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STUDY OF CYTOLOGICAL AND GENETIC DAMAGE INDUCED BY A FOOD COLORING DYE (TARTRAZINE)

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ABSTRACT

The Allium test is very good indicator for analyzing genetic damage by chemicals, Tartrazine (yellow 5) was chosen for this study to assay its genotoxic affect on *A. cepa* root tip cells. Various concentrations of dye (w/v) were prepared in distilled water and the highest concentration was 2 mg/100ml, The onion roots were soaked in various concentrations of dye for 48 hour. After that the cut root tips were fixed in 1:3 aceto-alcohols (Carnoy's fixative) and stored in 70% alcohol for microscopically studies. Staining was done in 2% aceto-carmine in 45% glacial acetic acid (v/v). According to the microscopic analysis only concentration 2 mg/100 ml showed abnormal division and decrease in mitotic index, where mitotic index in dye treated cells was 5,83% and showed significant decreasing in value compare with control (13,23%). Also several types of abnormal mitotic cells (AMC) were observed like c-mitosis, multipolar and Binucleus. And according to this study tartrazine are potential genotoxic agents in the environment.

KEYWORDS: Genetic damage, Tartrazine, c-mitosis, Binucleus, multipolar.

1. INTRODUCTION

Some food colors like tartrazine also known as Yellow 5 or E102 is used in many colored foods and drinks products, aspirin, vitamins and other substances (El Keredy, 2017), as well as pharmaceuticals and cosmetics. Mutagenic and toxicity of tartrazine action were determined by researches, Moreira Soares and his colleagues evaluated the potential in vitro cytotoxicity, genotoxicity and effects on DNA repair of human lymphocytes exposed to the dye (Soares et al., 2015). Also other researchers studied the mutagenic action of tartrazine and indigocarmine in a microbial model and in mice (Karpliuk et al., 1984). Onion (*Allium cepa* L.) with number of chromosomes (2n=16) has relatively large monocentric chromosomes and reasonable as test organism for the study of environmental mutagenesis (Moraes and Jordao, 2001; Patra and Sharma, 2002). Al-Sabti reported that onion root meristem cells are sensitive to genetic damage by chemical substances (Al Sabti, 1989). Many researchers used *Allium cepa* test to study chromosome damage, Peter and Tomaz reported the toxicity and genotoxicity effects of methyl methanesulphonate and they identified 15 categories of morphological aberrations like chromosome damage, chromatid damage and centromere damage (Peter and Tomaz, 2014). Tripathy and Rao evaluated the potential genotoxicity of orange red dye at various concentrations on growing root tip cells of onion by analyzing mitotic cell division and they observed many types of genotoxic effects like bridge in anaphase and abnormal uncoiling of chromosomes during metaphase and anaphase; and transverse orientation of chromosomes in spindle apparatus (Tripathy and Rao, 2015). The aim of this study To evaluate cytological and genetic damage that induced by food coloring dye (tartrazine) on root tip cells of onion during mitosis.

2. MATERIALS AND METHODS

Dye

Tartrazine (yellow 5) was selected for this study to assay its genotoxic affect on *A. cepa* root tip cells and was purchased from a local market of Amman.

Allium cepa test

Standard protocol (Tripathy and Rao, 2015) was followed with slight modifications; the bulbs of onion were purchased from local vegetable market of Amman. 11 onion bulbs presoaked in distilled water until the roots grew and became long 1 - 2 cm. Various concentrations of dye (w/v) were prepared in distilled water: 2 mg/100ml, 1,0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, and control. The onion bulbs were transferred from distilled water and presoaked in various concentrations of dye for 48 hour to

induce mitotic aberrations and to assess mitotic index by recording the dividing cells. After 48 hr, the cut root tips were fixed in 1:3 aceto-alcohols (Carnoy's fixative) (1 acetic acid : 3 ethanol) for 24 hours. Then stored in 70% alcohol for future use.

Staining and slide preparation

Squash preparation was adopted following the acid hydrolysis of cellulosic cell wall in 1 N HCl for 1 hour in room temperature. Staining was done in 2% aceto-carmine in 45% glacial acetic acid (v/v). Roots tips were squashed on a slide by light pressure carefully on cover slips. Then, The microscopic analysis using the 40X objective was used to calculate mitotic index and aberrant cells in metaphase, anaphase, and telophase cells were tested. The cells that showed fragments, bridges, laggards, c-mitosis and multipolar were considered aberrant cells.

The mitotic index was calculated as: MI= (Total dividing cells / Total observed cells)*100

3. RESULTS AND DISCUSSION

According to the microscopic analysis only concentration 2 mg/100 ml showed abnormal division and decrease in mitotic index, while lower concentration did not show any aberrant cells.

Mitotic Index

Table 1 shows the effect of tartrazine on mitotic index. In untreated meristematic cells, MI was registered to be 13.23%, While in dye treated cells (2 mg/100ml) MI was 5,83%, the results showed significant decreasing in MI value. Also the maximum number of dividing cells is (139) in control cells compare with (67) in treated cells. The observations of the current study are an indication of the genetic damage by tartrazine, which is evident from the lowering of the mitotic index and abnormal cells division. The decreasing of mitotic index could be have been accomplished by the inhibition of DNA synthesis at S-phase (Sudhakar et al., 2001); Epel found full inhibition of mitosis division when ATP level declining below the 50% of normal level (Epel, 1963); it may be assumed that the cell division process depended on energy, and some chemicals like tartazine effect on ATP synthesis. And according to Jain and Sarbhoy the chemical substances which impact sugar and ATP synthesis creating annoxia condition or by other ways spend much effect on chromosomes movement (Jain and Sarbhoy, 1988).

Table 1: Effect of Tartrazine on Mitotic index in root tip cells of *Allium cepa.*

Dye treatments	Total cells scored	Number of dividing cells	Mitotic index (MI)%
Control	1050	139	13,23%
2mg/100 ml	1149	67	5,83%

Genetic damage and aberrant cells

Several types of abnormal mitotic cells (AMC) were observed like c-mitosis, multipolar and Binucleus (Fig 1 and 2). C-mitosis was more appearance compare with other aberrant cells. Binucleus cells that were found at 2mg/100ml concentration could be due to the inhibition of cell wall development at final stage telophase (Sudhakar et al., 2001). Tartrazine showed effects like colchicine mode of action, colchicine inhibits the formation of spindle fibers and temporarily arrests mitosis division (Blakeslee and Avery 1937). Multipolar cells resulted from multipolar spindle and unequal separation of chromosomes, and many studies showed that pesticides have the same effect (Mohandas and Grant 1972).

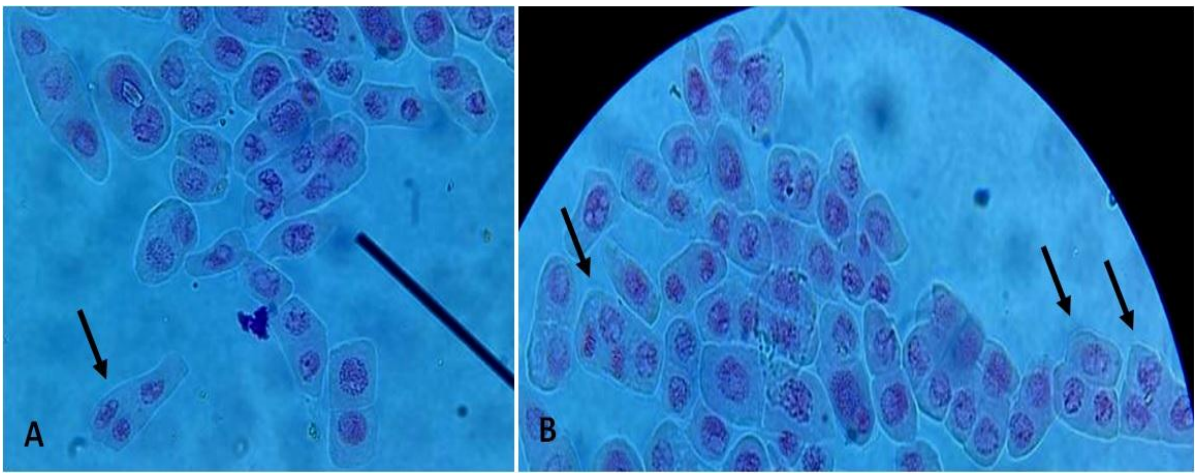

Figure 1: Effect of Tartrazine (2 mg/100 ml) on mitotic cells in root tip cells of Allium cepa. A and B showed multipolar.

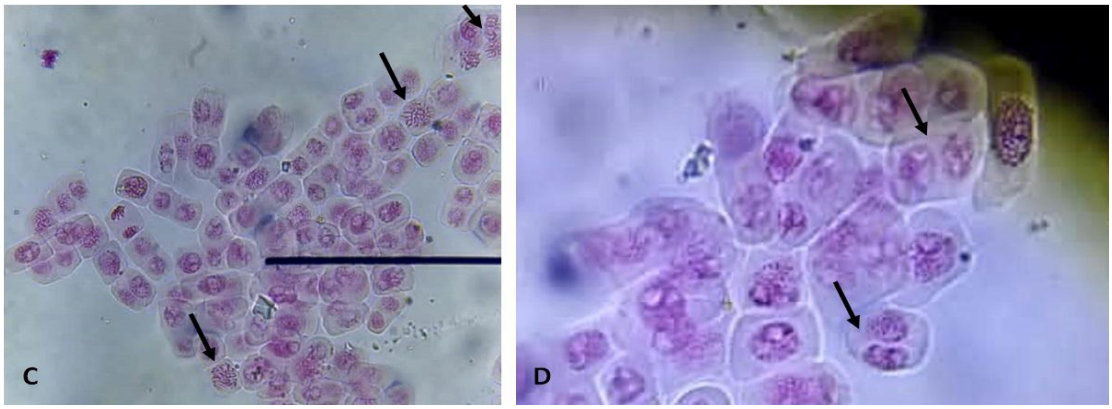

Figure 2: Effect of Tartrazine (2 mg/100 ml) on mitotic cells in root tip cells of Allium cepa. C: showed cmitosis, D: showed Binucleus.

4. CONCLUSION

From the above results it is clear that tartrazine are potential genotoxic agents in the environment.

5. FUNDING

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