

# Cytogenetic Assessment of Blackening Shampoo Used in Temporary Tattoo in the Philippines on Onion (*Allium cepa* L.) Roots

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## ABSTRACT

**Background:** Commercial blackening shampoos containing Para-Phenylenediamine (PPD) are widely used in the Philippines as alternatives to Henna (*Lawsonia inermis* L.) as a temporary tattoo ink due to their affordability and ease of application. Despite the known allergenic and genotoxic risks of PPD, including chromosomal aberrations and DNA damage, their cytogenetic effects remain understudied locally. **Objectives:** This study evaluated the cytogenetic impact of a commercial blackening shampoo on onion (*Allium cepa*) roots. **Materials and Methods:** Varying concentrations (50 ppm, 300 ppm, 1000 ppm) were used, with distilled water as a negative control and 300 mM H<sub>2</sub>O<sub>2</sub> as a positive control. Root length, mitotic index, and chromosomal aberrations were assessed after 48 hr of exposure. **Results:** Results showed concentration-dependent inhibition: higher concentrations reduced root length and the mitotic index, with the presence of aberrations, including condensed nuclei and vagrant chromosomes. **Conclusion:** These findings highlight potential health risks from PPD exposure in temporary tattoos, filling gaps in local literature on cytogenotoxicity. The study recommends caution for consumers and further research on animal and human exposure.

**Keywords:** *Allium cepa*, Blackening shampoo, Cytogenetic effects, Para-phenylenediamine.

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## INTRODUCTION

Henna (*Lawsonia inermis* L.), a deciduous shrub cultivated primarily in India, Sudan, Egypt, and Pakistan, yields a natural reddish-brown dye from its leaves, harvested multiple times annually (Dev, 2023; Ram and Shekhawat, 2011). Traditionally used for over 9,000 years in cultural rituals across South Asia, the Middle East, and Africa for adorning skin, hair, and textiles (Rubio *et al.*, 2022; Surjit *et al.*, 2023). Henna also symbolizes good luck and protection at events such as weddings and festivals (Rocamora, 2023). In the Philippines, henna is popular for temporary tattoos, often during festivals and special occasions, reflecting indigenous practices like batok (hand-tapped tattoos) that date back 4,000 years and convey social rank, spirituality, and heritage (Baret, 2024; Crisostomo, 2013; Rocamora, 2023; Wilcken, 2025).

However, natural henna's reddish hue and longer drying time have led to the widespread use of "black henna" alternatives, such as blackening shampoos, prized for their low cost, ease of application often requiring only water, and longevity on skin lasting 1-2 weeks (Arun Kumar, 2024; Baret, 2024; Bhuvanewari *et al.*, 2021). These products, primarily marketed as hair dyes, are repurposed for tattoos despite containing Para-Phenylenediamine (PPD), a synthetic oxidative chemical (Alarcón *et al.*, 2025). PPD, present at concentrations of 2-3.4% in dyes (exceeding safe limits in some cases), is classified as a skin sensitizer and as acutely toxic under EU regulations, although it is not definitively mutagenic or carcinogenic due to data gaps (Babić *et al.*, 2023; Chong *et al.*, 2016; Meyer and Fischer, 2015).

PPD poses significant health risks, including allergic contact dermatitis, blistering, scarring, and systemic effects like neurobehavioral changes and reproductive dysfunction via mechanisms such as oxidative stress, mitochondrial disruption, and DNA adduct formation (Chen *et al.*, 2025; Mukkanna *et al.*, 2017; Salami *et al.*, 2023). Genotoxic effects, including chromosomal aberrations and DNA damage, have been documented in animal models and *in vitro* assays (AlSeigini *et al.*, 2014; Chong *et al.*, 2016). Recent warnings from the Philippine FDA and US FDA highlight unauthorized blackening shampoos containing PPD, emphasizing risks of severe reactions, especially



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in children. Despite these concerns, blackening shampoos remain popular in the Philippines for temporary tattoos (Crisostomo, 2013; Rocamora, 2023).

The *Allium cepa* assay, a validated bioindicator with high chromosomal visibility ( $2n=16$ ) and an 82% correlation with mammalian systems, is ideal for screening for cytogenotoxicity (Bonciu *et al.*, 2018; Bosio and Laughinghouse IV, 2012; Fiskesjö, 2008; Khanna and Sharma, 2013; Mukherjee *et al.*, 2026). This study evaluates the cytogenetic effects of a commercial blackening shampoo on *A. cepa* roots, focusing on root length, MI, and aberrations, to inform consumer safety and address local data gaps on PPD-containing products.

## MATERIALS AND METHODS

### Research design

This study employed an experimental design using the *Allium cepa* root assay, modified from Nawalage and Pathiratne (2020), to assess cytogenetic effects. Bulbs were exposed to treatments in a controlled room temperature of 26°C, dark environment for 48 hr, with macroscopic (root length) and microscopic (mitotic index and aberrations) parameters evaluated.

### Participants and Sampling

Onion (*Allium cepa*) bulbs, weighing 17-40 g, were selected as the test organisms. A purposive sampling strategy was used, sourcing 25 bulbs from three vendors in Tigbauan, Iloilo, to ensure diversity from different farmers in Iloilo province. Inclusion criteria: healthy, uniform-sized bulbs with intact outer scales. Exclusion: damaged or sprouted bulbs. The sample size was justified in accordance with standard *Allium cepa* protocols, with five bulbs per treatment group (Fiskesjö, 2008). The research site was a laboratory in the Biology Department of the College of Arts and Sciences at West Visayas State University.

### Instrument

Data were collected using an Olympus CH30 compound microscope (low- and high-power objectives) for mitotic analysis. Root lengths were measured using Wadlow WVC2B15 stainless steel digital vernier caliper. Staining was performed with an acetocarmine solution, with validity assessed according to standard cytological protocols. The administration involved slide preparation, scoring 1000 cells per sample for mitosis, and interpreting aberrations in triplicate (Fiskesjö, 2008).

### Data collection

Onion bulbs were gently scraped to expose the root primordia and placed in distilled water in a dark room for 24 hr to initiate rooting. Five bulbs per group were then exposed to the following treatments: distilled water (negative control), 300 mM H<sub>2</sub>O<sub>2</sub> (positive control), and hair dye concentrations of 50 ppm, 300 ppm, and 1000 ppm (Akwu *et al.*, 2019). After 48 hr, the three

longest roots per bulb were measured. Three representative roots per treatment were harvested (1-2 mm tips), fixed in Carnoy's solution for 24 hr, stored in 70% alcohol at 4°C, macerated in 1N HCl for 5 min, stained with acetocarmine at 60°C for 1 hr, rinsed with 45% acetic acid, and mounted on slides. Ethical considerations: As a plant-based study, no animal ethics approval was required; however, environmental disposal followed local guidelines (Department of Environment and Natural Resources). Informed consent is not applicable.

### Data analysis

Root lengths were averaged across replicates, and growth restriction was assessed qualitatively. The Mitotic Index (MI) was calculated using the formula:

$$MI = \left( \frac{\text{Number of dividing cells}}{1000 \text{ observed cells}} \right) \times 100$$

Chromosomal aberrations were classified following the criteria described by Fiskesjö (2008). Statistical analyses were performed using SPSS version 23, with a significance level set at  $p=0.05$ . Homogeneity of variances was tested using Levene's test, and multiple comparisons were conducted using Tukey's *post hoc* test.

## RESULTS

The results on root growth in onion (*Allium cepa*), as shown in Table 1, varied significantly among treatments, indicating differential cytogenetic effects of the commercial blackening shampoo. The negative control (distilled water) exhibited the longest mean root length (22.27±9.3 mm), reflecting normal cell division and elongation under non-stress conditions. In contrast, the positive control (300 mM H<sub>2</sub>O<sub>2</sub>) produced the shortest roots (12.53±7.9 mm), confirming its strong inhibitory effect on root growth due to oxidative stress (Akwu *et al.*, 2019). Among shampoo treatments, 50 ppm resulted in a mean root length of 19.60±7.8 mm, which did not differ statistically from the control, suggesting minimal toxicity at this concentration. However, as the concentration increased to 300 ppm and 1000 ppm, root length decreased to 19.27±7.5 mm and 16.33±4.8 mm, respectively, indicating progressive inhibition of root growth. Statistical grouping indicates that 50 ppm is at the same significance level as the control, whereas 300 ppm and 1000 ppm show intermediate effects.

The mitotic index of onion (*Allium cepa*) root tips, as shown in Table 2, varied significantly among treatments, reflecting the cytogenetic impact of the commercial blackening shampoo. The negative control (distilled water) exhibited a high mitotic index of 32.89±2.6, indicating normal cell division under non-stress conditions. Similarly, the 50-ppm shampoo treatment showed the highest value (34.44±2.3), which was not statistically different from the control. In contrast, the positive control (300 mM H<sub>2</sub>O<sub>2</sub>) exhibited the lowest mitotic index (19.11±2.4), confirming its strong inhibitory effect on mitosis via oxidative stress (Akwu *et*

*al.*, 2019). Increasing the shampoo concentration to 300 ppm and 1000 ppm resulted in reduced mitotic indices ( $28.89 \pm 3.1$  and  $23.11 \pm 2.3$ , respectively), indicating intermediate inhibition compared to the control and positive control.

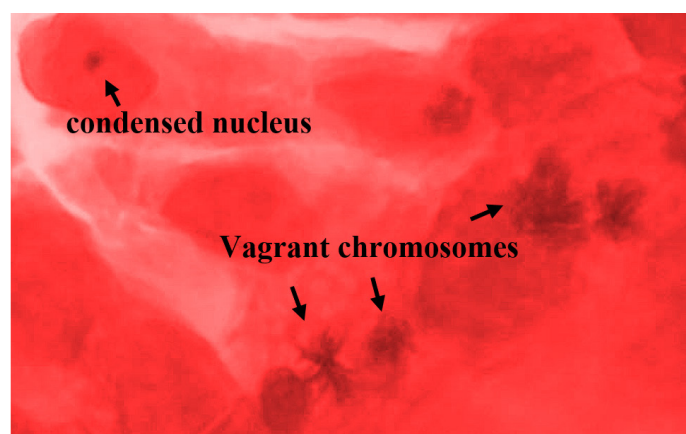
## DISCUSSION

The results show that root growth and mitotic activity in *Allium cepa* are reliable indicators of cytotoxic and genotoxic effects induced by the tested blackening shampoo containing PPD. Reduced root growth points to problems with cell division and elongation, consistent with earlier studies that use root growth as a primary indicator of phytotoxicity (Bonciu *et al.*, 2018; Bosio and Laughinghouse IV, 2012; Fiskesjö, 2008). The decrease in root length and mitotic index at higher shampoo concentrations suggests that meristematic activity is disrupted, possibly due to oxidative stress or blocked cell division. Toxicity assays indicate that root growth and mitotic activity are reliable indicators of cytotoxic and genotoxic effects. When root length decreases, it usually indicates that cell division and elongation are being blocked. This matches earlier studies that use root elongation as a key measure in phytotoxicity tests because it depends on active cell division (Al-Enezi and Aldawsari, 2022; Priac *et al.*, 2017; Sheridan *et al.*, 2022). The reduced growth also suggests the tested substance might change hormone balance or disrupt important metabolic pathways needed for root development, as seen in studies on auxin-cytokinin interactions and stress-related growth suppression (Al-Enezi and Aldawsari, 2022).

A large drop in the mitotic index at 300 ppm and 1000 ppm shows that normal cell division is being disrupted, possibly by affecting spindle formation or chromatin condensation (Al-Ahmadi, 2022; Graña, 2018). While chromosomal changes were not measured in detail, the presence of vagrant chromosomes and condensed nuclei suggests genotoxic effects, as shown in Figure 1. Chromosome stickiness may indicate changes in chromosomal proteins or DNA, and vagrant chromosomes suggest defects in the spindle apparatus, leading to errors during anaphase chromosome separation (Jayalal and Yatawara, 2024). These results align with the mechanisms in which changes in chromosome structure or number can damage genetic material, as described by Fiskesjö (2008).

The mitotic index (MI) is a clear measure of cell growth and a strong sign of cytotoxicity (Graña, 2018). A substantial decrease in MI relative to the control indicates that the test substance disrupts normal cell division, possibly by affecting spindle function, chromatin condensation, or DNA replication (Al-Ahmadi, 2022; Ristea and Zarnescu, 2024). Chromosomal abnormalities such as vagrant chromosomes, sticky metaphases, and condensed nuclei support this idea, although they were not measured in detail. Chromosome stickiness typically reflects changes in chromosomal proteins or DNA, and vagrant chromosomes exhibit spindle defects that cause errors during anaphase chromosome separation (Abubacker and Sathya, 2017; Jayalal and Yatawara, 2024). These findings are consistent with the mechanisms described by Fiskesjö (2008), in which changes in chromosome structure or number suggest toxicant effects on genetic material.

Similar results were reported by Nawalage and Pathiratne (2020), who found that herbal hair dyes reduced root growth and mitotic activity in *A. cepa* bulbs and exhibited greater cytotoxicity at higher concentrations. The effects of the tested shampoo are similar to those of oxidative dyes containing PPD, which have been linked to oxidative stress, DNA damage, and chromosomal instability in mammals (AlSeigini *et al.*, 2014; Salami *et al.*, 2023). Other studies have reported similar results when assessing the cytogenotoxicity of synthetic and herbal hair dyes, detergents, and agrochemicals using the *Allium cepa* test. Nawalage and Pathiratne (2020) showed that higher amounts of herbal hair dye reduced



**Figure 1:** Vagrant chromosomes and condensed nuclei observed under High-Power Objective (HPO) microscopy.

**Table 1: Average Root Lengths After 48 hr Exposure (mm).**

Treatments	Root length (mm)		
300 mM H <sub>2</sub> O <sub>2</sub>	12.53	±7.90	a
distilled water only	22.27	±9.28	b
50 ppm	19.60	±7.84	b
300 ppm	19.27	±7.50	ab
1000 ppm	16.33	±4.84	ab

Note: Means with the same letter are not significantly different ( $p > 0.05$ , Tukey's test).

**Table 2: Mitotic Index After 48 hr Exposure.**

Treatments	Mitotic Index		
300 mM H <sub>2</sub> O <sub>2</sub>	19.11	±2.35	a
Distilled Water only	32.89	±2.56	b
50 ppm	34.44	±2.26	b
300 ppm	28.89	±3.09	ab
1000 ppm	23.11	±2.32	ab

Note: Means with the same letter are not significantly different ( $p > 0.05$ , Tukey's test).

root growth and mitotic activity in onion bulbs. Similar patterns were also reported with streptomycin and dimethoate (Gupta *et al.*, 2025). These findings support the use of root elongation and mitotic index as reliable indicators of the safety of environmental chemicals. Other research has identified chromosomal changes, such as C-metaphase, anaphase bridges, and micronuclei, which exhibit clastogenic and aneugenic effects that can cause mutations and persistent genetic instability (Mukherjee *et al.*, 2026). These results suggest potential risks from chemicals such as Para-Phenylenediamine (PPD), a common hair dye ingredient that may be genotoxic under certain conditions (Babić *et al.*, 2023; Chen *et al.*, 2025). While earlier reviews reported that PPD is not genotoxic, new evidence suggests that its breakdown products and long-term exposure warrant further study (Chong *et al.*, 2016; Salami *et al.*, 2023).

## LIMITATIONS

This study did not measure chromosomal changes in detail or confirm the presence of PPD in the shampoo. Future research should use comet assays and advanced chemical tests, such as HPLC or LC-MS, to better assess genotoxicity and analyze chemical composition. This study used only qualitative methods to examine chromosomes. Additional genotoxicity assays, such as the comet assay or the micronucleus test, along with dose-response studies and molecular analyses of cell-cycle regulators, are recommended to further confirm the observed effects.

## CONCLUSION

The commercial blackening shampoo exhibits cytogenetic potential in *Allium cepa* root cells, with effects intensifying at higher concentrations. This confirms PPD's role in growth inhibition and chromosomal aberrations. The study suggests caution with PPD-based dyes, as prolonged skin exposure during temporary tattoos may pose health risks. Recommendations include regulatory testing of local products and public awareness campaigns on the hazards of PPD, particularly in the Philippines and other Asian countries. Furthermore, future studies on human dermal absorption and the long-term genotoxicity of these PPD-containing temporary tattoos are warranted.

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## ABBREVIATIONS

**FDA:** Food and Drug Administration; **HPLC:** High-Performance Liquid Chromatography; **LC-MS:** Liquid Chromatography-Mass Spectrometry; **MI:** Mitotic Index; **PPD:** Para-phenylenediamine; **ppm:** Parts per million.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

This research was self-funded by the author.

## AUTHOR CONTRIBUTIONS

**Edcel Jed Samson:** Conceptualization, methodology, investigation, data analysis, and writing - original draft, review, and editing.

## SUMMARY

This study demonstrates that blackening shampoo containing PPD exhibits clear cytogenetic potential in *Allium cepa*. The observed concentration-dependent reduction in root length and mitotic index, alongside chromosomal aberrations, suggests significant cytotoxic and genotoxic risks associated with its off-label use as a temporary tattoo ink.

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