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Original Research Article

In Silico ADMET Profiling and Toxicological Evaluation of Sudan IV Dye on *Allium cepa* (Onions) Root Meristems

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ABSTRACT

The widespread use of Sudan IV dye in the food industry raises serious concerns regarding its potential toxicity. This study therefore employed in-silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling to assess the toxicological potential of Sudan IV dye. Additionally, the cytotoxic and genotoxic effects of the dye were done using the root meristems of *Allium cepa*. ADMET prediction tools were employed to assess the physicochemical properties and toxicity profiles of the compound. For cytogenotoxic assessments, onion roots were exposed to 0.1%, 0.5%, and 1.0% concentrations of Sudan IV dye and ethanol for 24, 48, and 72 hours, with distilled water as the control for the study. Treated cells were fixed, hydrolyzed, squashed, and stained for microscopic observation at 1000x magnification. The ADMET profile prediction indicated a significant potential for bioaccumulation in adipocytes and highlighted risks of genotoxicity, carcinogenicity, mutagenicity, hematotoxicity, and nephrotoxicity. The dye significantly induced ($P < 0.05$) nine chromosomal abnormalities in *A. cepa* root tips especially after 72 hours of exposure. These induced abnormalities included sticky chromosomes, ghost cells, faulty polarity, chromosome bridges, C-mitosis, vacuolated cells, binucleate cells, fragmented chromosomes, and spindle disturbances. The highest root growth inhibition of 82.93% occurred with 1.0% Sudan IV dye treatment, while ethanol caused the least inhibition of 58.54%. Furthermore, Sudan IV dye significantly reduced the mitotic index across exposed durations, indicating disruption of normal cell division. This study demonstrates the toxicity of Sudan IV dye and suggests that its use especially in food products should be discouraged.

Keywords: In-silicon, Sudan IV, Cytotoxic, Genotoxic

Introduction

Food is essential for human survival by providing necessary nutrients for growth and overall health.[1] Therefore, the food industry has developed methods to enhance the appearance and sensory qualities of food, which has led to an increase in the use of food additives. The practice of improving the visual appearance of food dates back to ancient times, with ancient civilizations using plant-based additives like herbs and spices. [2-3] In modern times, synthetic additives are commonly used to enhance the taste, flavor, and appearance of food.[4] Consequently, this means that people can easily be exposed to harmful chemicals through the consumption of food containing adulterated additives.

Palm oil is one of the most common food additives used as an ingredient and agent for enhancing the quality, flavor, texture, and shelf-life of various food products. [5] About 3 billion people in 150 countries use products containing palm oil every day. [6]

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Also, approximately 90% of palm oil produced is consumed as food, while the remaining 10% is used in making soap and other products. [7] Sudan IV is used as a colorant in items like shoe polish, petrol, plastics, cosmetics, printing inks, as well as ingredients in animal feeds. [8] Consumers often regard the bright red color of palm oil as an indicator of high quality, particularly for food products.[9] Consequently, sellers often use Sudan IV dye to make palm oil look more attractive to customers. [10] In addition to this, Sudan IV dye is commonly applied to oil because it has a deep red color and dissolves well in oil. Sudan IV dye is a common contaminant in palm oil due to its fat-soluble properties, which allow it to blend easily into oil. [11-12] It is reported that a significant amount of palm oil produced in Nigeria is contaminated with this dye. [13] A study revealed that found that 57% of palm oil samples from major markets in Benin City were adulterated with varying concentrations of Sudan IV dye. [14] Given these alarming statistics, it is obvious that many people are likely consuming the dye daily through homemade and ready-to-eat meals.

The situation is particularly alarming because Sudan IV, like other Sudan dyes, is classified as a carcinogen.[15] To buttress this concern, a report revealed that the European Union has banned the use of Sudan IV dye in food products due to its hazardous nature. [16] Consequently, serious issues were raised concerning the widespread use of these dyes in Nigeria, highlighting their carcinogenic properties and the potential health risks they pose to consumers. [17] Similarly, the International Agency for Research on Cancer (IARC) classifies Sudan IV as a Group 3 carcinogen.[18] Some breakdown products of Sudan dyes are classified as category 2 carcinogens, which indicates that they could pose cancer

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risk under certain conditions.[19] As a result of these, countries like the United States, Canada, Australia, New Zealand, Ghana, India, China, and Japan have also banned Sudan dyes in food products. Despite the ban on azo dyes in food products, [14] noted that their use continues in Nigeria due to poor monitoring and compliance. This ongoing issue raises serious concerns, as a study showed that Sudan IV dye causes hemolytic anemia, blood cell lysis and inflammatory responses in the liver and kidneys of rats.[20] These alarming findings highlight the urgent need for effective monitoring and regulatory measures to address the continuous use of harmful substances in food products.

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) is indeed suitable for addressing issues related to the continued use of harmful substances in food products.[21] ADMET is a computational tool used in pharmacokinetics to evaluate the toxicity of chemicals and is widely applied in drug development. [22-23] Although ADMET analysis was originally developed for pharmaceuticals, [21] stated that the technique is now used to assess the safety of food additives, pesticides, veterinary drug residues, and mycotoxins. This makes ADMET a valuable tool for predicting the behavior and toxic effects of food contaminants. Regulatory bodies, such as the European Food Safety Authority (EFSA), use ADMET analyses to set Maximum Residue Limits (MRLs) for contaminants in foods. [24] By combining computational predictions with experimental data a clearer report of how contaminants may affect living systems will be provided. *A. cepa* assay is a valuable tool in toxicological assessments by providing a low-cost and reliable approach for monitoring food safety and environmental pollutants. [25]

Against this background, this study investigated the cytogenotoxic effects of Sudan IV dye using *A. cepa* L. as the model organism. *Allium cepa* is recognized as a sensitive, reliable, and cost-effective tool for screening potential environmental toxins.[26] The large chromosomes and well-defined cell cycle of *A. cepa* root tip cells make it easy to observe the effects of test substances on cell division and chromosomal abnormalities. [27] Findings from the *A. cepa* model correlate strongly with data from other plant models, such as *Lactuca sativa*. [28] The genotoxic effects observed in *A. cepa* root meristems also align well with findings from mammalian models. [25] Again, substances that cause significant genotoxicity in *A. cepa* have been shown to produce similar effects in rodents, [29] and human cell lines. [30] Similarly, the *A. cepa* assay serves as an essential biological tool for confirming the predictions of computational models, such as ADMET analysis, regarding the absorption, distribution, metabolism, excretion, and toxicity of chemicals.[31]

Combining these two methods provides a comprehensive approach to evaluating the safety of Sudan IV dye when ingested into the living system. These approaches ensure that both theoretical and practical aspects of toxicological assessment are thoroughly addressed. [31] Consequently, this dual methodology will not only deepen our knowledge of the potential health risks associated with the dye but also aid in the formulation of effective regulatory measures to ensure food safety. Specifically, the study aims at assessing the cyto-genotoxic effects of Sudan IV dye on *Allium cepa* (onion) root tip cells, while also utilizing ADMET analysis to evaluate the dye's pharmacokinetics and toxicity.

Materials and Methods

Collection of Samples

Healthy and uniform-sized purple *A. cepa* (onion) bulbs, with diameters ranging from 2.5 cm to 3.0 cm. [32] The onions were obtained from the Lokoja International Market in Kogi State (Latitude: 7° 50' 51.86" N, Longitude: 6° 44' 0.9" E) on the 15th of October 2023. The plant sample was identified at the herbarium of the Department of Botany at Federal University Lokoja and authenticated by Akanni Gbenga Theophilus using macroscopic methods. A specimen number, FUL-2024-022, was assigned to the sample. Additionally, 80% Sudan IV dye (Sigma-Aldrich), 99.8% absolute ethanol (BDH Chemicals, UK), 99.7% glacial acetic acid (BDH Chemicals, UK), and 36% hydrochloric acid (Sigma-Aldrich) were procured from Delux Chemical Store in Lokoja.

Development of Root from Onions

After removing the outer dry scales from the onion bulbs, the reduced stem was placed in freshly prepared distilled water to initiate the rooting process. This setup was kept in a dark cupboard, and the water was changed daily until roots developed. [33] Roots measuring up to 2 cm in length were selected for further study. [33]

Preparation of Different Concentrations of Sudan IV Dye

1 g of Sudan IV dye was dissolved in 100 mL of ethanol to produce 1% (w/v) stock solution. [34] This solution was stirred until the dye completely dissolved. To prepare different concentrations, 10 mL of the 1% stock solution was mixed with 90 mL of ethanol to make a 0.1% concentration. [34] For a 0.5% concentration, 50 mL of the stock solution was combined with 50 mL of ethanol. The 1.0% concentration was used directly from the stock solution without dilution. These concentrations are commonly used to contaminate palm oil. [13] Each prepared solution was mixed thoroughly to ensure even distribution of the dye before use in the study.

Transfer of Onion Roots into Treatments

Roots from the selected bulbs, measuring up to 2 cm in length, were transferred into beakers containing different concentrations of Sudan IV dye (0.1%, 0.5%, and 1.0%) by ensuring that the basal ends were submerged in the solutions. [27] To rule out any effects from ethanol, some roots of *A. cepa* were placed in ethanol separately, while roots in distilled water served as the control for the study. The roots were treated for 24, 48, and 72 hours in a dark, well-ventilated cupboard at room temperature (25 ± 2°C) to examine both short-term and long-term effects on root growth and cellular processes. [35] Each treatment was replicated five times and arranged in a Completely Randomized Design (CRD) to ensure statistical validity.

Root Growth Assessment

Twenty-five roots were randomly selected from five onion bulbs, and their initial lengths were measured with a meter rule before treatment. [36] After exposure to 0.1%, 0.5%, and 1.0% concentrations of Sudan IV dye, ethanol, and distilled water (control), the lengths were measured again after 24, 48, and 72 hours to evaluate the effects on root growth. The setups were kept in the dark, and the solutions were changed daily. [35] The growth in root lengths for each treatment was calculated by subtracting the initial measurements from the final lengths. The percentage of growth inhibition for the treated roots compared to the control was then calculated using the formula (equation 1). [37]

$$\text{Percentage Growth Inhibition PGI (\%)} = \frac{C0-C1}{C0}\ X100$$

Equation 1

C_0 = Length in control

C_1 = Length in treatments

Genotoxic Study

Onion root tips exposed to distilled water, ethanol, and various concentrations of Sudan IV dye for 24, 48, and 72 hours were excised between 8 and 9 AM Central African Time (CAT). [38] These root tips were then fixed in a freshly prepared Carnoy's fixative solution, which is a mixture of glacial acetic acid and absolute ethanol in a ratio 1:3 for 24 hours at 4 °C to preserve the cells in their natural state. [38] After fixation, the root tips were rinsed with distilled water and hydrolyzed in 1 N HCl at 60 °C for 10 minutes to soften them for easier squashing. [38] The hydrolyzed root tips were thoroughly rinsed with distilled water and placed on clean glass slides. The meristematic tips were carefully cut with a blade, discarding the remaining parts. Finally, two drops of aceto-carmine stain were applied to the meristems, which were then covered with coverslips. [38]

The samples were gently squashed using the flat end of a cylindrical search pin until a cloudy liquid was formed. [33] To remove any excess stain, the filter paper was softly pressed against the slides and cover slips. [32] Semi-permanent slides were produced by sealing the coverslip with nail polish after adding a drop of 45% acetic acid. [39] The prepared slides were then examined at magnifications of X 400 and X 1000 under

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the Olympus Microscope model CX21FS1 produced by Olympus Corporation in Japan. For each treatment, the best five slides were selected, analyzing a total of 200 cells per slide, resulting in a total count of 1000 cells per treatment at X 1000 magnification. The numbers of cells in different dividing stages of the cell cycle (prophase, metaphase, anaphase, and telophase) as well as interphase cells were counted for each treatment. [35] The quantity of cells showing each type of chromosomal aberration was recorded. [40]

Data Analysis

The percentages of cells exhibiting chromosomal abnormalities and the Mitotic Index (MI) for each treatment were calculated using the formulas (equation 2 and 3). [37]

$$Percentage\ of\ Aberrant\ Cell\ (PAC) = \frac{Total\ number\ of\ abnormal\ cells}{Total\ numberof\ cells\ examined}\ X\ 100 \quad Equation\ 2$$

$$Mitotic\ Index\ (MI) = \frac{Total\ number\ of\ dividing\ cell}{Total\ numberof\ cells\ examined}\ X\ 100 \quad Equation\ 3$$

The numbers of prophase, metaphase, anaphase, and telophase cells were summed to give the total number of dividing cells.

These data were analyzed using one-way Analysis of Variance (ANOVA) to identify significant differences between the control and treatment groups. Duncan Multiple Range Test was then used to compare means that showed significant differences ($p < 0.05$). The results were presented as mean ± standard deviation. All statistical analyses were performed using the SPSS version 23.0 software released in 2015. After the analysis, the percentages of aberrant cells and mitotic indices were displayed in bar charts.

Physicochemical Properties and ADMET Profile

Sudan IV Molecular Simplified Molecular Input Line Entry System (SMILE) was downloaded from PubChem Database [https://pubchem.ncbi.nlm.nih.gov/] with Compound CID: 62330 and was selected for ADMET studies in addition to other physiochemical analysis using ADMETlab 3.0 for further analysis [https://admetmesh.scbdd.com/service/evaluation/cal].

Results and Discussion

This study employed the ADMET profile of Sudan IV dye to predict its absorption, distribution, metabolism, excretion, and overall toxicity while using *A. cepa* root tips as a bioassay to evaluate its cytotoxicity and genotoxicity in living systems.

ADMET Profile of Sudan IV

Sudan IV dye exhibits a complex profile regarding its ADMET characteristics. The dye has a moderate molecular weight of 380.16 g/mol and a Topological Polar Surface Area of 69.67 Å², indicating favorable properties for absorption, but it exhibits poor permeability across Caco-2 (-4.57) and MDCK (-4.73) cells, with limited human intestinal absorption (0.0005) and high plasma protein binding of 98.82% (Table 1). This ADMET prediction profile indicates that Sudan IV has moderate molecular weight, topological polar surface area (TPSA), and neutral formal charge, which suggest favorable membrane permeability and potential oral bioavailability.[41] However, its high lipophilicity and poor water solubility imply a tendency to accumulate in lipid-rich tissues such as adipocytes, thereby limiting its availability in the bloodstream.

Table 1: Comprehensive Physicochemical Properties and ADMET Profile of Sudan IV Compound

Physicochemical Property		Absorption		Excretion	
MW	580.16	Caco2	-4.567	t0.5	0.558
Vol	407.938	MDCK	-4.732	cl-plasma	4.847
Dense	0.932	Pgp_inh	0.083	Toxicity	
nHA	5	Pgp_sub	1.58E-05	Ames	0.947
nHD	1	f20	0.477	Carcinogenicity	0.942
TPSA	69.67	f30	0.718	DILI	0.65
nRot	4	F50	0.993	EC	0.007
nRing	4	Hia	0.00049	EI	0.948
MaxRing	10	Distribution		Genotoxicity	0.999
nHet	5	PPB	98.819	Hematotoxicity	0.821
fChar	0	logVDss	0.411	Herg-10um	0.733
nRig	25	BBB	0.015	hERG	0.083
Flex	0.16	Metabolism		Nephrotoxicity-DI	0.78
logS	-6.23	CYP1A2-inh	0.983	Neurotoxicity-DI	0.982
logD	4.294	CYP1A2-sub	0.109	Ototoxicity	0.329
logP	5.297	CYP2C19-inh	0.003	Respiratory	0.813
Mp	132.132	CYP2C19-sub	2.76E-06	SkinSen	0.941
Bp	438.329	CYP2C9-inh	0.004		
Pka_acidity	11.464	CYP2C9-sub	0.032		
Pka_acidity	2.622	CYP2D6-inh	2.73E-06		
		CYP2D6-sub	0.529		
		CYP3A4-inh	1.09E-05		
		CYP3A4-sub	0.072		
		CYP286-inh	0.917		
		CYP286-sub	1.98E-09		
		CYP2CB-inh	0.998		

Key

High

Medium

Low

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Despite these characteristics that generally favour absorption, Sudan IV demonstrates poor absorption across intestinal barriers. This is evidenced by low predicted permeability values from Caco-2 and MDCK cell models, alongside a low Human Intestinal Absorption (HIA) value. Nevertheless, moderate fractional absorption rates indicate some systemic exposure to the compound under certain conditions. Additionally, its high Plasma Protein Binding (PPB) suggests prolonged retention in plasma, which may increase the risk of toxicity. Furthermore, limited permeability across the Blood-Brain Barrier (BBB) reduces exposure to the Central Nervous System (CNS), suggesting minimal neurotoxicity. [42-43]

The Cytochrome 450 values typically range from 0 to 1, and this study revealed that Sudan IV interacts with several CYP enzymes, acting as a strong inhibitor of CYP1A2 (0.98) and CYP2B6 (0.92) (Table 1). In addition to this, the compound demonstrates moderate substrate potential for CYP2D6 (0.53) and exhibits weak activity for other enzymes. This raises concerns regarding potential drug-drug interactions, especially for individuals taking medications metabolized by these enzymes. Study showed that 2, 3-Diaminophenazine showed similar inhibiting on CYP1A2 and CYP2B6 enzyme activities. [44] Although Sudan IV dye is rapidly eliminated from the body with a plasma clearance rate of 4.85 mL/min/kg and a half-life of 0.56 hours, its toxic effects can manifest quickly following exposure.[45]

Toxicity predictions indicated that Sudan IV poses a very high risk of genotoxicity (0.999), carcinogenicity (0.942), and Ames mutagenicity (0.947), along with significant hematotoxicity (0.821), neurotoxicity (0.982), and nephrotoxicity (0.780). These findings are concerning due to the substantial risks associated with genotoxicity, carcinogenicity, and mutagenicity of the dye. These findings align with studies showing that Sudan dyes, including Sudan IV, have been classified as Class-III carcinogens by the International Agency for Research on Cancer (IARC). [16] Additionally, Sudan IV dye is associated with hematotoxicity, neurotoxicity, and nephrotoxicity which raises substantial safety concerns.[46]

The ADMET profile further reveals that Sudan IV is weakly acidic and highly stable thermally, with a melting point of 132°C and a boiling point of 438°C. These properties suggest persistence in both environmental and biological systems. [47] Its structural rigidity characterized by four rings and 25 rigid bonds enhances binding specificity and may lead to harmful interactions with biological molecules such as proteins and DNA.[44] This indicates that Sudan IV could exhibit high toxicity along with potential genotoxic effects.

Cytotoxic and Genotoxic of Sudan IV

The effects of distilled water (control), ethanol and different concentrations of Sudan IV dye at 24 hrs, 48 hrs and 72 hrs durations of exposure are shown in Table 2. It was observed that the five phases of division responded significantly to different concentrations of Sudan IV dye and exposure durations (Table 2 and Figure 3). There is a significant increase ($p < 0.01$) in the number of cells at the five phases of the cell cycle for different concentrations of Sudan IV dye compared to the control and ethanol-treated roots at 24, 48, and 72 hours. Notably, more cells were observed in the interphase stage compared to other stages in this study (Table 2). This finding could be attributed to the arrest of cell division caused by the dye, which eventually resulted in the accumulation of cells in this phase. A delay in the transition from interphase to mitotic phases in non-dividing cells treated with the pesticide pendimethalin has been reported. [48] On the other hand, a significant reduction in the number of cells in the interphase stage was observed in ethanol-treated root tips when compared to the control at 48 and 72 hours of exposure. Ethanol was shown to induce cellular stress and apoptosis (cell death), which led to a decrease in the population of cells in the interphase stage. [49]

In this study, nine chromosome aberrations were induced in *A. cepa* root tips by the dye (Table 3 and Figure 4). These aberrations include sticky chromosomes, ghost cells, faulty polarity, chromosome bridges, C-mitosis, vacuolated cells, binucleate cells, fragmented chromosomes, and spindle disturbances. Furthermore, the progressive increase in the occurrence of these aberrations across the varying doses of the dye suggests a concentration-dependent relationship, where higher doses exacerbate the severity of chromosomal damage. An increase in the concentrations of chemical mutagens resulted in increasing frequencies of chromosomal abnormalities. [50] This observed genotoxic and mutagenic potentials of Sudan IV dye affirms the high genotoxicity and mutagenicity prediction by ADMET in this study.

In this study, it was observed that no chromosomal aberration was recorded in roots exposed to distilled water for 24 hours. However, at 48 hours of exposure to distilled water, a sticky chromosome was observed, while both sticky chromosomes and vacuolated cells were recorded at the 72-hour exposure duration. This finding revealed that even the control treatment can induce some level of chromosomal damage over an extended exposure period. The prolonged exposure of *A. cepa* root meristems to distilled water significantly induced multiple nuclei in cells. [51]

Ethanol induced sticky chromosomes at 24 hours, while at both 48 and 72 hours, sticky chromosomes and vacuolated cells were recorded. A study revealed that ethanol could induce oxidative stress thereby causing damage to cellular components. [52] However, the observation that these two abnormalities also appeared in roots exposed to distilled water (control) for 72 hours suggests that the chromosomal damages observed in the roots treated with Sudan IV dye were primarily due to the dye rather than the ethanol used as solvent. This observation supports the popular use of both water and ethanol as solvent for extracting active ingredients during genotoxic studies especially under short term study.

A significant correlation between the concentration of Sudan IV dye and the percentage of aberrant cells induced in *A. cepa* root tips, particularly at the 24-hour exposure duration was observed in this study (Figure 1). Increasing concentrations of synthetic dyes lead to a higher percentage of chromosomal aberrations in *A. cepa* root tip cells. [53] Interestingly, the trend observed at 72 hours, where no significant difference was noted in the percentage of aberrant cells induced by different concentrations of Sudan IV dye, revealed that even lower concentrations of the dye can induce significant genotoxic effects over extended exposure durations. Every concentration of Indigo carmine has been reported to induce chromosomal abnormalities. [54]

Table 4 shows the variation in root lengths and percentage growth inhibition of *A. cepa* exposed to ethanol and different concentrations of Sudan IV dye. Specifically, at 24 hours, the root lengths of *A. cepa* grown in ethanol and various concentrations of Sudan IV dye did not show significant differences compared to the control group. This suggests that short-term exposure to these substances may not immediately affect root growth, indicating a potential threshold for toxicity that may require longer exposure times to manifest its effects. However, significant differences in root length inhibition were observed at 48 and 72 hours, with ethanol-treated roots showing less inhibition compared to those treated with Sudan IV dye. This indicates that Sudan IV dye exhibits cytotoxic effects by severely inhibiting root growth especially at higher concentrations. The concentration-dependent effect of Sudan IV dye on root growth inhibition observed in this study is particularly noteworthy. As the concentration of the dye increased, the rate of inhibition of root growth also increased. Significant inhibition of the growth of *A. cepa* roots in response to increasing doses of the synthetic food dye erythrosine have been reported. [55]

The mitotic index (MI) quantifies the proportion of cells undergoing mitosis at a given.[56] In this study, the results of MI of the root cells revealed that at 24 hours, the MI was significantly higher in the ethanol-treated cells and the control group, while a significant decrease in MI was observed with increasing doses of Sudan IV dye (Figure 2). This indicates that Sudan IV dye has inhibitory effect on cell division *A cepa* root cells. A reduction in rates of cell division was reported in onion roots treated with toxic substances. [57] As the exposure duration increased to 48 and 72 hours, the MI remained highest in the ethanol-treated cells, which was statistically greater than those recorded in the control group. This suggests that ethanol has a stimulatory effect on cell division. However, the observed paradox between the significant growth inhibition rate in ethanol-treated roots and the stimulatory effects on mitotic indices suggests that ethanol may promote frequent cell division, resulting in the formation of new

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cells. Nevertheless, these new cells may not contribute to root elongation due to cell cycle arrest. Ethyl ethanol enhances cell division but significantly slows down the growth of roots in *A. cepa*. [58] In this study, the highest concentration of Sudan IV dye (1.0%) consistently resulted in the lowest mitotic indices across durations, indicating a dose-dependent cytotoxic effect of the dye.

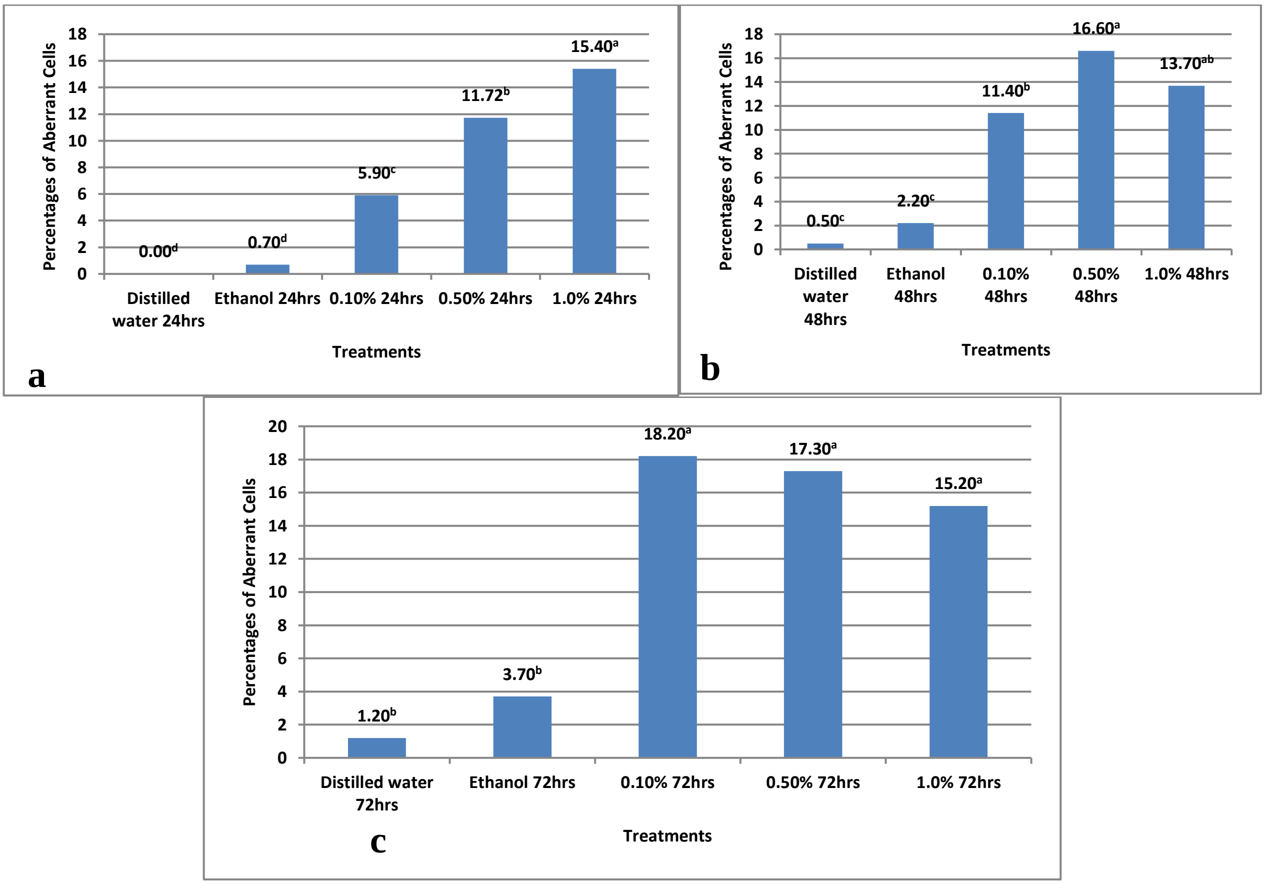

Figure 1: Percentage of Aberrant Cells Induced by Distilled water, Ethanol and Different Concentrations of Sudan IV Dye at (a) 24 hours (b) 48 hours (c) 72 hours

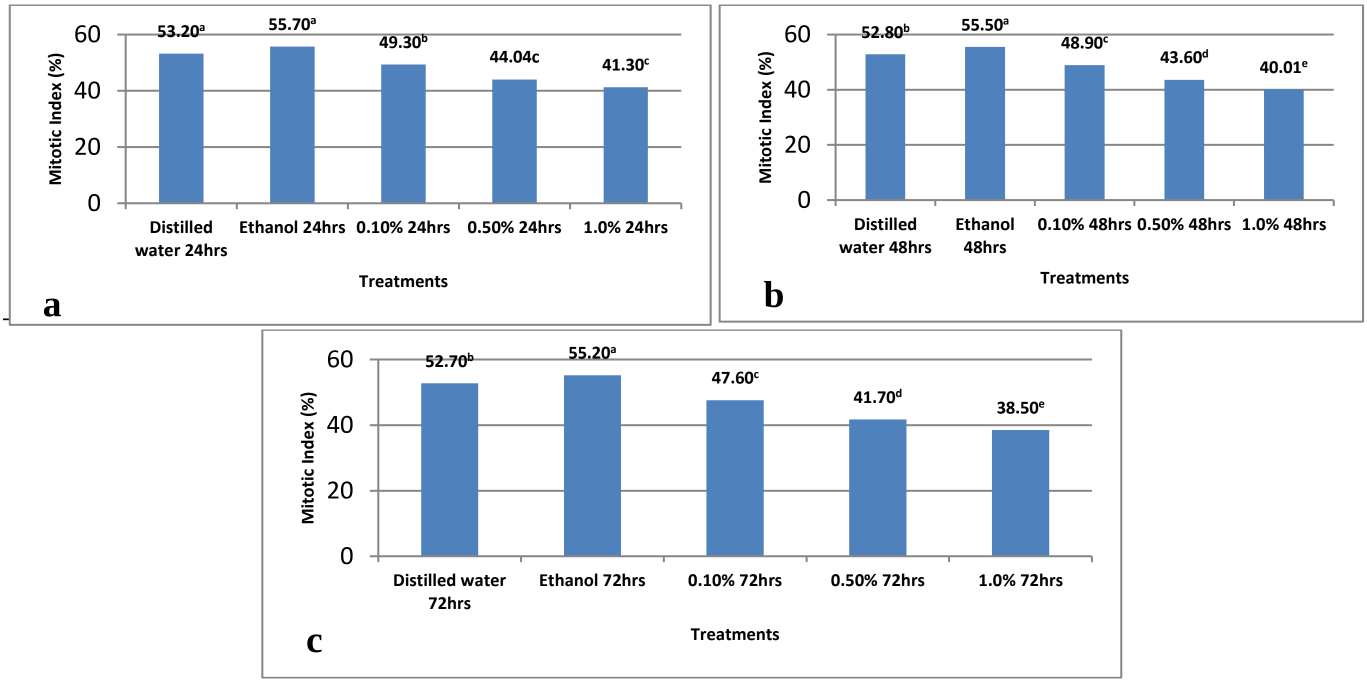

Figure 2: Mitotic Index for Distilled water, Ethanol and Different Concentrations of Sudan IV Dye at (a) 24 hours (b) 48 hours (c) 72 hours

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Table 2: Mitotic Phases of *Allium cepa* Root Tip Cells Grown with Distilled Water, Ethanol and Different Concentrations of Sudan IV Dye at Different Exposure Durations

Duration	**Concentration**	**Total Number of cells Examined**	**Average Number of cells Examined**	**Numbers of Cell in each Cell Cycles**				
				Interphase	**Prophase**	**Metaphase**	**Anaphase**	**Telophase**
24 hrs	Distilled water	1,000	200	93.60 ± 2.67^{d}	47.20 ± 2.59^{a}	29.80 ± 2.17^{a}	20.20 ± 2.77^{a}	9.20 ± 3.70^{a}
	Ethanol	1,000	200	88.60 ± 2.06^{d}	48.20 ± 4.02^{a}	31.20 ± 1.92^{a}	19.60 ± 5.03^{a}	11.00 ± 2.74^{a}
	0.10%	1,000	200	101.40 ± 5.55^{c}	37.80 ± 4.87^{b}	25.80 ± 4.87^{b}	15.80 ± 3.27^{a}	7.40 ± 2.97^{a}
	0.50%	1,000	200	111.80 ± 5.93^{b}	31.80 ± 3.19^{c}	17.20 ± 4.21^{c}	9.60 ± 2.61^{b}	6.00 ± 1.22^{b}
	1.0%	1,000	200	117.40 ± 2.97^{a}	28.00 ± 5.15^{c}	16.60 ± 3.65^{c}	3.60 ± 0.89^{c}	3.60 ± 1.14^{c}
P-value				0.000	0.000	0.000	0.000	0.002
48 hrs	Distilled water	1,000	200	94.40 ± 4.56^{d}	46.40 ± 4.72^{a}	28.40 ± 4.72^{b}	20.60 ± 1.82^{a}	9.20 ± 4.66^{a}
	Ethanol	1,000	200	89.00 ± 2.92^{e}	47.80 ± 4.44^{a}	30.60 ± 1.14^{a}	19.00 ± 2.65^{a}	9.20 ± 3.27^{a}
	0.10%	1,000	200	102.20 ± 1.92^{c}	34.00 ± 4.69^{b}	23.00 ± 4.69^{b}	12.80 ± 3.89^{b}	5.20 ± 1.79^{ab}
	0.50%	1,000	200	112.80 ± 3.83^{b}	29.00 ± 2.65^{b}	12.40 ± 2.79^{c}	6.80 ± 1.92^{c}	5.80 ± 1.92^{ab}
	1.0%	1,000	200	119.80 ± 2.59^{a}	28.20 ± 2.59^{c}	16.20 ± 3.70^{c}	4.60 ± 1.67^{c}	3.80 ± 1.30^{b}
P-value				0.000	0.000	0.000	0.000	0.022
72 hrs	Distilled water	1,000	200	94.60 ± 2.88^{d}	44.40 ± 2.79^{a}	30.00 ± 2.00^{a}	19.40 ± 3.05^{a}	9.20 ± 2.95^{a}
	Ethanol	1,000	200	89.60 ± 3.65^{e}	46.60 ± 3.05^{a}	28.60 ± 3.85^{a}	17.80 ± 4.21^{a}	10.00 ± 2.92^{a}
	0.10%	1,000	200	104.80 ± 1.48^{c}	30.00 ± 4.36^{b}	16.60 ± 4.98^{b}	8.80 ± 1.48^{b}	3.40 ± 1.14^{b}
	0.50%	1,000	200	116.60 ± 3.65^{b}	28.00 ± 2.55^{b}	10.20 ± 2.59^{c}	5.80 ± 3.11^{bc}	4.80 ± 2.05^{b}
	1.0%	1,000	200	123.00 ± 5.66^{a}	27.20 ± 1.79^{b}	12.00 ± 2.45^{c}	4.00 ± 1.87^{c}	3.40 ± 1.14^{b}
P-value				0.000	0.000	0.000	0.000	0.050

Data was expressed as mean ± SD.
Means with the same alphabets across treatments for each duration of exposure are not significantly different at $p \leq 0.05$

Table 3: Chromosomal Aberrations Induced in *Allium cepa* Root Tip Cells Grown with Distilled Water, Ethanol and Different Concentrations of Sudan IV Dye at Different Exposure Durations

Duration	**Conc.**	**Numbers of Chromosome Aberrations**								
		Sticky Chromosome	**Ghost Cells**	**Faulty Polarity**	**Chromosome Bridge**	**C-mitotic Cells**	**Vacuolated Cells**	**Binucleate Cells**	**Fragmented Chromosomes**	**Spindle Disturbance**
24 hrs	Distilled water	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}
	Ethanol	1.40 ± 1.95^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}
	0.10%	1.40 ± 1.34^{c}	1.00 ± 1.00^{b}	0.80 ± 0.44^{bc}	1.00 ± 1.00^{ab}	1.40 ± 0.35^{b}	4.40 ± 3.78^{b}	1.00 ± 0.70^{ab}	0.00 ± 0.00^{a}	0.80 ± 1.09^{ab}
	0.50%	6.60 ± 2.88^{a}	0.00 ± 0.00^{b}	1.60 ± 1.14^{ab}	2.40 ± 1.95^{a}	1.40 ± 1.95^{b}	8.80 ± 3.70^{a}	1.80 ± 2.49^{ab}	0.00 ± 0.00^{ca}	0.80 ± 1.05^{ab}

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	1.0%	4.20 ± 1.30^{b}	3.20 ± 1.30^{a}	2.40 ± 1.52^{a}	1.00 ± 1.00^{ab}	3.60 ± 0.59^{a}	12.20 ± 3.83^{a}	2.60 ± 1.14^{a}	0.40 ± 0.89^{a}	1.20 ± 0.84^{a}
	P-value	0.000	0.000	0.002	0.013	0.000	0.000	0.015	0.431NS	0.000
48 hrs	Distilled water	1.00 ± 1.41^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	Ethanol	3.20 ± 2.59^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	1.20 ± 1.30^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	0.10%	3.00 ± 2.45^{b}	2.40 ± 2.07^{a}	1.60 ± 2.06^{a}	2.00 ± 2.12^{ab}	2.80 ± 1.92^{a}	6.20 ± 3.11^{b}	1.80 ± 1.30^{a}	0.60 ± 1.34^{b}	2.40 ± 2.07^{a}
	0.50%	7.00 ± 2.74^{a}	3.40 ± 1.67^{a}	2.40 ± 0.89^{a}	2.80 ± 1.48^{a}	2.00 ± 0.70^{a}	8.80 ± 7.09^{ab}	2.80 ± 1.09^{a}	2.40 ± 1.67^{a}	1.60 ± 0.89^{a}
	1.0%	4.00 ± 1.22^{b}	3.40 ± 1.14^{a}	2.00 ± 1.00^{a}	1.00 ± 0.71^{bc}	1.60 ± 1.14^{a}	11.40 ± 2.07^{a}	2.60 ± 1.52^{a}	0.00 ± 0.0^{b}	1.40 ± 0.53^{ab}
	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
72 hrs	Distilled water	2.40 ± 2.07^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}	0.90 ± 0.01^{c}	0.00 ± 0.00^{c}	0.00 ± 0.0^{c}	0.00 ± 0.00^{c}
	Ethanol	4.80 ± 2.39^{ab}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}	2.60 ± 1.52^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	0.10%	3.40 ± 1.34^{b}	4.00 ± 0.71^{a}	2.60 ± 2.07^{a}	3.00 ± 1.58^{a}	3.60 ± 1.52^{b}	10.60 ± 2.30^{ab}	3.00 ± 0.71^{ab}	2.40 ± 1.14^{a}	3.80 ± 1.48^{a}
	0.50%	6.60 ± 3.36^{a}	3.60 ± 2.79^{a}	3.20 ± 1.30^{a}	1.40 ± 1.14^{b}	2.60 ± 1.52^{b}	8.80 ± 3.11^{b}	4.20 ± 1.92^{a}	2.40 ± 1.14^{a}	1.80 ± 1.79^{b}
	1.0%	3.20 ± 0.84^{b}	3.20 ± 1.64^{a}	2.80 ± 1.48^{a}	1.20 ± 0.45^{bc}	2.60 ± 0.55^{b}	12.40 ± 2.61^{a}	2.60 ± 0.55^{b}	1.20 ± 0.83^{b}	1.20 ± 0.45^{bc}
	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Data was expressed as mean ± SD.
Means with the same alphabets across treatments for each duration of exposure are not significantly different at $p \leq 0.05$
NS: not significantly at $p \leq 0.05$

Table 4: Effects of Ethanol and Different Concentrations of Sudan IV Dye on Relative Growth of Roots of *Allium cepa*

Concentration	Durations of Treatment					
	24 hours		**48 hours**		**72 hours**	
	IRL (cm)	RRI (%)	IRL (cm)	RRI (%)	IRL (cm)	RRI (%)
Control	0.16 ± 0.04^{a}	-	0.82 ± 0.08^{a}	-	0.80 ± 0.11^{a}	-
Ethanol	0.16 ± 0.05^{a}	0.00	0.74 ± 0.11^{b}	14.63	0.34 ± 0.13^{b}	58.54
0.10%	0.15 ± 0.03^{a}	6.25	0.60 ± 0.10^{c}	26.83	0.24 ± 0.05^{bc}	70.73
0.50%	0.15 ± 0.03^{a}	6.25	0.42 ± 1.30^{d}	48.78	0.16 ± 0.09^{c}	80.49
1.0%	0.14 ± 0.04^{a}	12.50	0.26 ± 0.05^{c}	68.29	0.14 ± 0.09^{c}	82.93
P-value	0.938^{ns}		0.000		0.000	

Data was expressed as mean ± SD.
Means with the same alphabets across treatments for each duration of exposure are not significantly different at $p \leq 0.05$
NS: not significant at $p \leq 0.05$
IRL: Increase in Root Length
RRI: Relative Root Inhibition

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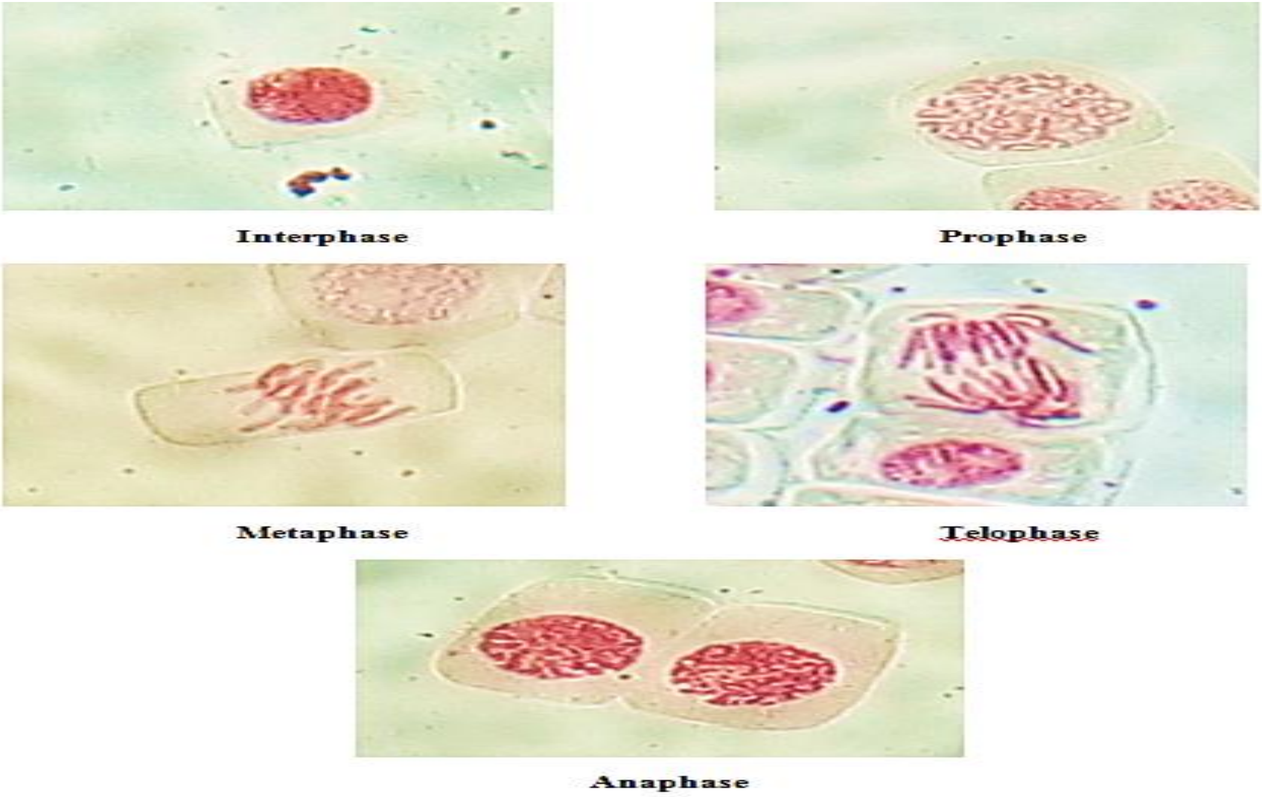

Figure 3: Normal stages of mitotic division from the treated *Allium cepa* root tip meristems

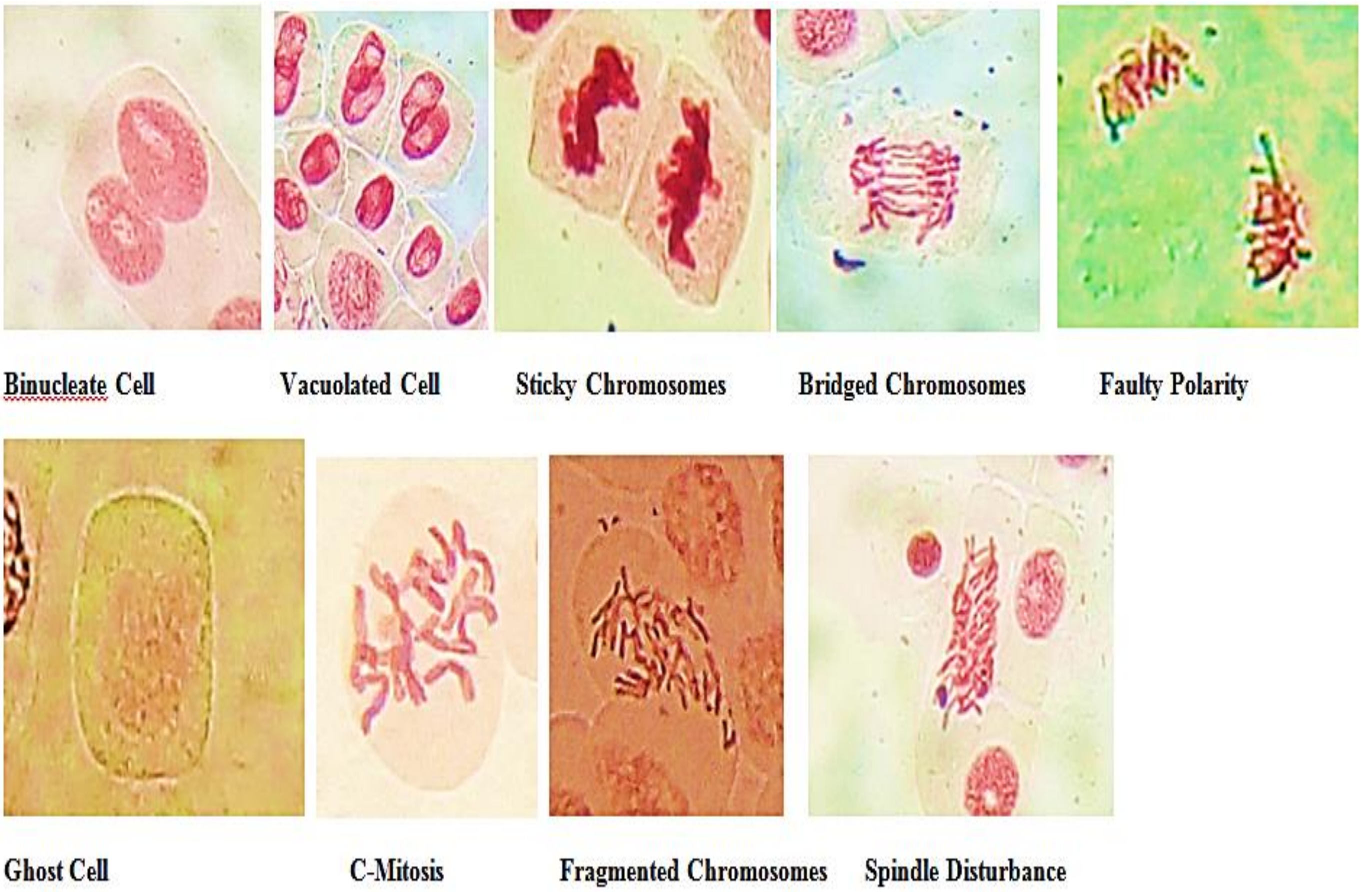

Figure 4: Abnormal Cells Induced in this study

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Conclusion

The ADMET profile of Sudan IV dye indicates a high potential for bioaccumulation in fat cells (adipocytes). Toxicity predictions show high risks of genotoxicity, carcinogenicity, mutagenicity, hematotoxicity, and nephrotoxicity, raising serious concerns about its safety. *A. cepa* assay revealed that Sudan IV dye induces chromosomal abnormalities such as sticky chromosomes, ghost cells, faulty polarity, chromosome bridges, C-mitosis, vacuolated cells, binucleate cells, fragmented chromosomes, and spindle disturbances particularly at 72 hours exposure duration. The cytotoxic effect of this dye is evident in the significant inhibition of root growth and decrease in mitotic index. Notably, the effect of this dye exhibited concentration- and duration-dependent effects on the root tip cells. Generally, this study unequivocally revealed that Sudan IV dye is toxic, and its applications in food products should be discouraged to protect public health. Therefore, regulatory measures should be implemented to limit human exposure to Sudan IV dye, especially in food products. The findings of this study can be applied in risk assessment models to evaluate the safety of different chemicals in food and other products.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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