Cytological and chromosomal damages induced by tartrazine and two classes (III and IV) of caramel food dyes

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ABSTRACT: Food colors such as tartrazine (E102), ammonia caramel (E150c), sulphite ammonia caramel (E150d) are widely used in the food sector. These additives are thought to be a source of long-term toxicity source. The goal of our research was to emphasize the cytotoxic and genotoxic effects of the three food colors at various concentrations (0.5%, 1 %, and 2%) using the *Allium cepa* test. The species is thought to be one of the best for assessing genotoxicity because of its low chromosomal number and lengthy chromosomes. The findings revealed that the three dyes have a cytotoxic impact, as seen by root growth inhibition after 120 h of incubation. The three food dyes had a genotoxic effect, as measured by a decrease in the mitotic index and an increase in the frequency of chromosomal aberrations such as chromosomal bridge, stickiness, and vagrant chromosomes, at both concentrations of 0.5% and 1%. At 2%, the mitotic index was reported as 0 and several cytological abnormalities (binucleate and micronucleated cells and fragmented nuclei) were noted. However, further *in vitro* and *in vivo* cytogenetic experiments treating cytotoxicity and genotoxicity of the three food dyes using alternative test models (animals, cell lines) will be needed to better understand their mechanisms of action.

Keywords: Tartrazine; Ammonia caramel; Sulphite ammonia caramel; Cytotoxicity; Genotoxicity.

1. INTRODUCTION

Natural dyes in food are not new. In fact, they have been used for centuries. It is motivated by a simple desire for a better presentation. However, supply issues, cost price increases, and, most importantly, an increase in dye requirements have led industries to seek more and more on the chemical side to compensate for nature's lack of collaboration. This is how synthetic dyes were created. According to statistics, over 10,000 different dyes and pigments are used in industry and more than 7×10^5 tons of synthetic dyes are produced globally each year [1,2]. These chemicals are commonly used in the textile, pharmaceutical, food, cosmetic, plastic, photographic, and paper industries [3]. Adding certain chemical substances to foods can have negative effects on health, because dyes have no nutritional value other than for aesthetic purposes. Not nutritious, culinary or technological [4].

Food poisoning caused by excessive amounts of additives is uncommon. Rather, chronic intoxications are to be feared, especially with chemicals that have a cumulative effect. Different effects characterize this medium- or long-term toxicity [5]. There is also indirect toxicity, which occurs when biochemical markers in vital organs are altered, or poisonous compounds are formed from substances found in meals [6].

Azo dyes are the largest group of synthetic dyes used in the world. For all members of this family, the name also implies the presence of one or more double bonds connecting two nitrogen atoms [7]. These two nitrogen atoms are linked to a varying number of aromatic nuclei that are somewhat replaced. These changes are crucial in the study of toxicity because they affect hydro or lipo-solubility [5,8]. Tartrazine (E102) is a chemically unique substance. It has a heterocyclic chemical structure, unlike other azos. This dye has been tested extensively in animals such as rats and mice over long periods of time and at high doses. There were no adverse effects or an increased incidence of tumors in these studies [9].

Tartrazine appears to cause the most allergic and intolerance reactions of any azo dye, particularly among asthmatics and those who tolerate aspirin [10]. Caramels have been widely used to add or restore a brown color to food for more than 100 years. Its most famous use is that of the coloring of cola drinks. They can also be found in alcoholic beverages, bakery goods, confectionery, meat and fish preparations, soups, sauces, and so on. Caramel colorants are classified into four classes (E150a, E150b, E150c, and E150d), each with slightly different chemical and functional properties (mist, flocculation, and separation) [11].

Caramel dyes are the result of a chemical reaction in which sugar is combined with acids, alkalis, sulphite compounds, or ammonium compounds under high temperature and pressure. This accelerates the caramelization process, resulting in a more intense color [11]. However, other compounds will be formed in parallel. For example, in caramel E150d, the mixture of sugar and ammonium sulphites produces a substance known as 4-methyl-imidazole [12,13]. This substance can be found in soy sauce, wine, black beers, soft drinks, and other foods that contain ammonia caramel and sulphite ammonia caramel [14]. 4-methylimidazole (4-MeI), a potent convulsive that was known at the time to cause neurotoxicity in rabbits, mice, and chickens at high doses [11].

There is no doubt that color influences consumer perceptions of food. It is also frequently associated with a particular flavor and the intensity of that flavor. However, dyes are highly contentious, with many people accusing them of causing allergies, food intolerance, and even more serious diseases. In this study, we focused on food colors, specifically those that are among the most commonly used additives in the production of soft drinks (Tartrazine (E102), ammonia caramel (E150c), and sulphite ammonia caramel (E150d)) using the *Allium cepa* test, which is regarded as a quick and effective test for determining the level of toxicity of certain substances.

2. MATERIAL AND METHODS

In this study, we used red onion bulbs (*Allium cepa*) purchased from the local market of Skikda. Before beginning the experiment, the old roots and the outer layer of the bulbs were removed. Tartrazine (E102, CAS: 1934-21-0), ammonia caramel (E150c, CAS: 8028-89-5), and sulphite ammonia caramel (150d, CAS: 8028-89-5) were provided by Coutex international. All other chemicals were from Sigma-Aldrich Chemicals, Germany.

2.1. Cytotoxicity assay

The onion bulbs were allowed to germinate in pots containing distilled water for two days (48 hours) (5 bulbs for each concentration). Bulbs with root sizes ranging from 1.5 to 2 cm were exposed to various concentrations of the three dyes (0.5%, 1%, and 2%). The incubation lasted 5 days, in the dark with the renewal of the solution every 24 hours. Root size is measured every 24 hours.

2.2. Genotoxicity assay

Bulbs with a root length ranging from 1.5 to 2 cm were incubated with different concentrations of the three food dyes (0.5%, 1%, and 2%). Incubation took place in the dark for 24 h.

2.3. Cytogenetic preparation

The roots of each treatment were recovered and fixed in a mixture (ethanol/acetic acid, 3v/1v) for 24 h before being transferred to 70% Ethanol and stored at 4 °C until use. The roots are hydrolyzed in HCl 1N at 60 °C for 10 min to prepare the slides [15]. After staining the roots with Schiff reagent for 20-30 min, the root tips were crushed in a drop of 45% acetic acid.

2.4. Parameters of genotoxicity

Normal and abnormal cells were counted from at least 10 roots collected from several bulbs (n=5, for each treatment), with over 600 cells counted in each root tip. The cells were examined using a Primostar microscope (Zeiss) at magnification x400.

Mitotic index:

$$IM(\%) = [(\text{Number of cells in division}) / (\text{Total number of cells})] \times 100 \qquad (1)$$

Phase index:

$$IP(\%) = [(\text{Number of cells in phase PMAT}) / (\text{Total number of cells})] \times 100 \qquad (2)$$

Index of chromosomal aberration:

$$IA(\%) = [(\text{Number of aberrant cells}) / (\text{Total number of cells})] \times 100 \qquad (3)$$

2.5. Statistical analysis

ANOVA test was used for all statistical analyses. The post-hoc Tukey test was used to examine differences in group averages using SPSS version 14.0 software. The results were reported as Mean and Standard Deviation, with significant differences at $p < 0.05$.

3. RESULTS

The cytotoxicity of the three food dyes was determined by measuring the root length (cm) of bulbs every 24 h for 5 days at various concentrations. Changes in shape, color of the roots, as well as root length, are indicators of toxicity. According to the results shown in Figure 1, there was a decrease in root growth over the 5 days (120h) period when compared to control. The root elongation of bulbs treated with tartrazine (0.5%, 1% and 2%) stopped on the third day of incubation (Figure 1A). Figure 1B, showed that ammonia caramel (E150c) inhibited root growth of bulbs compared to control, especially at the concentrations 1% and 2%, root elongation ceased on the third day of incubation. However, the lowest concentration of 0.5% had a slight inhibitory effect on bulb root growth. Sulphite ammonia caramel (E150d) has similar effects to tartrazine (E102), causing root growth inhibition at all concentrations and root elongation blockage beginning on the third day of incubation (Figure 1C). Among the above-mentioned signs of toxicity, root growth

inhibition induced by different concentrations of the three food dyes is considered as a sign of toxicity. But this inhibition was not significant compared to control (P>0.05).

The mitotic index, phase index, and chromosomal aberration rate were measured after 24 h of incubation to assess the genotoxicity of the three food dyes at different concentrations (0.5%, 1%, and 2%). The effects of tartrazine, ammonia caramel and sulphite ammonia caramel on the mitotic index were reported in Table 1.
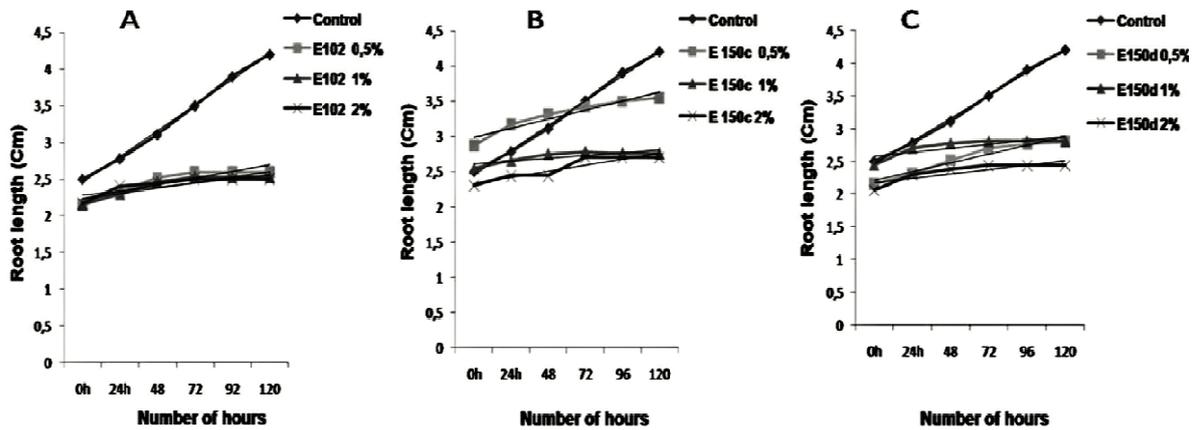

Figure 1. Root growth of *Allium cepa* after 5 days (120 h) of incubation with the three dye colors. Tartrazine (E102), ammonia caramel (E150c), sulphite ammonia caramel (E150d).

Table 1. Effect of food dyes on the frequency of mitotic index and chromosomal aberration index in *Allium cepa* cells.

Treatment (%)	AI (%)	MI (%)	Cells in division (%)			
			Prophase	Metaphase	Anaphase	Telophase
Control	0.53 ± 0.14	30,1 ± 6.42	12.4 ±1, 4	6.70 ± 3.31	8.0 ± 3.28	2.71 ± 1.1
E102 0.5%	6.82 ± 2.31*	34.6 ±9.81	13.7 ± 0.7	7.67 ± 0.71	9.9 3± 5.5	4.11 ± 3.2
E102 1%	6.6 ± 3.8*	30.8 ±9.91	15.2 ± 4.2	6.12 ± 4.0	8.15 ± 3.51	1.44 ± 0.9
E102 2%	0 ± 0	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0
E150c 0.5%	4.2 ± 1.9	23.8 ±8.20	14.8± 2.4	2.81 ± 0.72	4.9 1± 3.28	1.22 ± 0.8
E150c 1%	1.22 ± 0.51	9.51 ± 5.70	4.44 ± 0.6	3.93 ± 2.6	0.5 ± 0.72	0.6 2± 0.19
E150c 2%	0 ± 0	1.90±0.90*	1.88 ± 0.7	0 ± 0	0 ± 0	0 ± 0
E150d 0.5%	4.91 ± 0.92	36.3± 8.2	20.2 ± 1.3	6.71 ± 3.0	6.16 ± 2.61	3.81 ± 2.23
E150d 1%	3.06 ± 0.73	13.6 ±5.4	11 ± 1.2	0.99 ±1.4	0.98 ±1.2	0.33 ±0.95
E150d 2%	0 ± 0	0 ± 0*	0 ± 0	0± 0	0± 0	0± 0

MI: mitotic index, AI: aberration index, (mean ± SD, Tukey's test, *P<0.05 compared to control). Tartrazine (E102), ammonia caramel (E150c), sulphite ammonia caramel (E150d).

The results revealed that the three food dyes did not affect the rate of cell division (MI) at the concentration of 0.5%, although the rate of chromosomal aberration was increased compared to control. A decrease in the mitotic index and an increase in the rate of chromosomal aberrations at the concentration of 1% were noticed. For ammonia caramel, sulphite ammonia caramel, and tartrazine, the mitotic index (MI) of cells was reduced to 9.5%, 13%, and 30%, respectively, compared to control (30%). Whereas the mitotic index of cells treated with 2% was lowered to 0% for the three food dyes (there was no evidence of cell division. *Allium cepa* cells were inactive, and the cell cycle was fully inhibited at interphase, P<0.05).

Cytogenetic analysis of meristematic cells of *Allium cepa* treated with different concentrations of the three food dyes indicates the presence of different types of cell division abnormalities. The frequency of these abnormalities is expressed by the chromosomal aberration index, which varies depending on the concentration and the food dye. The results shown in Figure 2 indicate that the concentration 0.5% of tartrazine increases significantly the rate of chromosomal aberration in *Allium cepa* cells (6.28%, p<0.05). The aberration rate remains at the concentration of 1% high and significant compared to control (p<0.05). In the treatment, *Allium cepa* cells 0.5% of ammonia caramel and sulphite ammonia caramel increased the rate of chromosomal aberration, which was reported as 4.2% and 4.9% respectively, when compared to control (p>0.05).
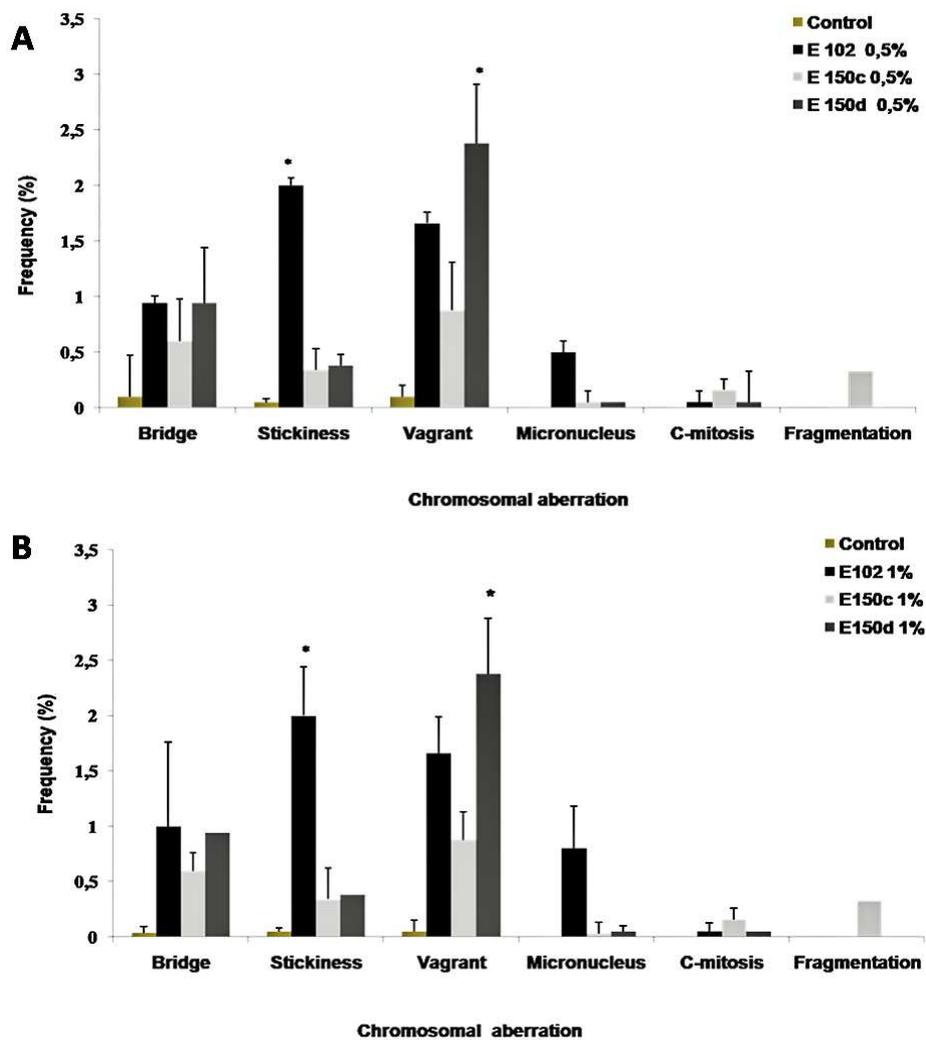

Figure 2. The frequency of each type of chromosomal aberration. *p<0.05, treated groups compared to control. Tartrazine (E102), ammonia caramel (E150c), sulphite ammonia caramel (E150d). A: treatment with the concentration 0.5%, B: treatment with concentration 1%.

At 1% the chromosomal aberration rate decreases strongly to 1.22% and 3% for ammonia caramel and sulphite ammonia caramel. However, tartrazine (E102) could produce more aberrant cells (P<0.05) than ammonia caramel and sulphite ammonia caramel at both concentrations of 0.5% and 1%. At the highest concentration (2%) the chromosomal aberration rate was reported as 0 for tartrazine and sulphite ammonia caramel and nearly 0 for ammonia caramel. This is mainly related to the absence of mitotic division at this

concentration, which allows suggesting that the three food dyes have a cytotoxic effect on the meristematic cells of *Allium cepa*.

The cytogenetic analysis showed several types of chromosomal aberration. Chromosomal bridge, stickiness, chromosome vagrant and micronucleus are the most common aberrations detected in *Allium cepa* cells after incubation, with the concentration 0.5% and 1% of the three food dyes. However, the frequency of each aberration changes from one dye to another (Figure 2). Tartrazine induces the appearance of the chromosomal bridge, stickiness (P<0.05), vagrant chromosome, and micronuclei.
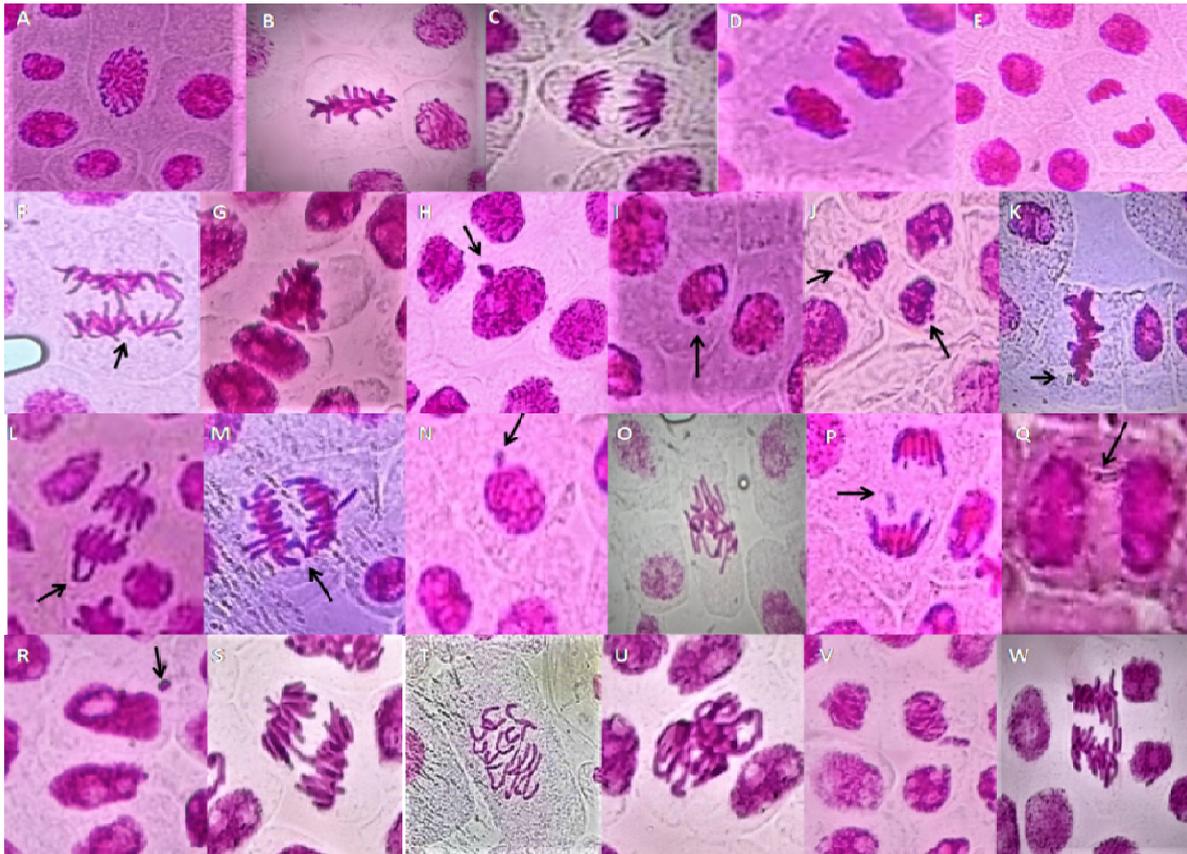

Figure 3. Chromosomal aberration induced by the three dye colorant at 0,5 and 1%. A-E: normal phases in control from prophase to cytokinesis. F-K: treatment with tartrazine (102), F: bridge, G: stickiness, H: micronucleus, I: chromatin bud, J: chromatin bud at telophase, K: fragmentation. L-Q: treatment with ammonia caramel (150d), L: vagrant, M: bridge, N: micronucleus, O: C-mitosis, P: fragmentation, Q: bridge at telophase. R-W: treatment with sulphite ammonia caramel (150d), R: micronucleus, S: anaphase bridge, T: C- mitosis, U: stickiness, V: isolated chromosome, W: anaphase bridge and vagrant.

Ammonia caramel and sulphite ammonia caramel induce vagrant chromosome (P<0.05, for E150d), chromosomal bridge, stickiness. However, the frequency of these chromosomal aberrations in cells treated with ammonia caramel was very low in comparison to tartrazine and sulphite ammonia caramel (Figure 2 and Figure 3). Other types of aberrations, including fragmentation and C-mitosis, were also observed. Besides, we noticed that the increase in chromosomal aberration rate was inversely related to dye concentration. At 1% a minor decrease in the aberration rate was observed but at 2%, a complete absence of chromosomal aberrations was observed (Figure 4), which is directly related to the rate of mitotic division (0%).
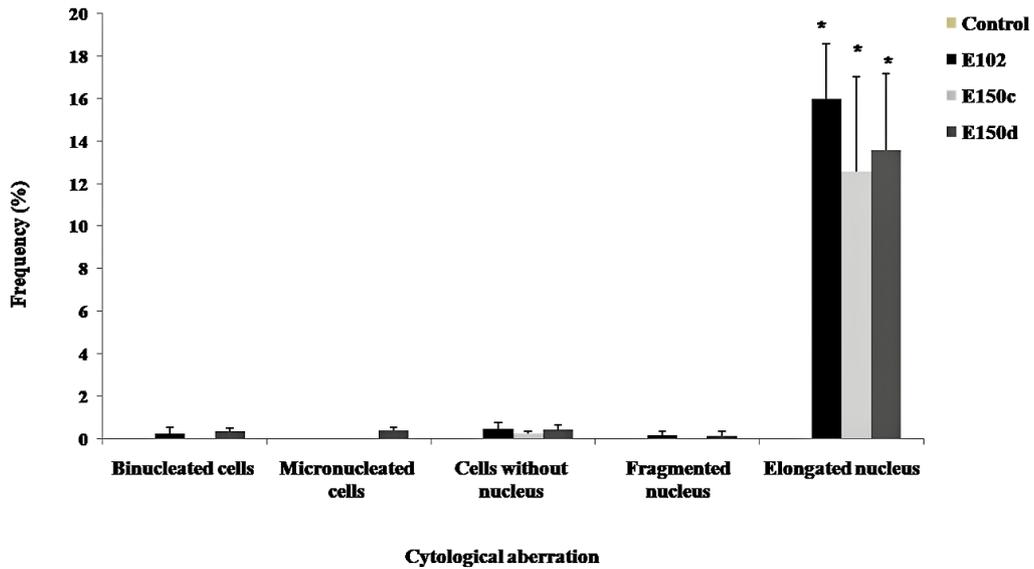
Figure 4. The frequency of each type of cytological alterations in the concentration 2%. *p<0.05, treated groups compared to control. Tartrazine (E102), ammonia caramel (E150c), sulphite ammonia caramel (E150d).
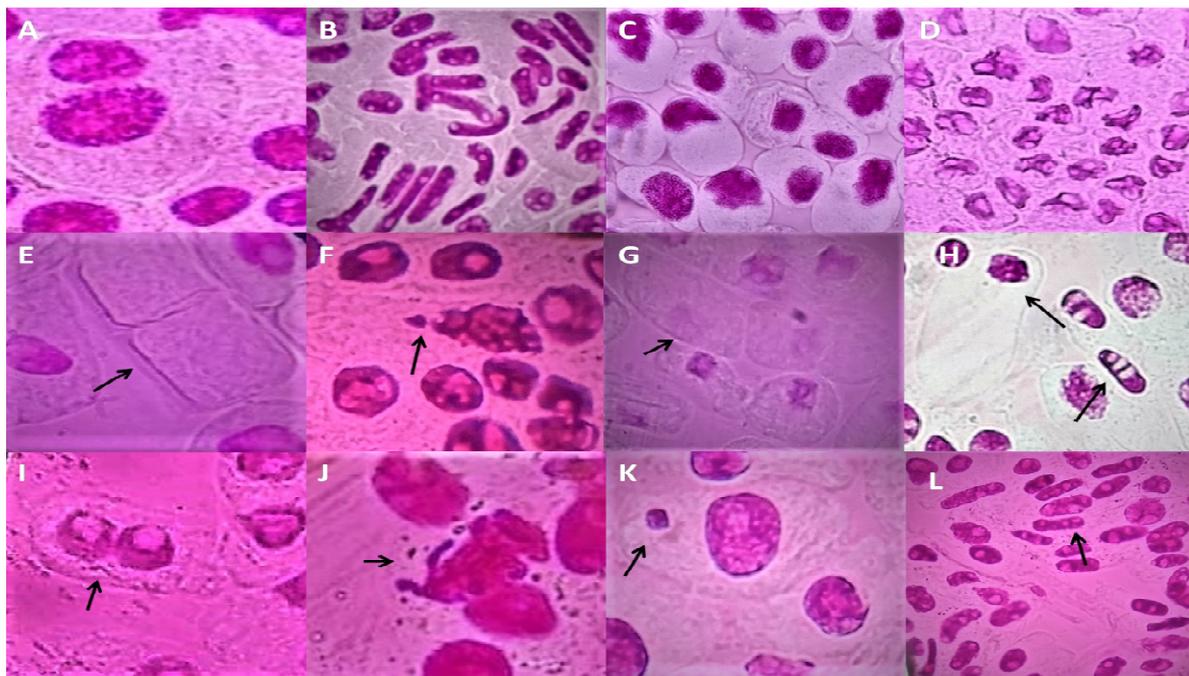

Figure 5. Nuclear lesions and cellular aberration induced by the three colorants at 2%. A-F: treatment with tartrazine (E102), A: binucleated cell, B elongated nucleus, C and D: nucleus shape change, E: cells without a nucleus, F: fragmented nucleus. G-H: treatment with ammonia caramel (E150c), G: cells without nucleus, H: giant cell and elongated nucleus with lesions. I-L: treatment with sulphite ammonia caramel (E150d), I: binucleated cell, J: fragmented nucleus, K: micronucleated cells, L: elongated nucleus with nuclear lesion.

The presence of numerous cytological alterations in the cell cycle was enhanced when cells were treated with 2% (p<0,05) (Cells without nucleus, fragmented nucleus, binucleated cells, micronucleated cells and elongated nucleus). An elongated nucleus with nuclear dissolution was the most prevalent type for the three food dyes (Figure 5).

4. DISCUSSION

The *Allium cepa* test has frequently been used to determine the cytotoxicity and genotoxicity of various substances. It is regarded as a quick and inexpensive test for determining the level of toxicity by taking three parameters into account: inhibition of root elongation, effect on mitotic index, and study of chromosomal aberrations [16].

The purpose of this study was to assess the genotoxicity of three commonly used food dyes, tartrazine, ammonia caramel, and sulphite ammonia caramel. According to the cytotoxicity test results, the three food dyes at different concentrations (0.5%, 1%, and 2%) inhibited root growth during the first two days of incubation, and root growth ended on the third day of incubation accompanied by a decrease in the mitotic index at the concentrations 1% and 2% and an increase in the rate of chromosomal aberrations at concentrations of 0.5% and 1%. dyes. A decrease in the mitotic index of less than 20% compared to control can be lethal to the organism, while a decrease of less than 50% is generally lethal [17]. It could due to an inhibition of DNA synthesis (blocking the replication phase) or a blockage of the cell cycle's G2 phase, preventing the cell from entering mitosis [18].

Our results are in agreement with those obtained by others [19,20], showing that tartrazine induces chromosomal aberrations and causes significant DNA damage. For ammonia caramel and sulphite ammonia caramel, the lowest concentration of 0.5% increased the rate of chromosomal aberration in cells when compared to control, but not significantly ($p > 0.05$). The chromosomal aberration rate decreases strongly at concentrations of 1%. Most of the toxicity and genotoxicity studies have yielded contradictory results, which vary depending on the genotoxic test used. Many studies have shown the safety of caramel colors [21, 22]. Inversely, our results indicated that both ammonia caramel and sulphite ammonia caramel induce chromosomal aberration. A low concentrations (0,5 and 1%), similar results showing the ability of caramel to induce chromosomal aberrations have already been published [23,24].

These findings are consistent with previous *in vitro* findings using *Allium cepa* test, suggesting that synthetic food colors have a genotoxic effect manifested as an increase in chromosomal aberration accompanied by a decrease in the percentage of the mitotic index [25,26]. Our results revealed that tartrazine has more ability to produce chromosomal aberrations compared to ammonia caramel and sulphite ammonia caramel. At the highest concentration (2%) the chromosomal aberration rate was recorded as 0 for the three food dyes. This is mainly related to the absence of mitotic division at this concentration, which allows to suggest that the three food dyes can have a cytotoxic effect on the cells of *Allium cepa*. These data are consistent with those of the study [10], in which cell death was observed at the highest dose, clearly indicating the cytotoxic impact of sunset yellow on meristematic cells of *Brassica campestris*.

Several types of chromosomal aberrations are considered in the different phases of cell division. These chromosomal aberrations are changes in chromosomal location and structure caused by chromosomal material rupture or exchange. Most cell aberrations are fatal, but there are many related aberrations that can cause genetic, somatic, or hereditary effects [27].

Stickiness, vagrant chromosomes, and anaphasic bridges are the most common chromosomal abnormalities found in our study. The presence of sticky chromosomes is associated with the highly toxic effects of a specific chemical [28]. A clastogenic effect is indicated by chromosomal bridges and fragments, whereas an aneugene effect is indicated by vagrant chromosomes, stickiness, and C-mitosis [29]. Anaphasic chromosomal bridges could be induced by unequal reciprocal translocation or by the presence of dicentric

chromosomes. These bridges are responsible for structural chromosomal mutations [30]. Another type, C-mitosis was observed in the meristematic cells (with E150c, p>0,05), and resulted from the inhibition of spindle formation during metaphase.

The induction of chromosomal fragmentation, microtubule assembly disorders, and cell death can all be linked. The presence of sticky chromosomes, notably after incubation with tartrazine (E102), may be due to the inhibition of specific proteins (histone proteins) involved in chromosome condensation and segregation [30].

At the highest concentration (2%) of the three food dyes, the results showed no cell division (Figure 5). Similar results were obtained, when treating *Allium cepa* cells with the concentration of 4% of E127, E129 and E133 resulted in a highly significant decrease in rate division [31]. At the same concentration (2%), elongated cells with nuclear dissociation were the most abundant types. Many authors suggest that nuclear dissociation is related to an inhibitory effect on DNA replication during the S-phase of the cell cycle [32,33].

It is clear that the concentrations of the three food dyes (0.5% and 1%) not only interfere with the cell cycle, but also affect chromatin organization and DNA replication, resulting in the appearance of various chromosomal aberrations. Our findings are in agreement with previous data [34-36], indicating the genotoxic effect of many food dyes using species such as *Vicia faba*, *Allium cepa, Brassica campestris, Foeniculum vulgare*, these plants provide excellent cytogenetic systems with a wide range of genetic parameters, genetic mutation, mitotic and meiotic chromosomal aberrations, and DNA damage.

5. CONCLUSION

Based on the results of the study, it is quite clear that the three dyes commonly used to color food have cytotoxic and genotoxic effects These effects are manifested as root growth inhibition, a decrease in the mitotic index, the induction of chromosomal aberrations, and nuclear lesions. Additionally, tartrazine and sulphite ammonia caramel were aneugenic, inducing significant vagrant chromosomes, whereas ammonia caramel did not induce any significant genotoxicity.

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