive compounds, including caffeine and secondary metabolites (Raghunath *et al.*, 2023). The concentrated stock solution provided a practical and reproducible basis for further dilution with distilled water to obtain final concentrations of 25% v/v, 50% v/v, 75% v/v, and 100% v/v before use. These concentration levels enabled evaluation across a broad exposure range, from low to extremely high extract concentrations, facilitating assessment of dose-response association effects.

### *Allium cepa Bioassay*

Inter-bulb variability was minimized based on obtaining the *Allium cepa* bulbs from a single cultivar and lot and storing the samples under identical conditions before use. Only healthy, undamaged bulbs of uniform size (1.0–1.5 cm diameter) and weight (7±3 g) were selected. In total, 3 onion bulbs were used

for each concentration of coffee extract as well as for the positive control (0.4 mg/mL caffeine) and were maintained at a constant temperature (25 ± 2 °C). A 0.4 mg/mL caffeine solution was prepared by accurately weighing 40 mg of analytical-grade caffeine and dissolving it in distilled water, with the final volume adjusted to 100 mL to ensure complete dissolution. This concentration has been reported widely in another study to inhibit cell proliferation and enhance the toxicity of cells (Meisaprow *et al.*, 2021). In the present study, the separate treatments were immersed in each concentration made from a fresh solution for 24 and 48 hours. These exposure durations were selected to represent acute (24 hours) and prolonged (48 hours) treatments. The root cytotoxicity assay used different concentrations (0%, 25%, 50%, 75%, or 100% v/v) of each extract. For cytological analysis, roots were pooled from 3 bulbs per treatment. Subsequently, the emerged rootlets were cut and transferred to a fixative solution (ethanol-to-glacial acetic acid ratio of 3:1) for 24 hours at 4°C. Roots were selected randomly and root tips (1–3 mm long) were hydrolyzed in 1 N hydrochloric acid at room temperature for 5 minutes and then stained with aceto-orcein for 5 minutes. After staining, the tips were squashed on a glass slide and each slide was viewed under a light microscope (Olympus CX 23) using a 400× magnification. Under the microscope, 500 cells were considered per slide for three slides in each treatment to determine the mitotic phase for the mitotic index percentage (%MI) and the chromosomal aberration percentage, based on equations (1) and (2), respectively.

$$\text{MI \%} = \frac{\text{Number of cells in mitotic phases}}{\text{Total number of cells counted}} \times 100 \quad (1)$$

$$\text{Chromosomal aberration \%} = \frac{\text{Number of cells with chromosomal aberrations}}{\text{Total number of cells counted}} \times 100 \quad (2)$$

*Statistical Analysis*

The data from all treatments were analyzed using SPSS software program version 29 for statistical analysis. Differences between treatments were analyzed using a one-way analysis of variance, followed by Duncan's multiple range test for post-hoc comparisons at the 5% threshold ($p < 0.05$) to compare the averages. All treatments were conducted in triplicate, with the data presented as mean ± standard deviation (SD) values (Freund & Wilson, 2003).

## Results

*Effect of Coffee Bean Extract on Onion Root Length*

The onion root growth was evaluated. Based on the results, the negative control roots after 24 hours of exposure were white, long and fleshy (Fig. 1A), with a greater root length than 0.4 mg/mL for the caffeine-positive control group (1.07 ± 0.23 cm and 0.33 ± 0.12 cm, respectively) (Table 1). Treatment with 25% coffee bean extract reduced the mean root length to 0.70 ± 0.10 cm, while the higher concentrations (≥50%) produced further reductions; root lengths at 50%, 75% and 100% did not differ significantly from the caffeine-positive control (Table 1). Morphologically, the roots exposed to all concentrations of the coffee bean extract were brownish, shortened and thin at both 24 and 48 hours (Fig. 1B–E), whereas the roots in the caffeine-positive control were white but shortened and fleshy (Fig. 1F). Extending exposure to 48 hours resulted in a significant increase in root length for the negative control (2.17 ± 0.15 cm vs. 1.07 ± 0.23 cm at 24 hours), while roots treated with 25–100% coffee bean extract remained comparable to the positive control (Table 1). Based on these results, the constituents of the coffee bean extract (potentially the caffeine) inhibited *A. cepa* root elongation in a concentration-dependent and time-dependent manner and induced characteristic morphological alterations.

***Table 1:****Length of Onion Roots After 24- And 48-Hours Exposure to Coffee Bean Extract*

| Exposure (Hours) | Concentration (% V/V) | Root Length (cm) |
|---|---|---|
| 24 | 0 | 1.07 ± 0.23[b] |
| | 25 | 0.70 ± 0.10[c] |
| | 50 | 0.37 ± 0.06[d] |
| | 75 | 0.33 ± 0.06[d] |
| | 100 | 0.27 ± 0.06[d] |
| | 0.4 mg/mL caffeine | 0.33 ± 0.12[d] |
| 48 | 0 | 2.17 ± 0.15[a] |
| | 25 | 0.47 ± 0.06[d] |
| | 50 | 0.17 ± 0.06[d] |
| | 75 | 0.30 ± 0.00[d] |
| | 100 | 0.20 ± 0.00[d] |
| | 0.4 mg/mL caffeine | 0.33 ± 0.06[d] |

*Note: Values (mean ± SD) with different lowercase superscripts in column indicate significant (p<0.05) differences*

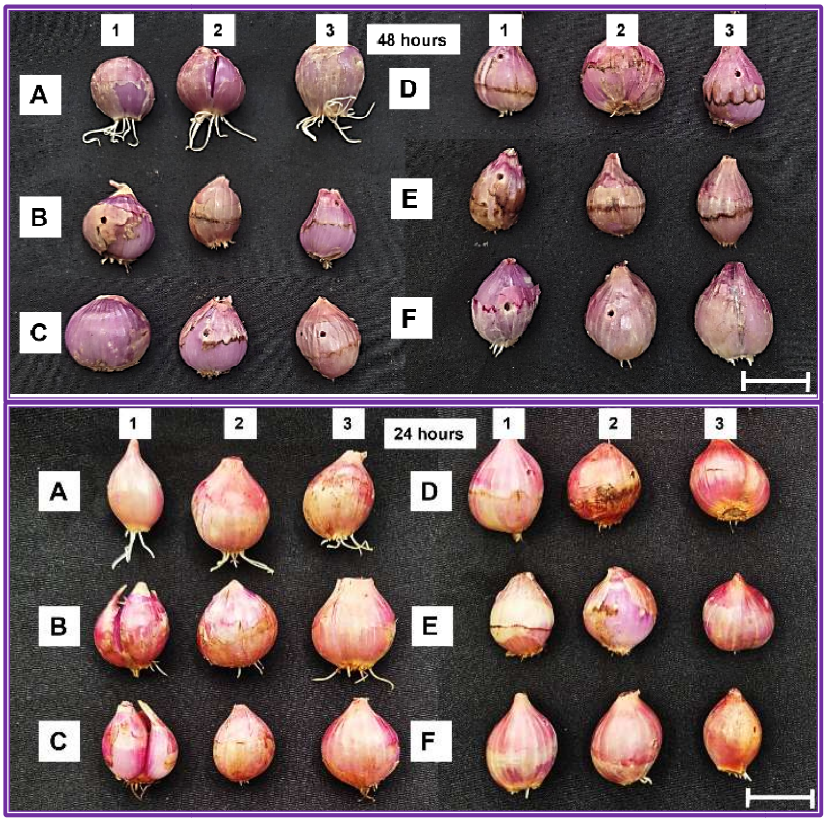


***Figure1:****Onion Bulb Roots After Growing For 24 And 48 Hours In: (A) Distilled Water, (B) 25% Coffee Bean Extract, (C) 50% Coffee Bean Extract, (D) 75% Coffee Bean Extract, (E) 100% Coffee Bean Extract (F) 0.4 Mg/Ml Caffeine (Scale Bar = 1 Cm)*

*Mitotic Index and Chromosomal Aberration Percentages of Onion Root Cell*

In the cytotoxicity assay, the MI% for the negative and the positive controls after 24 hours of exposure were 13.73 ± 0.58% and 7.00 ± 1.00%, respectively (Table 2). The treatments at 25%, 50%, 75%, and 100% concentrations were significantly different from the controls. All treatments reduced MI% compared to the negative control, with no significant difference observed for the 50%, 75%, and 100% treatments (Table 2), suggesting a plateau in cytotoxicity at higher concentrations. However, the MI% was reduced more after 48 hours of exposure than after 24 hours, indicating a time-dependent inhibition of cell proliferation. In addition, there were significant differences among the 25%, 50%, 75%, and 100% treatments (Table 2).

The aberrant cells were categorized as anaphase bridge, telophase bridge, fragmented chromosome, c-mitosis, sticky chromosome, and binucleated cell. Compared to the negative control, there was a

significant increase in the percentage of chromosomal aberrations in the 50% treatment after 24 hours of exposure. In addition, the 50% treatment affected onion bulb root germination, resulting in the formation of shorter roots compared to the controls (Fig. 1A and B). The lowest rate of aberrant cells was in the 25% treatment, and it increased to its highest level in the 100% treatment, which was like the positive control. After exposure for 48-hours exposure, all treatments had a significant percentage of chromosomal aberrations compared to the controls. The reduction in bridge formation at 50% concentration may be attributed to activation of cell cycle checkpoints and the elimination of severely damaged cells prior to mitosis. In contrast, the increased frequency of bridges at the 75% and 100% concentrations suggested that the DNA damage exceeded the cellular repair capacity and checkpoint control, allowing damaged cells with structural chromosomal abnormalities. These findings suggested that at moderate concentrations, the genotoxic effects may be underestimated due to the inhibition of mitotic progression in the damaged cells. In contrast, the higher concentrations appeared to induce extensive chromosomal abnormalities that became clearly detectable at the cytological level. The concurrent reduction in the MI and the increased frequency of chromosomal bridges at the elevated concentrations and longer exposure durations collectively confirmed that the treatments exerted both cytotoxic and genotoxic effects in a concentration-dependent and time-dependent manner. Notably, the number of binucleated cells was higher than other chromosomal aberrations (Table 2).

***Table2:*** *Mitotic Index and Chromosomal Aberrations of A. cepa after 24- and 48-Hours Exposure to Coffee Bean Extract*

| Ex (hrs) | Concentration (% v/v) | MI% | AB | TB | FC | CM | SC | BC | CA % |
|---|---|---|---|---|---|---|---|---|---|
| 24 | 0 | 13.73±0.58$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{e}$ | 0.00±0.00$^{e}$ |
| | 25 | 11.00±1.25$^{b}$ | 0.07±0.12$^{b}$ | 0.00±0.00$^{b}$ | 0.13±0.12$^{bc}$ | 0.00±0.00$^{c}$ | 0.13±0.12$^{bc}$ | 5.53±0.30$^{d}$ | 5.87±0.30$^{e}$ |
| | 50 | 8.40±0.20$^{c}$ | 0.20±0.2$^{ab}$ | 0.13±0.12$^{b}$ | 0.33±0.23$^{bc}$ | 0.20±0.20$^{ab}$ | 0.33±0.12$^{bc}$ | 6.27±0.61$^{cd}$ | 7.60±0.20$^{d}$ |
| | 75 | 7.73±0.31$^{c}$ | 0.60±0.4$^{a}$ | 0.33±0.12$^{b}$ | 0.40±0.20$^{b}$ | 0.07±0.11$^{bc}$ | 1.20±0.40$^{a}$ | 7.13±0.83$^{bc}$ | 9.73±0.42$^{c}$ |
| | 100 | 7.40±0.35$^{c}$ | 0.60±0.2$^{a}$ | 0.80±0.53$^{a}$ | 1.07±0.11$^{a}$ | 0.93±0.92$^{a}$ | 1.20±0.81$^{a}$ | 7.60±0.60$^{b}$ | 12.13±1.86$^{b}$ |
| | 0.4 mg/mL caffeine | 7.00±1.00$^{c}$ | 0.33±0.12$^{ab}$ | 0.27±0.30$^{b}$ | 0.47±0.30$^{b}$ | 0.80±0.20$^{ab}$ | 1.20±0.20$^{ab}$ | 19.07±0.50$^{a}$ | 21.73±0.90$^{a}$ |
| 48 | 0 | 14.73±1.22$^{a}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{f}$ | 0.00±0.00$^{f}$ |
| | 25 | 9.60±0.35$^{b}$ | 1.27±0.30$^{bc}$ | 1.13±0.30$^{b}$ | 1.40±0.20$^{cd}$ | 0.87±0.30$^{c}$ | 1.67±0.30$^{bc}$ | 6.40±0.80$^{e}$ | 12.73±0.42$^{e}$ |
| | 50 | 8.40±0.60$^{c}$ | 0.47±0.30$^{cd}$ | 0.33±0.30$^{bc}$ | 2.53±0.50$^{bc}$ | 1.13±0.50$^{bc}$ | 2.93±0.80$^{ab}$ | 8.60±0.80$^{d}$ | 16.00±0.72$^{d}$ |
| | 75 | 7.27±0.12$^{d}$ | 2.00±0.60$^{ab}$ | 1.93±0.75$^{a}$ | 3.47±1.62$^{ab}$ | 1.93±0.83$^{ab}$ | 4.00±1.92$^{a}$ | 10.86±0.50$^{c}$ | 25.20±1.44$^{c}$ |
| | 100 | 5.80±0.20$^{e}$ | 2.67±0.99$^{a}$ | 2.33±0.61$^{a}$ | 4.53±0.30$^{a}$ | 2.73±0.30$^{a}$ | 2.80±0.87$^{ab}$ | 12.53±0.90$^{b}$ | 27.60±0.8$^{b}$ |
| | 0.4 mg/mL caffeine | 3.80±0.35$^{f}$ | 1.33±0.23$^{bc}$ | 0.67±0.23$^{bc}$ | 3.33±0.90$^{ab}$ | 2.07±0.50$^{a}$ | 3.47±0.50$^{ab}$ | 22.67±1.17$^{a}$ | 33.53±0.61$^{a}$ |

*Note: Values (mean ± SD) with different lowercase superscripts in column indicate significant ($p < 0.05$) differences. Abbreviations: Ex(hrs)=Exposure (hours), MI% = mitotic index percentage, AB = anaphase bridge, TB = telophase bridge, FC = fragmented chromosome, CM= c-mitosis, SC = stickiness chromosome, BC = binucleated cell, CA% = Chromosomal aberrations (%)*

*Mitotic Phases and Types of Chromosomal Aberration in Cell*

Based on the results, most chromosomal aberrations in cells were observed during interphase, metaphase, anaphase, and telophase, with very few in prophase (data not shown). The extract from the coffee beans caused various types of abnormal cell division (anaphase bridge, stickiness in metaphase, stickiness in anaphase, fragmented chromosome, c-mitosis, telophase bridge, and binucleated cell) across the five different concentration treatments (Fig. 2F–2L). During mitotic division, the prophase, metaphase, anaphase, and telophase had lower values as the concentration of coffee extract increased (Fig. 2B–2E).

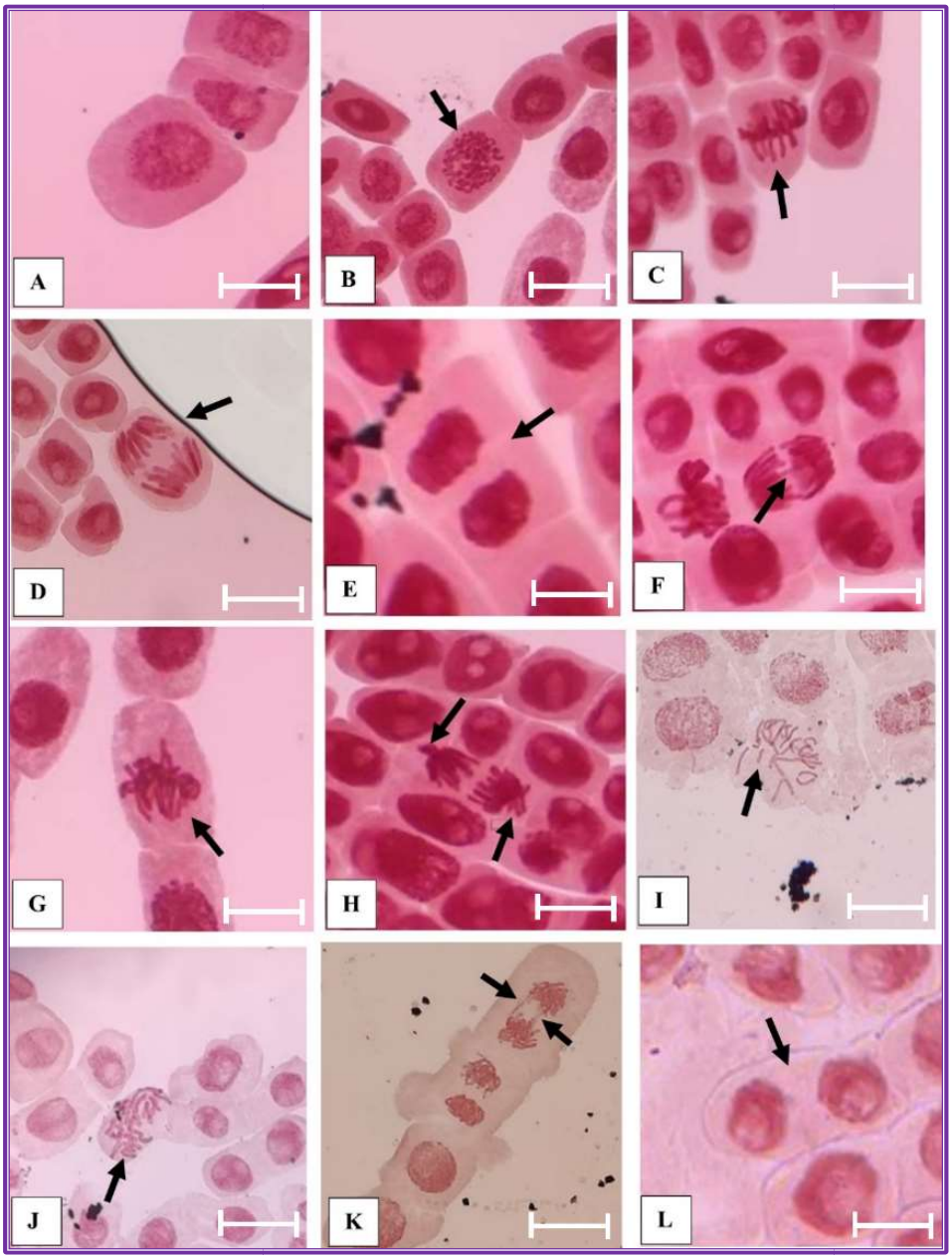


***Figure2:****Characteristics Of Normal (A–E) And Abnormal Cell Division (F–L) In Onion Root Tip Cells At 400× Magnification Indicated By Black Arrows: (A) Interphase, (B) Prophase, (C) Metaphase, (D) Anaphase, (E) Telophase, (F) Anaphase Bridge, (G) Stickiness In Metaphase, (H) Stickiness In Anaphase, (I) Fragmented Chromosome, (J) C-Mitosis, (K) Telophase Bridge, And (L) Binucleated Cell (Scale Bar = 0.03 Mm)*

## Discussion

The consumption of coffee containing caffeine has increased among individuals worldwide (Freitas *et al*., 2024). The current study assessed its cytotoxicity using *A. cepa* as an *in vivo* model to evaluate the potential adverse effects of caffeine. *A. cepa* is considered a suitable plant for the detection of genotoxic substances due to its large chromosomes, which are easily observed under a light microscope (Nicuță *et al.*, 2025). Chromosomal structural changes in *A. cepa* can reflect genotoxicity, and its chromosomes exhibit morphological similarities to those of mammalian cells (Firbas& Amon, 2014). The current study showed that cytotoxicity observed at the root tips of *A. cepa* increased in a dose-dependent manner.This finding was consistent with another report where herbal extracts tested on *A. cepa* cells had a reduced mitotic index in a concentration-dependent manner (Madić *et al.*, 2019;

Osigwe *et al.*, 2025). In the current study, the mitotic index after 24 hours of exposure to the coffee bean extract at concentrations of 50%, 75%, and 100% (v/v) was not significantly different from that observed with caffeine exposure that was used as the positive control. Other studies have suggested that caffeine can alter the cell cycle checkpoint, leading to impaired cell cycle progression and reduced cell proliferation (Ito *et al.*, 2003; Porta *et al.*, 2003; Bode & Dong, 2007).

The chromosomal aberrations in the root cells after 24 hours of exposure to coffee bean extract were consistent with studies by Farhan and Tawfiq (2016) and George and George (2017) that reported caffeinated soft drinks could induce various chromosomal aberrations—chromosomal bridges, fragments, bi-nucleated cells, and sticky chromosomes—in *A. cepa*. The preservation of genomic stability in healthy cells requires precise DNA replication and equal distribution of genetic material between daughter cells during each cycle of cell division. In the current study, chromosomal bridges were observed in the treatment group, indicating a disruption in genome integrity. Such chromosomal anomalies are known to cause DNA breakage and the formation of micronuclei, which may disrupt gene dosage and contribute to genomic instability—factors that are often associated with cancer development (Bizard& Hickson, 2018).

Additionally, the formation of chromosomal aberrations in cells exposed for 48 hours was higher than in the treatment for 24 hours, suggesting that the duration of caffeine exposure influenced genomic instability. This was consistent with a study by Khan *et al.* (2020), which reported that after exposure to food dyes for 48 hours, the mitotic index was greater than after exposure for only 24 hours. Furthermore, another study reported that caffeine inhibited DNA repair mechanisms and disrupted cell cycle checkpoint regulation, potentially resulting in mutations and programmed cell death (Ferguson & Philpott, 2008). Therefore, caffeine from the coffee extract may have induced chromosomal aberrations in the onion root tip cells, which was consistent with the results of Bonciu *et al.* (2022), who reported that commonly consumed caffeine-containing beverages, such as Coca-Cola®, could exert cytotoxic and genotoxic effects. Their study revealed that increasing concentrations of Coca-Cola® led to a strong cytogenotoxic effect in the meristematic cells of *A. cepa*, including a decrease in MI% (from 12.5% to 3.5%) and an increase in the occurrence of various chromosomal and nuclear abnormalities (from 12.6% to 23.2%), such as bridges, laggards, stickiness, and disrupted nucleus. The presence of caffeine generally affected aberrant cells, which were predominantly binucleated cells caused by cell plate synthesis. This process resulted in the formation of cell walls that were either developed normally, curved, or branched, giving rise to aberrant daughter cell walls (Manandhar *et al.*, 1996). In addition, more cells with sticky chromosomes may indicate highly toxic effects and possibly lead to cell death (Donghua *et al.*, 1996). Furthermore, chromosomal bridges in anaphase and telophase can create tension that causes the kinetochores at the ends of the bridges to move away from the newly formed daughter nuclei, which may result in misformed micronuclei or abnormally shaped nuclei in the daughter cells (Stewénius *et al.*, 2005; Pampalona *et al.*, 2010).

While caffeine consumption is generally safe for healthy adults, it may be harmful for certain vulnerable populations, manifested as impairments in cardiovascular function, sleep, and substance use (Temple *et al.*, 2017; Mateo *et al.*, 2026). The reported caffeine levels in the arabica coffee extracts obtained through the boiling, drip-filtering, and espresso brewing processes were 65.58 ± 9.83, 55.58 ± 10.61, and 64.25 ± 11.56 mg/g, respectively (Hutachok *et al.*, 2021). High doses of caffeine can negatively affect mood and cognitive performance (Ruxton, 2008). The recommended daily caffeine intake for adults is 400 mg (two-to-three cups of coffee); however, for sensitive or susceptible individuals, such as pregnant women and children, the suggested amounts are ≤300 mg and ≤2.5 mg, respectively, to reduce the risk of adverse health effects (Wikoff *et al.*, 2017; Reyes & Cornelis, 2018). However, a study in mice that consumed caffeine (0.3 and 1.0 mg/mL) during pregnancy and lactation demonstrated genotoxic effects, as evidenced by the comet assay and micronucleus test, in both female mice and their offspring. Additionally, offspring exhibited reduced early-life body weight, suggesting that maternal caffeine intake may adversely affect genetic stability and development (Magenis *et al.*, 2023). The current study recorded an increase in chromosomal aberrations in *A. cepa* exposed to caffeine, suggesting that this substance may affect genomic integrity during cell division.

These findings highlight the importance of minimizing caffeine intake to reduce potential health risks. Hutachok *et al.* (2021) examined roasted arabica coffee extracts at concentrations between 3.125 and 1,000 µg/mL, with incubation periods of 24 and 48 hours, using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to assess cell viability. Based on their results, the coffee extracts did not produce any harmful effects on breast cancer cells or normal white blood cells; furthermore, they enhanced the viability of white blood cells with longer incubation periods. Therefore, the results from the treatment for 48 hours in the present investigation suggest that the impacts of higher levels (100–200 mg/mL) of arabica coffee extract should be tested on human cells to assess their effects at 24 and 48 hours, providing insights into the possible impact of long-term coffee extract consumption.

*Limitations and Future Scope*

A limitation of the present study is the absence of physicochemical characterization of the *C. arabica* bean extract, including the identification and quantification of bioactive compounds such as caffeine. In addition, the study did not investigate the underlying mechanisms insights into the observed cytotoxic effects. Future research should address these limitations by incorporating comprehensive chemical characterization and conducting detailed mechanistic investigations.Additionally, further studies using model systems that are more relevant to human biology are needed to elucidate the underlying mechanisms of toxicity and to assess the potential implications of these findings for human health.

## Conclusion

The 200 mg/mL concentration of arabica bean extract produced significant cytotoxicity on *A. cepa* cells after 24 and 48 hours. The lowest cytotoxic effect was at the 25% (v/v) dilution of 200 mg/mL coffee extract, which correlated with the highest mitotic index. Furthermore, prolonged exposure (48 hours) resulted in a significant decrease in the mitotic index. Therefore, based on the current results, while arabica coffee is commonly consumed, its long-term impact may lead to cytotoxicity, particularly at high concentrations (50–100%), likely due to the high caffeine content. Therefore, this research should provide a starting point for further investigation into the genotoxic and cytotoxic effects of caffeine on human cells.

## Conflict of Interest

The authors declare that they have no competing interests.

## Acknowledgment

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