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RESEARCH PAPER

Chromosomal instability and abnormal mitochondrial activity induced by two synthetic food colorants

Aswathi Pokkadath, Reshmi Chembrammal* & John Ernest Thoppil

Aswathi Pokkadath
e-mail: aswathipokkadath@gmail.com

Reshmi Chembrammal *
e-mail: reshmibalan824@gmail.com

John Ernest Thoppil
e-mail: jethoppil@gmail.com

Cell and Molecular Biology Division, Department of Botany, University of Calicut, Malappuram, Kerala, India

* corresponding author

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ABSTRACT

The artificial colorants act as a potent cytotoxic agent at the consumer level, whose analysis is inevitable. The investigation is mainly focused to analyze the toxicity of two commonly used synthetic food colorants (Brilliant blue and Fast green) with the help of *Allium cepa* root tip assay. The frequency of aberrations and the concentrations of both the colorants were found to be positively correlated in the chromosome studies. The integrity of the cell membranes, which is a potent parameter to detect cellular damage was detected using Evans blue staining. 2,3,5-triphenyl tetrazolium chloride (TTC) method was also adopted to determine the damaging effects of the synthetic molecules on the mitochondrial oxidative properties in the root tip cells. Both food colorants showed prominent membrane damage and mitochondrial activity reduction in the test material.

Keywords: chromosomal abnormality, membrane damage, metabolic inhibition, food colorants, brilliant blue, fast green

РЕЗЮМЕ

Поккадат А., Чембраммал Р., Топпил Дж.Э. Нестабильность хромосом и аномальная митохондриальная активность, индуцируется двумя синтетическими пищевыми красителями. Искусственные красители действуют как мощный цитотоксический агент на уровне потребителя. Исследование в основном направлено на анализ токсичности двух широко используемых синтетических пищевых красителей (Brilliant blue и Fast green) с помощью анализа кончика корня *Allium cepa*. Было установлено, что частота аберраций и концентрация обоих красителей положительно коррелируют при исследовании хромосом. Целостность клеточных мембран, которая является информативным параметром для выявления клеточных повреждений, была обнаружена с помощью окрашивания синим Эвана. Метод 2,3,5-трифенилтетразолия хлорида (TTC) был также использован для определения повреждающего действия синтетических молекул на окислительные свойства митохондрий в клетках кончика корня. Оба пищевых красителя показали значительное повреждение мембран и снижение митохондриальной активности в исследуемом материале.

Ключевые слова: хромосомные аномалии, повреждение мембран, ингибирование метаболизма, пищевые красители, блестящий синий, быстрый зеленый

Переведено редколлегией

Synthetic food colorants are one of the major ingredients in fast-food and soft drinks and it can cause many lifestyle diseases like diabetes, variation in blood pressure, cholesterol accumulation and cancer. These attractive synthetic colorants are extensively used in the production of beverages and medicines. Humans, especially young children are always attracted to food and drinks bearing pleasant colors. So, addition of attractive colors can enhance the delicious value and the sweetness of food and drink for the consumers (NIN, 1994).

Synthetic certified colorants are the most popular type of food colorings, as they are brighter, more uniform, better characterized, and of higher tinctorial strength, encompass a wider range of hues, and are less expensive than colors derived from nature (Griffiths 2005). Most of the synthetic dyes are originally resulting from coal tar, commonly called coal-tar dyes and it contain azo group. Azo dyes are compounds characterized by the presence of one or more azo groups (–N=N–) and constitute the most important class of dyes in the textile industry (Kunz et al. 2002). Some authors revealed that tests with microorganisms and mammalian cells show that azo dyes are toxic compounds (Michaels et al. 1985, Amin et al. 2010). All the cytotoxic effects reported for azo dyes might be due to the direct action of dyes on the cells, especially by the formation of metabolites resulting from azo bond reduction (Lin & Leu 2008). These metabolites can react with the DNA molecule, damaging both its structure and function (Oliveira et al. 2010). Food colors are the key source of food intoxication and surveys have been conducted to determine the presence of non-permitted food colors in different food items (Koutsogeorgopoulou et al. 1998).

Colors are often used in soft drinks, various kinds of toffees, ice-creams, jams, jellies etc, by the big manufacturers as well as street vendors. It has been suggested that consumption of foods containing color additives could sometimes

lead to harmful effects (NIN 1994). The present study was intended to realize the toxic potential of synthetic food colorants, like fast green and brilliant blue with core importance to their cytotoxicity, membrane damage, and mitochondrial function.

MATERIAL AND METHODS

The synthetic food colorants, fast green (CAS No. 2353-45-9) and brilliant blue (CAS No. 3844-45-9), in pure form were purchased from the local market whereas *Allium cepa* bulbs were purchased from Tamil Nadu Agricultural University and planted for fresh roots. Evans blue (CAS No. 314-13-6) and TTC (CAS No. 298-96-4) were purchased in pure form from the HiMedia chemical laboratory.

Cytotoxicity in *Allium cepa*

For making fresh stock solution, 0.1 g of both colorants were dissolved in 100 ml of distilled water. Different concentrations of the colorants, viz, 0.005, 0.01, 0.05 and 0.1 % (w/v) were prepared. Distilled water and hydrogen peroxide (0.1 %) were taken as the negative control (NC) and positive control (PC) respectively. Mitotic index and the percentage of abnormal cells are the two important parameters, which are calculated using the following formulae:

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Abnormality Percentage} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

Rooted bulbs were treated with various concentrations of colorants at different time intervals such as half an hour, 1, 2 and 24 h. They were washed thoroughly and immediately fixed in modified Carnoy's fluid (1 acetic acid: 2 alcohol) for 1 h. The root tips were washed and hydrolysed in 1N hydrochloric acid for 5–10 minutes to separate the cells during squashing. The root tips were then washed and stained with 2 % acetocarmine for 4 h. Destained with 45 % acetic acid, squashed and mounted on clean microslides. Slides were observed under the microscope, Leica DM 2000 LED, Germany.

In situ visualization of cell death and metabolic activity

For the visualization of cell death, root tips were treated with different concentrations for 24 h. The roots were stained using 0.25 % (w/v) Evans blue solution for 15 min and followed by washing with distilled water (Baker & Mock 1994). Roots with dead cells were macro-photographed for the qualitative estimation. Afterwards ten root tips with equal length (10 mm) were excised and soaked in 3 ml of N, N-dimethylformamide for 1 h at room temperature. The absorbance of Evans blue was detected at 600 nm.

The metabolic activity was analysed by placing the treated bulbs in 0.5 % 2,3,5-triphenyl tetrazolium chloride (TTC) and kept at 35 ± 1°C for 15 min in the dark. For qualitative estimation root tips with equal length (10 mm) were excised and placed. Subsequently root tips were washed with distilled water and photographed. The colored compound released by soaking with 95 % ethanol was used to measure the absorbance at 490 nm (Shimadzu, Japan).

Statistical analysis

The data obtained throughout the work were subjected to statistical analysis using IBM SPSS Statistics Version 20. The data obtained were analysed in One-Way ANOVA followed by the Duncans Multiple Range Test (DMRT) to confirm the variability of data and validity of results. All the values were expressed in mean ± standard error. The statistical significance was determined with $p < 0.05$, which is considered significant.

RESULTS

Severe cytotoxicity was observed in *Allium cepa* root tips treated with the selected two synthetic colorants. Both clastogenic and non-clastogenic abnormalities were induced by different concentrations of the food colorants (Figs 1, 3). A dose dependent reduction mitotic index was observed. The abnormality percentage of the treated roots was found to be higher than that of negative control, which revealed the toxicity of both food colorants (Tables 1, 2).

In the Evans blue staining technique, both colorants gave prominent cell death which was dose dependent (Figs 2A, 4A). In both the food colorants, maximum cell death was observed in the concentration of 0.1 %. The expedient color gradation reveals that the percentage of cell viability in brilliant blue is less than that in fast green.

The effect of mitochondrial activity decreases, when the *A. cepa* roots were treated with brilliant blue and fast green. In both colorants, positive control showed highest inhibition of metabolic activity. Positive control shows 0.0327 ± 0.0005 and 0.0424 ± 0.0003 cell viability in the treatment of brilliant blue and fast green respectively. Highest stain uptake can be observed in the lowest concentration (0.0125 %), so when concentration increases stainability decreases. The intensity of stain uptake in *A. cepa* roots are very less because the roots show drastic cell death when they were treated with food colorants (Figs 2B, 4B)

DISCUSSION

The cytotoxic effect of selected synthetic food colorants (Brilliant blue and fast green) was evidenced by an incredible lowering of mitotic division in *Allium cepa* root tip meristematic cells and shows a considerable rise in abnormality. Cytotoxic studies gave so many cytogenetic aberrations, pinpointing cell death. Treated cells showed cytoplasmic disintegration, nuclear vacuolation, cytoplasmic shrinkage, receding of cytoplasm, nuclear fragmentation, cytoplasmic vacuolation, enucleated cells, ghost cells, cytoplasmic breakage and nuclear disintegration. A similar food colorant induced mitotic inhibition was reported earlier (Prajitha & Thoppil 2016).

The *Allium* model used to possess the ability to interact with mutagenic agents during its cell cycle and also to determine the cytotoxic and genotoxic effects of harmful chemicals (Fiskesjo 1997, Nithyameenakshi et al. 2006). The pulverisation of chromosomes is due to the premature condensation of chromosomes as a result of the action of chemical

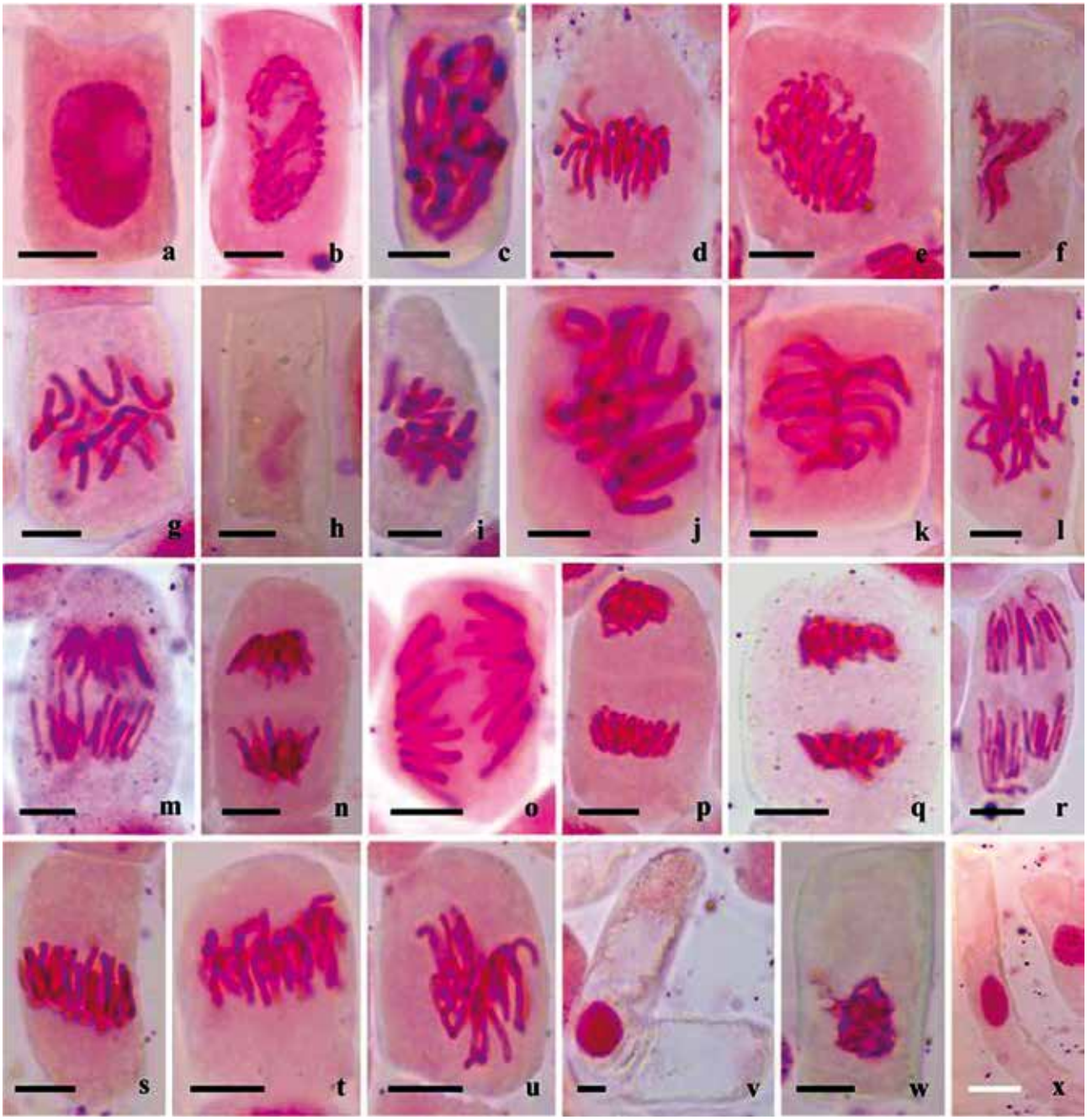

Figure 1 Cytological aberrations induced in *Allium cepa* root meristem with the effect of brilliant blue: a – double nuclear lesion at interphase, b – lesion at prophase, c – chained metaphase, d – chromosome clumping at metaphase, e – chromosome gapsat early metaphase in a polyploid cell, f – chromosome disintegration, g – cytostasis at metaphase, h – ghost cell formation at metaphase, i – hypercondensation at metaphase, j – pole to pole metaphase, k – pole to pole sticky metaphase, l – stellate metaphase, m – chromosome bridges at anaphase, n – coagulated anaphase, o – equitorial separation at anaphase, p – shift in MTOC during anaphase, q – sticky anaphase, r – unequal separation at anaphase, s – unipolar movement at anaphase in a hypoploid cell, t – shift in equitorial plate, u – tropokinesis, v – hyperchromasia at interphase showing cytoplasmic vaculation, w – nuclear fragmentation and extrusion of chromatin, x – strap cell showing cytoplasmic shrinkage; scale bar – 10µm

substances found in the extract (Knuutila et al 1981). The normal organization of chromatin in the nucleus and chromosome segregation is genetically controlled (Franklin & Cande 1999). Chromosome stickiness was one of the most frequently scored abnormality and it was observed in almost all the treatments in the present study. Stickiness is due to the disturbances in the nucleic acid metabolism of the cell (Darlington 1942). Stickiness has been interpreted by many to be the result of depolymerisation of DNA (Franklin & Cande 1999), partial dissolution of nucleoproteins (Kaufmann 1956), breakage and exchange of basic folded fibre unit of chromaids (Klasterska et al. 1976) and stripping of protein covering of DNA in chromosomes (Stephen 1979). It may be due to the action of the extract on the protein, which forms an integral part of chromosomes (El-Sadek 1972).

The sticky nature of chromosomes is probably due to the heterochromatinisation resulting in denaturation of nucleic acid and thus making the chromosome contour adhesive (Grundmann 1966). Induction of stickiness is sometimes manifested as the cytotoxic effect of the chemical

Table 1. Effect of different concentrations of Brilliant blue on *Allium cepa* root meristem at different time periods

Treatment	Time period (h)	No. of dividing cells/1000	No. of abnormal cells/1000	Mitotic index % ± SE	Abnormality % ± SE
Negative	1/2	833	123	83.3333 ± 0.72648[a]	14.7153±0.03426[c]
	1	861	95	86.1667 ± 1.48137 [a, b]	11.1612±0.14473[b]
	2	885	97	88.5000 ± 0.57735[b]	10.3735±0.30153[a]
	24	891	92	89.1667 ± 0.44096[b]	9.8885±0.34389[a]
Positive	1/2	316	617	31.6667 ± 1.66667[a]	61.7000 ± 1.37961[a]
	1	333	604	33.3333 ± 303333[a]	60.4000 ± 0.95394[a]
	2	350	665	35 ± 2.88675[a]	66.5667 ± 3.19235[a]
	24	366	646	36.6667 ± 4.40959[a]	64.6667 ± 1.07445[a]
0.005%	1/2	686	385	68.6667 ± 2.02759[a]	38.5333 ± 0.93868[a]
	1	776	391	77.6667 ± 1.45297 [b, c]	39.1000 ± 2.08167[a]
	2	823	465	82.3333 ± 1.45297[d]	46.5667 ± 1.44952[b]
	24	723	466	85 ± 2.64575[d]	46.6333 ± 1.44491[b]
0.01%	1/2	653	440	65.3333 ± 2.40370[a]	44.0333 ± 2.19418[a]
	1	743	473	74.3333 ± 1.45297 [b, c]	47.3000 ± 1.05987 [a, b]
	2	791	524	79.1667 ± 0.60093 [c ,d]	52.43333 ± 1.08985[b]
	24	693	521	82.6667 ± 2.60342 [d]	52.1667 ± 1.67564[b]
0.05%	1/2	635	569	63.5 ± 2.36291[a]	56.9000 ± 0.15275[a]
	1	723	571	72.3333 ± 1.45297 [a ,b]	57.1333 ± 0.77960[a]
	2	776	544	77.6667 ± 0.60093[b]	54.4667 ± 1.97005[a]
	24	666	541	81.3333 ± 2.90593[b]	54.1333 ± 2.17128[a]
0.1%	1/2	631	572	63.1667 ± 3.65529[a]	57.2000 ± 1.50997[a]
	1	653	576	65.3333 ± 0.33333 [a, b]	57.6667 ± 1.39204[a]
	2	761	596	76.1667 ± 0.72648 [a, b]	59.6333 ± 1.63333[a]
	24	628	600	77.5 ± 5.20416[b]	60.0000 ± 3.65923[a]

SE, standard error. Means within a column followed by the same letters are not significantly different ($p<0.05$) as determined by DMRT

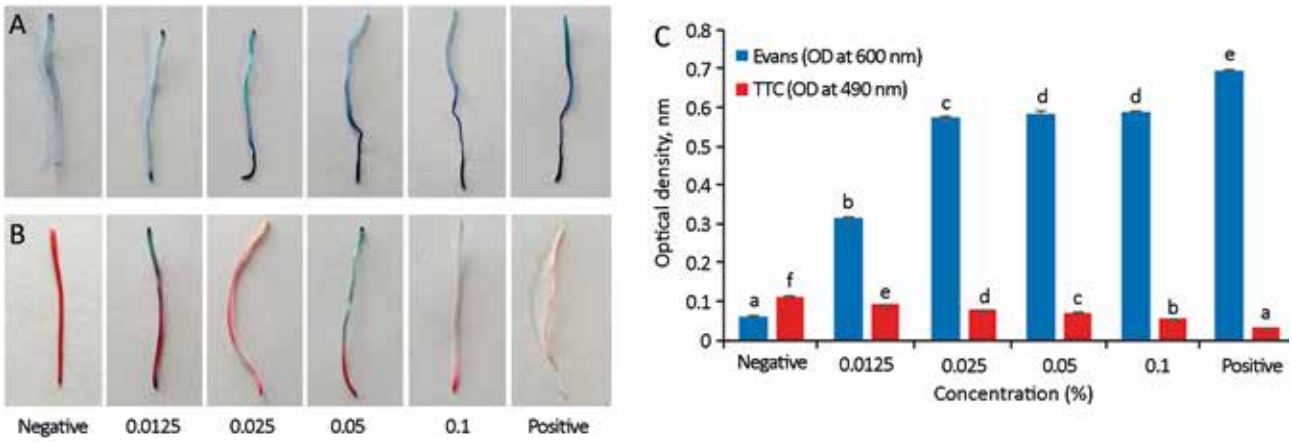

Figure 2 Effect of food colourant (Brilliant blue) on cell viability and mitochondrial function (C). A – Evans blue staining technique: effect of food colorant (brilliant blue) on cell viability; graph and figures showing a significant dose-dependent increase in celldeath. B – TTC staining technique; graph and figures showing dose-dependent decrease in mitochondrial activity. Same letters above columns in the histogram mean no significant difference ($p<0.05$) as determined by DMRT

substances (Panda & Sahu 1985). Electron microscopic studies demonstrated stickiness as a chromatid type aberration (McGill et al. 1977, Klasterska et al. 1976). Stickiness may result from the entanglement of chromatin fibres, which fail to condense properly in preparation for mitosis (McGill et al. 1977). There could be some substances present in the food colorant, which affect the DNA structure in the *A. cepa* root tip meristem perhaps resulting in physical depolymerisation of DNA. This together with or without partial dissolution of nucleoprotein could account for stickiness of chromosomes (Mercykutty & Stephen 1980).

In Evan's blue staining method, a considerable increase in cell death was observed. Cell death is a marker of cytotoxicity, and it was confirmed by Evan's blue staining method on the basis of its penetration to non-viable cells (Panda et al. 2011). Intensity of dye absorbed by root cells was directly propotional to the cell death (Fig. 4); which could be seen within a few minutes after the treatment based on the result reported by (Achary et al. 2008)

A significant inhibition of metabolic activity can be observed in the TTC assay. The cleavage of triphenyl tetrazolium chloride into red colored triphenyl formazan derivative by living cells is the principle of the TTC assay. The reduction of TTC only take place in metabolically active cells, the level of activity is a measure of the viability of the cells (Prajitha & Thoppil 2017). The present study confirms the cytotoxic nature of the synthetic food colorants. So, the usage of food colors may lead to many lifestyle diseases.

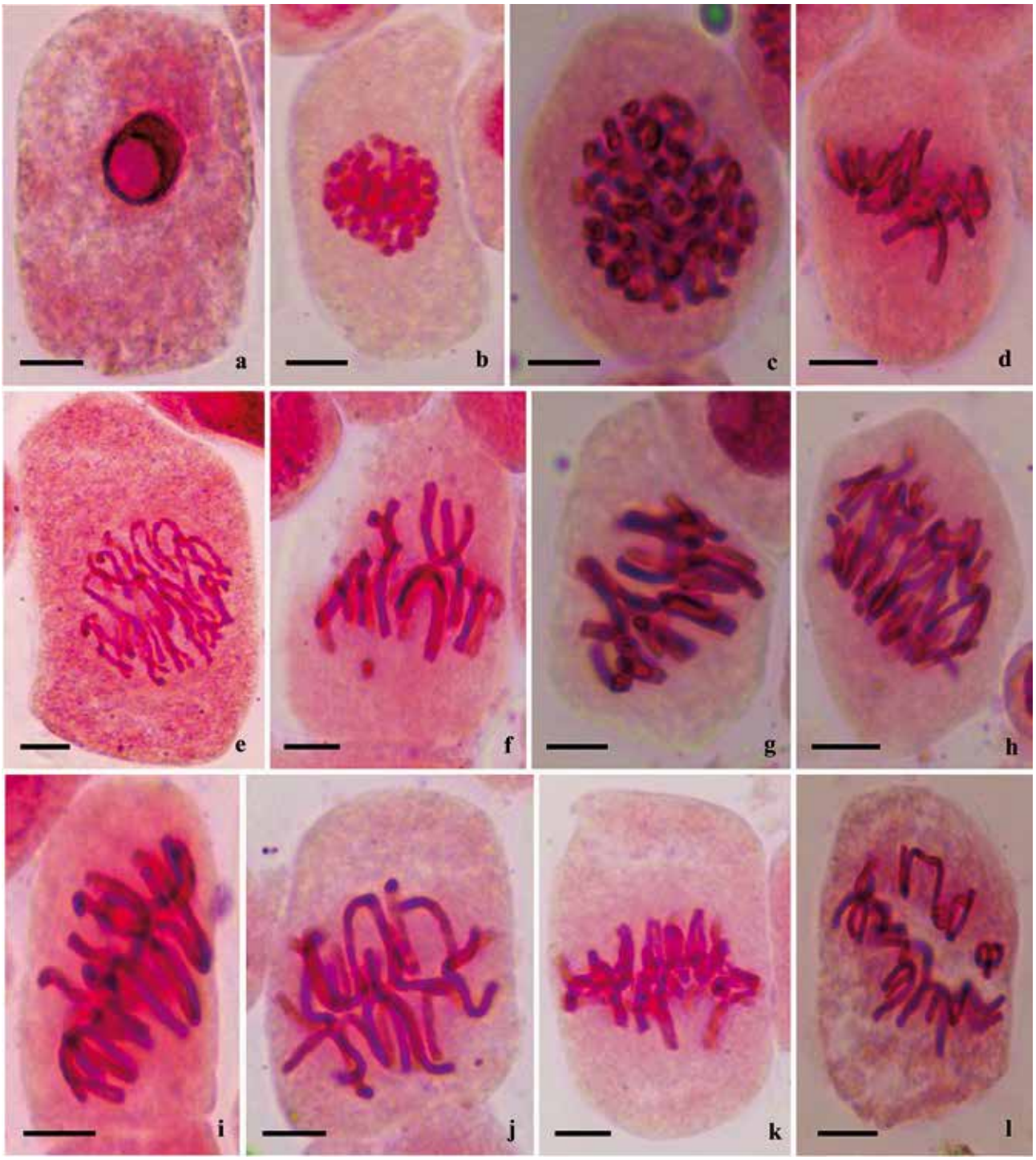

Figure 3 Cytological aberrations induced in *Allium cepa* root meristem with the effect of fast green; a – single nuclear lesion, b – formation of lesion at prophase, c – ball metaphase, d – pulverised metaphase in a hypoploid cell, e – chromosome erosion at early metaphase, f – chromosome fragment at metaphase in a hypoploid cell, g – diagonal metaphase in a hypoploid cell, h – diagonal stathmo anaphase showing multiple bridges, i – diagonal sticky metaphase, j – disturbed metaphase, k – pulverised metaphase, l – scattered metaphase showing chromosome erosion; scale bar – 10μm

CONCLUSION

Synthetic food colors are one of the major ingredients in the modern fast-food items. So today the availability of such food colors is increasing day by day. Brilliant blue and fast green are the two important food colors used to prepare blue and green colored food items respectively. The present study is mainly focused on the cytotoxic activity of these two food colorants. Cytotoxic study showed the considerable decrease of mitotic index which may reveal the toxicity of the colorants. So, the frequent usage of such synthetic food colors may lead to several lifestyle diseases.

Table 2. Effect of different concentrations of Fast green on *Allium cepa* root meristem at different time periods

Treatment	Time period (h)	No. of dividing cells/1000	No. of abnormal cells/1000	Mitotic index % ± SE	Abnormality % ± SE
Negative	1/2	800	93	80.0000 ± 0.28868[a]	11.2750±0.20353[b]
	1	815	90	81.5000 ± 0.57735[a]	11.2676±0.24649[b]
	2	840	89	84.0333 ± 0.77531[b]	10.1517±0.22539[a]
	24	853	85	85.3333 ± 0.72648[b]	9.5049±0.23298[a]
Positive	1/2	308	624	30.8333 ± 1.36423[a]	62.4333 ± 1.54524[a]
	1	335	608	33.5000 ± 0.76376[a, b]	60.8333 ± 1.09291[a]
	2	350	671	35.0000 ± 0.86603[b, c]	67.1000 ± 3.23316[a]
	24	380	652	38.0000 ± 1.60728[c]	65.2333 ± 1.01050[a]
0.005%	1/2	710	391	71.0000 ± 2.30940[a]	39.1667 ± 1.09291[a]
	1	791	401	79.1667 ± 1.58990[a, b]	40.1667 ± 2.20479[a]
	2	838	473	83.8333 ± 1.58990[b]	47.3333 ± 1.52461[b]
	24	758	472	75.8333 ± 4.10623[a, b]	47.2667 ± 1.46211[b]
0.01%	1/2	658	455	65.8333 ± 2.20479[a]	45.5667 ± 2.18810[a]
	1	735	484	73.5000 ± 0.86603 [a, b]	48.4333 ± 0.88380[a, b]
	2	805	527	80.5000 ± 1.04083[b]	52.7333 ± 1.07445[b]
	24	720	525	72.0000 ± 4.53689 [a, b]	52.5000 ± 1.75024[b]
0.05%	1/2	658	578	65.8333 ± 2.20479[a]	57.8667 ± 0.40552[a]
	1	766	575	76.6667 ± 0.88192[b]	57.5333 ± 0.8667[a]
	2	815	549	81.5000 ± 0.76376[c]	54.9333 ± 1.98522[a]
	24	845	547	84.5000 ± 0.86603[c]	54.7000 ± 2.18251[a]
0.1%	1/2	741	579	74.16667 ± 3.00463[a]	57.9667 ± 1.53876[a]
	1	738	579	73.8333 ± 0.72648[a]	57.9333 ± 1.44491[a]
	2	825	602	82.5000 ± 2.50000[a]	60.2667 ± 1.61795[a]
	24	705	606	70.5000 ± 6.78847[a]	60.6333 ± 3.62231[a]

SE, standard error. Means within a column followed by the same letters are not significantly different ($p<0.05$) as determined by DMRT

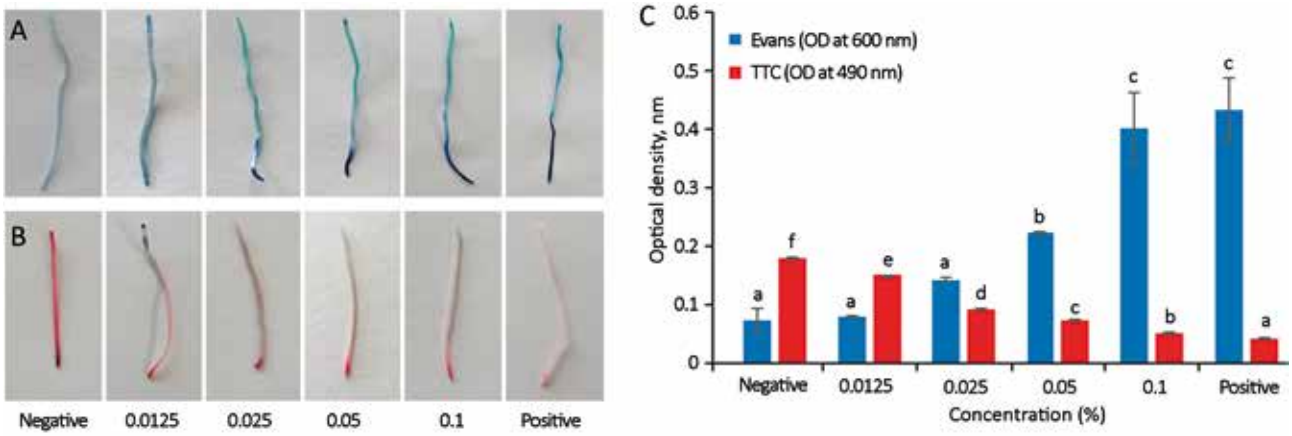

Figure 4 Effect of food colorant (fast green) on cell viability and mitochondrial function (C). A – Evans blue staining technique: effect of food colorant (fast green) on cell viability; graph and figures showing a significant dose-dependent increase in cell death. B – TTC staining technique; graph and figures showing dose-dependent decrease in mitochondrial activity. Same letters above columns in the histogram mean no significant difference ($p<0.05$) as determined by DMRT

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LITERATURE CITED

Achary, V.M.N., S. Jena, K.K. Panda & B.B. Panda 2008. Aluminium induced oxidative stress and DNA damage in root cells of *Allium cepa* L. *Ecotoxicology and Environmental Safety* 70(2):300–310.

Amin, K.A., H.A. Hameid & A.H.A. Elsttar 2010. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. *Food and Chemical Toxicology* 48(10):2994–2999.

Baker, C.J. & N.M. Mock 1994. An improved method for monitoring cell death in cell suspension and leaf disc assays using Evan's blue. *Plant Cell, Tissue and Organ Culture* 39(1):7–12.

Darlington, C.D. 1942. Chromosome chemistry and gene action. *Nature* 149(3768):66–69.

El-Sadek, L.M. 1972. Mitotic inhibition and chromosomal aberration induced by some arylasonic acids and its compounds in root tips of maize. *Egyptian Journal of Genetics and Cytology* 1:218–224.

Fiskesjo, G. 1997. *Allium* tests for screening chemicals: evaluation of cytological parameters. In: *Plants for Environmental Studies* (W. Wang, J.W. Gorsuch & J.S. Hughes, eds), pp. 307–333, CRC Lewis Publishers, New York, USA.

Franklin, A.E. & W.Z. Cande 1999. Nuclear organization and chromosome segregation. *Plant Cell* 11(4):523-534.

Griffiths, J.C. 2005. Coloring foods and beverages. *Food Technology* 59(5):38–44.

Grundmann, E. 1966. *General Cytology*. Edward Arnold Ltd, London.

Kaufmann, B.P. 1956. Cytochemical studies of changes induced in cellular materials of ionizing radiations. *Annals of the New York Academy of Sciences* 59(4):553–566.

Klasterska, I., A.T. Natarajan & C. Ramel 1976. An interpretation of origin of sub-chromatid aberrations and chromosome stickiness as a category of chromatid aberration. *Hereditas* 83(2): 153–169.

Knuutila, S., M. Siimes & P. Vuopio 1981. Chromosome pulverization in blood diseases. *Hereditas* 95(1):15–24.

Koutsogeorgopoulou, L., C. Maravelias, G. Methenitou & A. Koutselinis 1998. Immunological aspects of the common food colorants, Amaranth and Tartrazine. *Veterinary and Human Toxicology* 40(1):1–4.

Kunz, A., P. Peralta-Zamora, S.G. Moraes & N. Duran 2002. Degradation of reagent dyes for the system iron metal/hydrogen peroxide. *New Journal of Chemistry* 25:78.

Lin, Y.H. & J.Y. Leu 2008. Kinetics of reactive azo dye decoloration by *Pseudomonas luteola* in a biological activated carbon process. *Biochemical Engineering Journal* 39(3):457–467.

McGill, M., S. Pathak & T.C. Hsu 1974. Effects of ethidium bromide on mitosis and chromosomes: a possible material basis for chromosome stickiness. *Chromosoma* 47(2): 157–166.

Mercykutty V.C. & J. Stephen 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium* test. *Cytologia* 45(4):769–777.

Michaels G.B. & D.L. Lewis 1985. Sorption and toxicity of azo and triphenyl methane dyes to aquatic microbial populations. *Archives of Environmental Contamination and Toxicology* 4(1):323–330.

NIN 1994. *Studies on newer adulterants and contaminants Annual Report 1993–94 of the National Institute of Nutrition*. Indian Council of Medical Research, Hyderabad.

Nithyameenakshi, S., P.R. Jeyaramraja & S. Manian 2006. Cytotoxicity of fungicides Azoxystrobin and Difenoconazole in root tips of *Allium cepa* L. *Asian Journal of Cell Biology* 1(1): 65–80.

Oliveira, G.A., E.R. Ferraz, F.M. Chequer, M.D. Grando, J.P. Angeli, M.S. Tsuboy, J.C. Marcarini et al. 2010. Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. *Mutation Research* 703(2):200–208.

Panda, B.B. & V.K. Sahu 1985. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphate insecticide 'Fensulphothion'. *Cytobios* 42:147–155.

Panda, K.K., V.M.N. Achary, R.P. Krishnaveni, V.K. Adhi, S.N. Sarangi, S.N. Sahu & B.B. Panda 2011. *In vitro* biosynthesis and genotoxicity bioassay of silver nanoparticles using plants. *Toxicology In Vitro* 25(5):1097–1105.

Prajitha V. & J.E. Thoppil 2016. Apoptotic induction via membrane/DNA damage and metabolic inactivation by synthetic food colorants in *Allium cepa* root meristem. *Turkish Journal of Biology* 40(4):922–933.

Prajitha V. & J.E. Thoppil 2017. Cytotoxic and apoptotic activities of extract of *Amaranthus spinosus* L. in *Allium cepa* and human erythrocytes. *Cytotechnology* 69(1):123–133.

Stephen, J. 1979. Cytological causes of spontaneous fruit abortion in *Haemanthus katherinae* Baker. *Cytologia* 44(4): 805–812.