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Studies on Mitodepressive Effect Of Indigocarmine

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Abstract

In the present study the effect of an artificial food dye Indigocarmine on mitotic index of *Chara vulgaris* was studied. The plants of *C. vulgaris* were treated with different concentrations of dye like 100 ppm ,250 ppm ,500ppm ,750 ppm and 1000 ppm for 2h,4h and 6h duration. The mitotic index was decreased in most of the concentrations as compared to the control. The dye showed a mitodepressive effect ,especially in higher concentrations

Key words : *Indigocarmine, Chara vulgaris , mitotic index ,mitodepressive*

Introduction

A number of food additives are being extensively used in modern food industry to serve different purposes. The use of artificial food colours has became a necessity now-a- days. A broad range of synthetic food colours are widely used in different food items to restore the colour loss during processing and ensuring uniformity of colours in the product. The synthetic food colours are usually chemicals. Hassan (2010) and Mahfoz *et.al.* [2010)] . did a number of excellent studies on the chemical nature of dyes and its toxicity.

Indigocarmine is one of the permitted food dye in India. It is used in canned fruits, milk products, jam, jellies, candies, dry –premix foods and pharmaceuticals etc. A number of scientists Gaunt *et.al*. (1969), Price *et.al*.(1978) and Giri *et.al* (1986) did various investigators on Indigocarmine. However no systematic studies have been made to evaluate the toxicity of this dye on mitotic cycle. Since the study of effect of several chemicals on plant mitosis may provide valuable information in relation to possible cytotoxicity in human being, so an effort has been made in the present study to evaluate the mitodepressive effect of Indigocarmine on mitotic index of *Chara vulgaris.*

Materials and Methods

Indigocarmine is also known as Indigotine or FD and C blue No.2 (C. I. 1971), Food blue 1(No. 73015 EEC No. E -132). It is a disodium salt of 1- indigotin 5 5' – disulphonic acid. It is a permitted dye in India with maximum ADI of 5 mg / kg body wt. FAO / WHO (1994).

Stock solution of Indigocarmine was prepared by dissolving 1 gram of dye in 1000 ml of distilled water. By dilution of stock solution different concentrations of dye 100 ppm, 250 ppm ,500 ppm,750 ppm and 1000 ppm was prepared.

The test material *Chara vulgaris* (n=28) was thoroughly washed in tap water and identified according to monograph and iconograph of Wood and Imahori (1965). The treatment of indigocarmine was given to

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young plants of *C. vulgaris* for 2h,4h and 6h. The treated fertile tips were fixed in Carnoy's fluid (1:3 acetic acid : alcohol) and transferred to 70 % alcohol for preservation. For control the plants with fertile tips were immersed in double distilled water. Smearing of spermatogenous filaments were prepared following iron –alum acetocarmine method given by Godward (1948) .Mitotic index was calculated by using with following formula

$$\text{Mitotic index \%} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells counted}} \times 100$$

To estimate SD and SE a statistical analysis was done.

Results and discussions

The obtained results are presented in Table – 1.The number of cells in interphase and different stages of cell cycle and the mitotic index value (MI) obtained from *Chara vulgaris* cells ,when treated with different concentrations of Indigocarmine for different duration is indicated in the table . According to Smaka kinel *et.al.* (1996) mitotic index is an acceptable measure of cytotoxicity for all living organisms .It is obvious from the Table -1 that the mitotic index was decreasing compared to the control in all experimental variants. The value of mitotic index in control was 43.4 %.The aqueous solution of Indigo carmine of 100 ppm concentration decrease the MI (41.9 %) in 2h and 43 % in 4h duration treatment as compared to control (43.4 %). In 6 h treatment of the dye there is slight increase in value of MI (43.5 %). When 250 ppm concentration of dye was treated for 2h , there is again mild increase in value of MI (43.6%) .After that there is continuous reduction in the value of mitotic index, when treated with 500 ppm, 750 ppm and 1000 ppm concentration of Indigo carmine . The lowest percentage of mitotic index (39.3 %) was reported by 1000 ppm concentration of dye, when given for 4 h duration . Decrease in MI is the indicator of cytotoxic and mitodepressive nature of dye.The mitodepressiveness may be caused either by obstruction in the onset of prophase or by an arrest of one of the mitotic phases.

Table -1 clearly indicates that the total number of dividing cells were more in control (527 cells) as compared to treated cells. The number of dividing cells were more in low concentration and reduced in higher concentration of dye. According to Sudhakar *et.al* .(2001) the decrease in mitotic index may be due to inhibition of DNA synthesis at S phase . Yildiz and Arikan (2008) stated that the inhibition of mitotic activities is often used for tracing cytotoxic substances .Out of the four mitotic stages(prophase, metaphase ,anaphase ,telophase) ,the number of cells were maximum in prophase followed by metaphase and anaphase .The least number of dividing cells were observed in telophase . Increase in prophase was also observed by Rencuzogullari *et. al.* (2001),Turkoglu (2007) and Onyemobi *et.al.* (2012) in *Allium cepa* root tips ,when treated with different food preservatives.

The highest number of cells in prophase (129) were found by aqueous solution of 100 ppm ,when given for 4 h, while lowest number of cells in prophase (87 cells) were reported in 500 ppm concentration for 6 h treatment .Telophase showed highest number of cells (80 cells) by aqueous solution of 500 ppm for 4 h and lowest number of cells (48 cells) were recorded in aqueous solution of 1000 ppm when given for 6 h.

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Mutagenic effect of some food colourings included indigo carmine was done by Karpliuk *et.al.* (1984) and found that doses of these dyes applied in food industry are fairy safe. A short term toxicity study in pigs was done by Gaunt *et.al.* (1969) ,he found no effect on growth, urine and serum analysis or organ weight ,when indigocarmine was fed at level of 150,450,1350 mg/kg/day for 90 days. Indigo carmine was found to cause a reduction in sperm density at high doses in swiss albino mice by Dixit and Goyal (2013) According to Zippelius *et.al.* (2013) indigocarmine damages human chondrocytes in a time and concentration dependent manner.

Conclusion

The present investigation suggests that such studies should be conducted continuously for further investigation as indigocarmine is frequently used artificial food colour in different food products including pharmaceuticals.

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