Toxicological Bioassay of Paracetamol Using *Allium cepa* as a Bioindicator

**ABSTRACT**

This study aimed to evaluate the cytotoxic effects of Paracetamol on onion root tip cells. Three experimental treatments were conducted using Paracetamol concentrations of 500, 250, and 125 mg/L, along with a control (distilled water). After 96 hours of exposure, root growth, mitotic index, and the frequency of chromosomal abnormalities in *Allium cepa* were assessed. The relative growth of the roots of *A. cepa* was influenced in each treatment, showing a significant decrease from 5.74 0.27 to 1.58 0.13. Microscopic analysis revealed a substantial reduction in the mitotic index, with a recorded value of 8.76 ± 0.7 at the *A. cepa* 500 mg/L treatment. Furthermore, a total of 10 distinct chromosomal abnormalities were identified, indicating a significant structural impact on chromosomes induced by Paracetamol exposure.

The results demonstrate that *Allium cepa* serves as an effective model for toxicological assessments, as all tested concentrations exhibited significant effects. These findings suggest that prolonged exposure to high Paracetamol concentrations may irreversibly impact genome integrity, posing ecological risks. Further analytical validation and metabolite characterization are essential.

***Keywords:*** *Paracetamol, Allium cepa, Cytotoxicity, Self-medication*

1. INTRODUCTION

As lives getting faster, there's an increased reliance on immediate relief from health issues. Many people have turned to self-medication for minor ailments, opting for over- the- counter (OTC) drugs to treat their health problems. Self-medication has been defined as “the taking of drugs, herbs or home remedies on one's ow initiative, or on the advice of another person, without consulting a doctor” (Hernandez-Juyol and Job-Quesada, 2002).Commonly used medication includes analgesics, cough syrups, laxatives, antibiotics, antihistamines, vitamins and antacids, as they are easily accessible, without a prescription.

Paracetamol also known as acetaminophen or N-acetyl β-aminophenol, is one of the mild analgesics & antipyretic easily available at all pharmacies and are considered as an OTC drugs. Its effectiveness in providing quick pain relief and its strong safety profile have led many people to trust it blindly. During the COVID-19 pandemic, paracetamol became one of the most commonly used treatments for symptom relief, as prescribed by health professionals (Mostafa et al., 2022; Pandolfi et al., 2022). It was also frequently used for self-medication during the quarantine period (Faqihi and Sayed, 2021). This increased demand led to a rise in paracetamol consumption, ultimately resulting in a shortage of the drug (Romano et al., 2021). Paracetamol is commonly used for its ability to relieve pain and inflammation. However, paracetamol poisoning can occur even at recommended therapeutic doses. It can also result from multiple therapeutic or supratherapeutic doses (Lubel et al., 2007; Chidiac et al., 2023).

Paracetamol is rapidly absorbed in the duodenum (Bartholom ét al., 2015), crosses the blood- brain barrier and is uniformly distributed throughout the central nervous system (Klotz, 2012; Shazma, 2018). Its metabolic pathway involves three key organs: the liver, intestine and kidneys.

At therapeutic doses, Paracetamol is typically metabolized by the cytochrome P450 system, resulting in the formation of N-acetyl-p-benzoquinone imine (NAPQI) (Fig 1: Adapted from Moyer et al., 2011).However, in cases of overdose, this process depletes cellular glutathione levels in the liver, as NAPQI quickly reacts with glutathione. This reaction leads to oxidative stress, causing cellular damage & death. Paracetamol is a dose dependent fatal hepatoxic agent and can cause acute hepatocellular injury leading to centrilobular necrosis (Hinson et al., 2010), this injury to centrilobular necrosis is due to elevated level of NAPQI (Shan et al., 2019; Guengerich, 2021). Its overdose can also cause renal failure & depletion of glutathione (Abraham, 2005). Paracetamol has emerged as the leading cause of acute liver failure (ALF), a serious condition characterized by elevated plasma, aminotransferase levels, specifically ALT and AST (Hinson et al., 2010).

It was reported that paracetamol metabolic protein adduct seems to cause disruption of Electron Transport Chain (ETC) and thus formation of Reactive oxygen species (ROS) in mitochondria (Jaeschke et al., 2012; Jaeschke and Ramachandran, 2024).

It was found that the toxicity of p-aminophenol and p-benzoquinone (by products of paracetamol synthesis) is significantly greater than that of the drug itself, the potential environmental risks could increase during the formation of higher molecular weight intermediates. These metabolites may accumulate and reach elevated concentrations (Chuanzhou et al., 2016; Chacón et al., 2022).

Over the years, using bioindicators with plants seeds has been recognized as an effective scientific technique for assessing cytotoxicity, demonstrating high sensitivity and ease of cost. Currently, among the higher plant species used for evaluating environmental contamination, the most commonly utilized are *Allium cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris*, and *Hordeum vulgare* (Grant, 1994). Among these, *A. cepa* is particularly favored for assessing chromosomal damage and mitotic cycle disturbances due to its advantageous chromosome characteristics, such as large chromosome size and a relatively low chromosome number (2n = 16) (Fiskesjo, 1985). Additionally, this test system has demonstrated high sensitivity in detecting environmental chemicals.

The *Allium cepa* test has demonstrated high sensitivity and strong correlation with other test systems, including mammalian models. A study evaluating 148 chemicals using the *Allium* test reported that 76% produced positive results (Grant, 1982). Based on these findings, the author recommended adopting the test as a standard method for detecting chromosome damage induced by chemicals. It was also reported that the sensitivity of the *A. cepa* test was almost identical to that observed in algae and human lymphocyte test systems (Fiskesjo, 1985). A later study found an 82% correlation between the *A. cepa* test and carcinogenicity tests conducted on rodents (Rank and Nielsen, 1994). The findings also suggested that the *A. cepa* test was more sensitive than both the Ames test and the Microscreen test in detecting genotoxic effects.

This study evaluates the possible cytotoxic effects of Paracetamol using *Allium cepa* (Javed et al., 2011; Leston, et al., 2013; Özlem, 2017; Azzazy, 2020; Sandra et al., 2020; Mercado et al., 2020).



2. material and methods

**2.1. Collection of Plant Material:**

The onion bulbs were used as bioindicators to identify cytological changes. They were purchased from local market and were exposed to hydration to stimulate the cell growth so that root meristematic cells would elongate then they are subjected to the Paracetamol treatment. Three treatments were carried out with the following Paracetamol concentration (500, 250, 125mgL-1) and a control (distilled water).

**2.2. Root Growth**

After 96hrs of exposure to each concentration, the root growth of *Allium cepa* was evaluated. The bulb achieving a root length greater than 1mm were taken into account (modified procedure by Salazar et al., 2020). The relative root growth percentage, following formula was applied: (Thijs, et al., 2019)

**2.3. Mitotic index**

After treatment period (after 96hrs), slides were prepared by Acetocarmine squash preparation. Approximately 4000 cells were randomly analyzed in both control and treated group of onion bulbs. The mitotic index (MI) was determined to assess the presence or inhibition of cell division. It is defined as the ratio of the no. of cells undergoing mitosis to the total no. of cells (Datta et al., 2018). The following formula was used for the calculation:

**2.4. Cell Abnormalities:**

*A. cepa* bioassaywas conducted to assess the rate of abnormalities. The following formula applied in methodology outlined by Salazar & Maldonado Bayona (2020)was used: (Salazar et al., 2020)

**2.5. Statistical Analysis:**

The data are expressed as mean S.E. and statistical analysis was performed by using t- test.

3. results and discussion

**3.1. Root Growth:**

As mentioned above, that the *A. cepa* bulbs were firstly exposed to hydration than they were subjected to Paracetamol, so some variations are possible. Consequently, the extent of impact will depend on the chemicals involved & the duration of exposure (Salazar et al., 2019). The relative growth of the roots of *A. cepa* was influenced in each treatment, showing a significant decrease (Table 1). This is because the roots are typically the first tissue exposed, where the toxicants inhibit their elongation & proliferation, ultimately comprising the plant’s growth & reproductive potential (Talukdar and Talukdar, 2014).

|  |  |  |
| --- | --- | --- |
| Paracetamol Concentration (mgL-1) | Root Length (cm)  *A. cepa* | Relative Root Growth Percentage (RRG)  *A. cepa* |
| Control | 5.74 0.27 | ------ |
| 125mgL-1 | 3.88 0.2 | 67.59 |
| 250mgL-1 | 2.47 0.21 | 43.03 |
| 500mgL-1 | 1.58 0.13 | 27.52 |

**Table 1: Root Growth & Relative Growth Percentage of Allium cepa**

Other studies have shown similar statistically significant findings in radicle elongation, demonstrating that the degree of root inhibition is directly proportional to paracetamol concentration.

**3.2. Mitotic index & Mitotic inhibition:**

The mitotic index (MI) is a quantitative measure used to evaluate cell division in an organism (Kato and Haskins, 2022). Thus, if the MI is lower than that of the control treatment, it indicates that the drug is impacting mitosis.

|  |  |  |
| --- | --- | --- |
| Paracetamol Concentration (mgL-1) | Mitotic index (MI)  *A. cepa* | Mitosis inhibition  (%)  *A. cepa* |
| Control | 16.46 0.81 | ------ |
| 125mgL-1 | 11.57 0.7 | 29.70 |
| 250mgL-1 | 10.82 0.63 | 34.26 |
| 500mgL-1 | 8.76 0.7 | 46.78 |

**Table 2: Mitotic index (MI) & Mitosis inhibition Percentage of Allium cepa**

In this instance, microscopic analysis of the cells shows a significant decrease in the mitotic index, with values of 8.76 0.7 at the 500mgL-1 treatment of *A. cepa*.

Measuring mitosis inhibition offers important insights into the relationship between damage and the concentrations applied. The mitotic inhibition reached 46.78% in *A.cepa*, indicating a concerning decline of nearly 50% compared to the control treatment, as the control did not induce any inhibition in the *A. cepa*.

After 96hours of exposure, at paracetamol concentrations of 250 mg L⁻¹ and 500 mg L⁻¹, mitotic inhibition reaches (Jing, et al., 2009) 26% and 46.78% respectively, in *A. cepa* (Table 2), which is nearly 50% relative to the control treatment—an indication of significant inhibition. Measuring mitotic inhibition provides crucial insight into the correlation between cellular damage and the concentrations applied. No mitotic inhibition was observed in the control group, suggesting that increased paracetamol concentration intensifies the disruption of the cell cycle.

**3.3. Cell Abnormality Index:**

A total of 10 types of chromosomal abnormalities were observed, indicating a significant structural impact on chromosomes induced by paracetamol exposure (Table 3, Fig. 2). In *A. cepa*, all detected abnormalities appeared at paracetamol concentrations of 125, 250 and 500 mg L⁻¹. These root anomalies are likely due to two primary factors: first, paracetamol is absorbed by bioindicators and once internalized, it disrupts normal cell division (Fig 2.) and induce cytotoxic effects; second, as a low molecular weight compound, paracetamol exhibits a strong affinity for root tissues, enhancing uptake and accumulation predominantly in roots.

Nuclear abnormalities, often characterized by structural changes in interphase nuclei, are increasingly studied as indicators of environmental chemical toxicity (Leme et al., 2009). This study observed a pronounced increase in nuclear abnormalities in root tip cells exposed to Paracetamol. Root tip cells exposed to Paracetamol displayed prominently condensed nuclei with smaller or faint nucleoli and cytoplasm disintegration.

Chromosomal aberrations, defined by structural changes or changes in chromosome number, can arise either spontaneously or due to chemical or physical exposure (Russel, 2002; Obe et al., 2002). Indicators of aneugenic action include chromosome adherence, chromosomal loss/delay, multipolarity and disrupted anaphase due to mitotic spindle disruptions, whereas chromosomal bridges and breaks in anaphase and telophase suggest clastogenic effects (Sandra et al., 2010; Farhana and Malik, 2013).

The most common abnormality in this bioassay was the presence of micronucleus (Fig. 3, Table 3). Overall, treatment with 500 mg L⁻ resulted in the highest frequency of abnormalities. The relative abnormality rate, which considers both the micronucleus index and abnormality percentage (Table 4), indicating that paracetamol induces irreversible DNA damage. This DNA and chromosomal damage likely relate to cellular death mechanisms and carcinogenesis pathway.

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**Table 3. Chromosomal abnormalities in *A. cepa***

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Paracetamol Conc. (mgL-1) | Multipolar | Micronuclei | Hyperchromasia | Sticky metaphase | Split chromosome | Binucleated cell | Irregular  Anaphase | Anaphase  Bridge | Polar Slip | Cells without a nucleus |
| Control | 1.4 ± 0.5 | 0 | 0 | 0 | 0.8 ± 0.01 | 0 | 1.6 ± 1.2 | 1.6 ± 0.6 | 0 | 2.4 ± 0.5 |
| 125mgL-1 | 10.4 ± 0.8a | 9.6 ± 1.2a | 31.2 ± 0.8a | 5 ± 1a | 5.4± 1.2a | 0 | 2.8 ± 0.5 | 7.2 ± 1a | 0.8 ± 0.2 | 11.6 ± 1.2a |
| 250mgL-1 | 12.8 ± 1.2a | 12 ± 1ab | 34.2 ± 1.8a | 8.4 ± 0.6a | 5.8 ± 0.6a | 4 ± 0.7a | 3.2 ± 0.7a | 12.6 ±1.2a | 2.2 ± 0.2a | 13.8 ± 1.8a |
| 500mgL-1 | 15.2 ± 1.6ab | 18 ± 0.5 abc | 41.2 ± 2.4abc | 10.4 ± 0.5ab | 6.6 ± 0.8a | 14.8 ± 1.1ac | 6.8 ± 1ab | 14.8 ± 1ab | 7.4 ± 0.6ab | 19.8 ± 2.4ab |

**Means with different letters show significant differences according to t-test (*P* ≤ 0.05) .**

|  |  |  |
| --- | --- | --- |
| Paracetamol Concentration (mgL-1) | Micronuclei Index (%) | Relative Rate of Abnormality (%) |
| Control | 0 | 0.8 |
| 125mgL-1 | 0.96 0.2 | 8.6 |
| 250mgL-1 | 1.2 0.5 | 10.9 |
| 500mgL-1 | 1.8 0.72 | 15.5 |

**Table 4: Micronuclei index (%) & Relative Rate of Abnormality (%) of Allium cepa**

4. Conclusion

The present study aimed to evaluate the cytotoxic effects of Paracetamol using *A. cepa*. Observations included inhibited root growth, abnormalities in the mitotic process, and a concerning micronucleus index across all tested concentrations. These results highlight the potential for constant exposure to Paracetamol, particularly at higher concentrations, to cause irreversible ecological damage by compromising genome integrity. However, despite testing a wide range of Paracetamol concentrations, it remains essential to analytically validate these concentrations and characterize the active metabolites of the pharmaceutical compound.

**DISCLAIMER (Artificial intelligence)**

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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