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ABSTRACT

Formalin is a well-known aqueous solution of formaldehyde, commonly used as a sterilant and disinfectant in hospitals, laboratories, and industries. It is a known carcinogenic and genotoxic toxicant. This study examines the cytogenotoxic effects of various concentrations of formalin using the Allium cepa assay. Healthy Allium cepa bulbs were exposed to different concentrations of formalin (0.1%, 0.5%, 1%, 2%, and 5% v/v) with distilled water serving as control for 48 hours. Root growth inhibition, mitotic index (MI), and chromosomal aberrations (CAs) were microscopically assessed by staining with aceto-orcein. Formalin exposure reduced root growth and mitotic index in a concentration-dependent manner. Root growth was suppressed by more than 80% in the 5% formalin group compared to control. The mitotic index was considerably reduced, recording 12.4% and 2.3% in control and 5% formalin, respectively ($p < 0.05$). Various chromosomal abnormalities were observed, including stickiness, laggards, bridges, vagrant chromosomes, and micronuclei formation. Aberrations were concentration-dependent, with the highest frequency at 2% and 5% formalin solutions. Formalin demonstrated potent concentration-dependent cytotoxic and genotoxic effects in Allium cepa root meristems. These plant-based findings underscore the genotoxic potential of formalin and highlight the utility of the Allium cepa assay as a reliable bio-indicator for genotoxicity screening of chemical sterilant.

Keywords: Formalin, Cytogenotoxicity, Allium cepa, mitotic index, chromosomal aberrations

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INTRODUCTION

Formalin is an aqueous solution with about 37-40% formaldehyde, which is very commonly used as a sterilant and tissue preservative in hospitals, laboratories, and mortuaries because of its wide-spectrum antimicrobial action [1,2]. Nevertheless, formalin is linked to various health hazards such as irritation of the respiratory tract, sensitization of the skin, and cancer [3]. Formaldehyde is a Group 1 carcinogen in the list of the International Agency for Research on Cancer (IARC) [4]. Formaldehyde has mutagenic and cytogenotoxic effects arising from occupational and environmental exposures, where formaldehyde exposure has been shown to cause DNA-protein crosslinking, chromosomal damage, and oxidative stress effects [5,6]. As such, its possible cytogenotoxic risk at different concentrations is essential to evaluate its safety profile. The Allium cepa assay is a sensitive, inexpensive, and internationally validated test system for detecting Cytogenotoxicity [7,8]. It measures endpoints including root growth inhibition, mitotic index, and chromosomal aberration, which are valid markers of DNA and cell division dysfunction [9]. This study investigates the cytogenotoxic effects of varying concentrations of formalin using the Allium cepa assay to provide experimental data for risk assessment.

MATERIALS AND METHODS

Test Material:

Commercial formalin solution (37–40% formaldehyde in water, stabilized with methanol) was diluted to obtain test concentrations of 0.1%, 0.5%, 1%, 2%, and 5% (v/v). Distilled water was used as the negative control. Although the commercial formalin used was stabilized with methanol, a separate methanol control was not included because the methanol content in the final diluted test concentrations was negligible (less than 0.1% v/v) and thus considered unlikely to contribute significantly to the observed biological effects. Distilled water was therefore deemed adequate control for this study.

Plant Material:

Onion (Allium cepa L.) bulbs (2-3 cm in diameter) that appeared in good health and of uniform size were purchased in a local market. Bulbs were dried in the sun and 48 hours later, all outer scales were removed and dried roots were trimmed.

Experimental Procedure:

Bulbs were placed in glass beakers containing test concentrations or control water, ensuring that the basal portions were fully submerged. The exposure time was 48 hours at room temperature.

Root Growth Inhibition Test:

Five longest roots were measured in length and the mean values obtained. Relative root growth inhibition when compared to control was determined.

Cytological Analysis:

Excision of root tips (1-2 cm) occurred and was fixed in ethanol: acetic acid (3:1) 24hours after exposure, hydrolyzed 1N HCl at 60^{o}C for 5 min, and stained with 2% aceto-orcein. Microscopic examination of squash preparations was carried out as previously described [7,9].

Mitotic Index (MI):

At least 1,000 cells per slide were scored for each treatment. MI was calculated as: MI (%) = (Number of cells in Mitosis / Total number of observed cells) x 100 [8,9].

Chromosomal Aberrations (CA):

Cells in division were screened for structural and spindle abnormalities, including stickiness, bridges, laggards, vagrants, multi-polarity, and micronuclei [8,9].

Statistical Analysis:

Data were expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was used ($p < 0.05$ considered significant).

RESULTS

Root Growth Inhibition: A significant reduction in root growth was observed following exposure to formalin, which exerted its effect in a concentration-dependent manner. Root lengths decreased from 4.8 ± 0.3 cm in the control to 0.9 ± 0.2 cm at 5% formalin ($p < 0.05$).

Table 1 shows the effect of varying concentrations of formalin on root growth of Allium cepa after 48 hours exposure. Values are expressed as mean ± SEM (n = 4). A concentration-dependent reduction in root length was observed, with significant inhibition at higher concentrations ($p < 0.05$ vs control).

Mitotic Index: A dose-dependent reduction in MI was observed. The control had a mitotic index of 12.4 ± 0.8%, while 5% formalin showed 2.3 ± 0.4% with $p < 0.05$.

Table 2. Effect of formalin exposure on mitotic index of *Allium cepa* root meristem cells. Values are expressed as mean ± SEM (n = 4). A significant, concentration-dependent decrease in mitotic activity was observed ($p < 0.05$ vs control).

Table 1:	
Formalin Concentration (%)	Root Length (cm) (Mean ± SEM)
Control	4.80 ± 0.30
0.1	4.72 ± 0.29
0.5	4.41 ± 0.27
1.0	4.00 ± 0.25
2.0	3.24 ± 0.23
5.0	0.90 ± 0.20

Table 2:	
Formalin Concentration (%)	Mitotic Index (%) (Mean ± SEM)
Control	12.40 ± 0.80
0.1	12.20 ± 0.78
0.5	11.40 ± 0.70
1.0	10.40 ± 0.60
2.0	8.40 ± 0.50
5.0	2.30 ± 0.40

Chromosomal Aberrations:

Diverse aberrations were also noticed on exposure to formalin. Stickiness and bridges were predominant, followed by laggards and vagrant chromosomes. Micronuclei formation was noted at higher concentrations (≥ 2%). The frequency of aberrations increased with concentration, with maximum effect at 5% formalin.

DISCUSSION

The Allium cepa assay demonstrated that formalin exerts both cytotoxic and genotoxic effects in a concentration-dependent manner on plant root meristem cells. The inhibition of root growth reflects cytotoxicity at the level of cell elongation and division, consistent with known aldehyde toxicity in plant systems [10].

This marked decrease in mitotic index is indicative of cell cycle arrest, which may be attributed to DNA-protein crosslinking and disruption of spindle fiber assembly by formaldehyde [11]. Stickiness and bridges are chromosomal aberrations that signal DNA condensation errors and clastogenic activity [12]. Spindle apparatus interference is suggested by the presence of laggards and vagrants, while micronuclei formation confirms genotoxic activity in the plant root meristem cells [13]. These findings are consistent with

previously reported genotoxic effects of formaldehyde in plant test systems [14,15].

The Allium cepa bioassay proved to be a reliable and cost-effective biomonitoring tool for identifying the cytogenotoxic potential of chemical sterilant such as formalin. However, it is important to note that these findings are limited to a plant-based model and should not be directly extrapolated to human or animal biological systems.

Limitations of study

The study used a plant-based model, which may not fully replicate human biological responses

Only acute exposure (48 hours) was assessed

Molecular mechanisms were not directly investigated

CONCLUSION

Formalin demonstrated concentration-dependent cytotoxic and genotoxic effects in Allium cepa root meristems, as evidenced by significant root growth inhibition, reduced mitotic index, and increased frequency of chromosomal aberrations. These plant-based findings confirm the genotoxic potential of formalin within the Allium cepa model system. Given the limitations of this study, including its plant-based design and acute exposure period, these results should be interpreted within the context of the Allium cepa bioassay and not extrapolated to human or animal systems without further investigation.

Conflict of interest

The authors declare no conflict of interest

Author Contributions: Boniface A. Onoja and Adeoye O. Oyewopo conceptualized and designed the study. Boniface A. Onoja conducted the investigation. Elijah A. Adepoju performed the statistical analysis. Bernard A. Alabi and Boniface A. Onoja drafted the manuscript. All authors reviewed and edited the manuscript. Adeoye O. Oyewopo supervised the study.

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