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CITOTOXICITY ASSESSMENT OF FOOD DYES BY BIOASSAY WITH *Allium cepa* L

AVALIAÇÃO DE CITOTOXICIDADE DOS CORANTES ALIMENTARES POR BIOENSAIO COM *Allium cepa* L

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RESUMO: Os corantes alimentares são uma classe de aditivos que são adicionados aos alimentos para fornecer ou alterar cores tornando os produtos mais atraentes para os consumidores. Contudo, o consumo de corantes excede a ingestão diária aceitável podendo causar os efeitos adversos à saúde. Este estudo analisa a citotoxicidade de quatro corantes alimentares em bioensaio com *Allium cepa* (cebola). As três concentrações de soluções foram preparadas em água destiladas 15 mg, 30 mg e 60 mg/100mL para os testes dos tartrazina (E102), vermelho 40 (E129) e azul brilhante (E133) e de 5 mg, 10 mg e 20 mg/100mL para amarelo crepúsculo (E110). Antes de serem tratadas, as cebolas limpas e descascadas foram colocadas em recipientes com água destilada por 24 horas para estimular o crescimento das raízes. O ensaio foi realizado em 96 horas. As cebolas tratadas com água destilada foram utilizadas como controles. Ao final dos experimentos, foi mensurado o tamanho das raízes, oito bulbos para cada tratamento. Os resultados mostraram que os corantes provocam a inibição de crescimentos de raízes das cebolas em relação aos controles. As inibições variam entre 22% a 48%, apenas a concentração de 15 mg/100mL de azul brilhante e de 5 mg – 10 mg/100mL de amarelo crepúsculo que não provocam as inibições em crescimento radicular. Foi observada que uma diminuição periódica do comprimento radicular conforme o aumento da concentração dos corantes. Com esses resultados indica a importância de evitar ou reduzir o consumo de alimentos processados que contêm corantes sintéticos.

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Palavras-chave: Allium cepa. Citotoxicidade. Corantes Alimentares.

ABSTRACT: Food dyes are a class of additives that are added to foods to provide or change colors, making food products more attractive to consumers. However, the consumption of dyes exceeds the acceptable daily intake can cause adverse health effects. This study analyzed the cytotoxicity of the food dyes in *Allium cepa* (onion) bioassay. The three concentrations of solutions were prepared in distilled water 15 mg, 30 mg, and 60 mg/100mL to test tartrazine (E102), red 40 (E129) and brilliant blue FCF (E133). The concentrations of 5 mg, 10 mg, and 20 mg/100mL were prepared to test sunset yellow FCF (E110). Before being treated, the cleaned and peeled onions were placed in containers with distilled water for 24 hours to stimulate the root growth. The inhibition test was carried out for 96 hours. Onions treated with distilled water were used as the control. The length of the roots was measured, eight bulbs for each concentration. The results showed that the dyes caused the inhibition on onions root growth compared to the control group. Inhibitions vary from 22% to 48%, only the concentration of 15 mg/100mL of E133 and the concentrations of 5 mg – 10 mg/100mL of E110 that did not reduced the root growth. It was observed that the root length decreased as the concentrations increased. With these results it indicates the importance of avoiding or reducing the consumption of processed foods that present the presence of synthetic food dyes.

Keywords: *Allium cepa*. Cytotoxicity. Food Colors.

INTRODUCTION

Food additives are substances that are intentionally added to foods for various reasons, such as improving taste, texture, and appearance, as well as preserving food. According to Polonia and Peres (2009), food colors are a class of additives that are added to foods to provide or change colors making products more attractive to consumers. ANVISA (1997) legalized eleven artificial colors used in Brazil: tartrazine (E102), sunset yellow FCF (E110), azorubine (E122), amaranth (E123), ponceau 4R (E124), erythrosine (E127), red 40 (E129), patent blue FCF (E131), indigotine (E132), brilliant blue FCF (E133) and fast green FCF (E143). These dyes, some are banned in other countries. For example, amaranth, azorubine or erythrosine are prohibited in the United States (Carocho et al., 2014). Foods that are easily recognized by the uses of dyes are candies and gums, jellies, and artificial juices. The dyes E102, E110, E129 and E133 are more used in the foods most consumed by children such as crackers, cookies, corn chips, cereals and cookies (Teixeira, 2018).

The adverse effects of food additives are related to the dose and the cumulative (Dengate; Ruben, 2002). This means that when the person ingests additives highly and frequently, the greater the possibility of exposure to toxins. Therefore, a quantitative dose application study can be valuable in assessing the toxicity potential of food additives (Coon, 1961). The consumption of dyes exceeds the acceptable daily intake (ADI) can cause adverse health effects, such as allergies, stomach problems, hyperactivity in children and are toxic to human lymphocytes (Carocho et al., 2014, McCann et al. 2007). ADI is a numerical value of

additives in mg/kg of body weight that can be consumed daily without presenting health risks. Brazilian legislation requires that the food industries list all additives on food labels (Anvisa, 2002), however, the amount of additive used is not necessarily declared. Some studies have shown that dye concentrations have exceeded the maximum limit in gelatin, powdered juice, chewing gums and colored cereals (Prado; Godoy, 2007; Piasini, 2014).

Toxicity is defined as a property inherent to the substance that produces harmful effects to exposed organisms for a certain time that produces adverse effects such as: inhibition of the reproduction and growth of the tested organism or mortality and immobility (Arraes; Longhin, 2012). The use of biological tests to evaluate bioactivity of plant extracts and compounds has often been applied for the identification and monitoring of potentially toxic substances (Iganci, 2006). According to Grant (1999), the bioassay with plants has been considered quite sensitive and simple in monitoring the cytotoxic effects (toxic in living cells) of chemical compounds. *Allium* cepa has been indicated as an efficient test for the evaluation of cytotoxicity due to its properties of kinetic proliferation (Gomes et al., 2013). Its reduced number of large chromosomes (2n = 16) and changes in cells facilitate the observation of results resulting from the action of chemical compounds (Rodrigues et al., 2016).

The objective of this study was to evaluate the cytotoxicity of the four main food colors (tartrazine yellow (E102), sunset yellow FCF (E110), red 40 (E129) and brilliant blue FCF (E133) in bioassay with *Allium cepa* L.

MATERIAL AND METHODS

Material

Food dyes were purchased at the local party chain in Aparecida de Goiânia which include tartrazine yellow (lot 10013, expiration date 24/01/2021), sunset yellow CFC (lot 22004, expiration date 03/07/2021), red 40 (lot 14014, expiration date 05/17/2021) and brilliant blue (lot 11014, expiration date 03/22/2021) from the Gran Chef brand (Top2K Comércio Varejista Ltda., São Paulo) (Figure 1).

Preparation of different concentrations of dyes

The limits allowed by Brazilian legislation for the use of tartrazine, red 40 and brilliant blue dyes are 30g/100g while sunset yellow limit is 10 mg/100g (Prado; Godoy, 2007). Three different concentrations will be prepared in 100 ml distilled water: C1 = 30 mg/100mL, C2 = 15 mg/100mL (half of C1) and C3 = 60 mg/100mL (twice as much as C1) for the testing of tartrazine dyes, 40 red and brilliant blue. For the sunset yellow dye test, concentrations of C1 = 10

mg/100mL, C2 = 5 mg/100mL (half of C1) and C3 = 20 mg/100mL (twice C1) will be prepared.

Bioassay with *Allium cepa* L.

To carry out the study, equal-size *Allium cepa* (onions) were selected and purchased from the local store. The onions were kept cold and dry until the experiment. Before use, the onions were carefully peeled, and the brown tunics were removed without destroying the primordial root. Clean and healthy onions were placed in a container with distilled water for 24 hours to encourage root growth until they reach about 1.0 cm in length (Ozkara et al., 2015; Gomes et al., 2013). After this period, the best bulbs for the experiment will be chosen and treated with different concentrations of food dyes (8 bulbs for each treatment) at room temperature for 96 hours, with root growth being measured every 24 hours (Figure 2) (Longhin, 2008). In this test, onions treated with distilled water will be used as controls.

Statistical analysis

The mean ± SD (standard deviation) of the root length for the treatments group and the control were calculated. The data were analyzed statistically by measuring SD and one-way analysis of variance (ANOVA) and post hoc Dunnett's test. The test was performed to compare the significant differences between the treatments group to a single control group at p value less than 0.05.

Figure 1: a) Dyes used in the experiment: bright blue (E133), red 40 (E129), and tartrazine (E102).

Figure 2: Equal-size bulbs *Allium cepa* without any treatment were placed in a container with distilled water for 24 hours to stimulate the root growth.

RESULTS AND DISCUSSION

According to Morrison et al. (2012), azo group dyes have a naphthalene ring attached to a second benzene ring by an azo bond (N = N). These rings can contain one, two or three

sulfonic groups. This group of dyes represents the class of synthetic dyes most used by the food industry, mainly brilliant blue, red 40, tartrazine yellow and sunset yellow. These four dyes are controversial in relation to their toxic activity and attract the interest of toxicologists and allergists (Gomes et al., 2013).

The average length of the root for the control group was 4,208 ± 0732 cm (Table 1). The reduction of the means of the root length were significant for dyes treatments of E102, E129, and E133 at 15, 30, and 60 mg/100 mL except at 15 mg of E133. At this treatment, there was positive effect on the root length than the control whereas the treatment increased the roots growth to 4.967 ± 0.570 cm. The *Allium* test on E110 at 5 and 10 mg/100 mL did not affect the root growth. All treatments group means are significantly different from the control mean at $p < 0.05$, performed by Dunnett's test.

Table 1 showed that the dyes analyzed caused significant inhibitions in root growths of onions compared to the control in the doses and exposure times evaluated. Inhibitions range from 25 to 40% in brilliant blue tests, 37 to 41% in red 40 tests, 26 to 40% in tartrazine yellow tests and 22% in sunset yellow. In an applied toxicity test, small concentrations of 5 mg and 10 mg/100mL of sunset yellow and 15 mg/100mL of brilliant blue did not cause inhibitions. It was observed that the root lengths were progressively reduced by increasing the dyes concentrations.

Sunset yellow food coloring (E110) is synthesized from coal tar and azo paints. According to Sardi et al. (2010), it can cause anaphylactic shock, angioedema, vasculitis and inhibition of thromboxane synthesis in people sensitive to its composition; may increase aggressive behavior in children and trigger severe allergic reactions in people with sensitivity to acetaminophen, acetylsalicylic acid and sodium benzoate. The study by Gomes et al. (2013) revealed that sunset yellow and tartrazine produced a cytotoxic effect on the activity of the meristematic cells of the root of *Allium cepa*. The effect of tartrazine on *Allium cepa* at concentrations of 0.1, 1, 3 and 5 mg /ml^{-1} were also studied by Lerda (2017). The analysis was focused on root growth, the frequency of mitosis in a meristematic zone and chromosomal aberrations. According to the author, tartrazine reduces root growth and the frequency of mitotic cells in meristematic zones and increases the frequency of aberrant cells. Tartrazine inhibits the longitudinal growth of the root due to its concentration and blocks the cell division cycle at a stage prior to mitosis.

Table 1: The means of *Allium* roots length treated with different dyes concentrations for 96 hours. The mean length of the root for all treatments were significantly different from the mean of the control.

Test substance	E number	Concentrations (/100 mL)	Root length (cm) Mean± SD*	Inhibition (%)
Control		Distilled water	4.208 ± 0.732	-
Tartrazine	E102	15 mg	3.100 ± 0.312	26
		30 mg	2.804 ± 0.359	33
		60 mg	2.537 ± 0.549	40
Red 40	E129	15 mg	2.485 ± 0.380	41
		30 mg	2.646 ± 0.358	37
		60 mg	2.187 ± 0.322	48
Brilliant blue FCF	E133	15 mg	4.967 ± 0.570	-
		30 mg	3.137 ± 1.087	25
		60 mg	2.525 ± 0.451	40
Sunset yellow	E110	5 mg	4.112 ± 0.727	2
		10 mg	4.154 ± 0.567	1
		20 mg	3.279 ± 0.727	22

*SD: Standard deviation.

Red dye cytotoxicity, mutagenicity or carcinogenicity 40 has not been found in the scientific literature. However, the results indicate high toxicities in three evaluated doses, inhibitions of 41% at a concentration of 15 mg/100mL, 37% at a concentration of 30 mg/100mL and 48% at a concentration of 60 mg/100mL (Table 1). The similar result was presented by Oliveira et al. (2013). According to them, the red dye 40 promoted a significant reduction in cell division and induced the appearance of aberrations of the anaphasic and telophasic bridge and micronucleated cells.

In relation to the brilliant blue dye, it has a basic structure composed of three aryl radicals, usually phenolic groups, linked to a central carbon atom. It has sulfonic groups that provide high solubility in water, which is one of the main attractions for the food industry. Prado and Godoy (2007) reported that this additive has the potential to promote cell division changes in rodent thyroid cells, the function of releasing a large amount of iodine in the body of these animals. In that study, brilliant blue was toxic at doses of 30 mg and 60 mg/100mL, while the dose of 15 mg /100mL did not inhibit the root growth of *Allium cepa* (Table 1). Other authors, Oliveira et al. (2013), showed that the brilliant blue did not cause a significant reduction in the applied doses, 0.4 and 4.0mL, in the exposure times of 24 and 48h.

CONCLUSION

The results indicate that the synthetic dyes were cytotoxic to the onion cells at the doses and exposure times evaluated, except for the small doses of sunset yellow and brilliant blue. It

was observed that a periodic decrease in root lengths as the concentration of dyes increased. This work reinforces the importance of the *Allium cepa* bioassay test since it presents similar results with another research. However, the frequency of cytotoxicity for each dye depends on the doses applied and the plant parts and methods used. With these results it indicates the importance of avoiding or reducing the consumption of processed foods that contain synthetic dyes.

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