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Genotoxic effects of Food Dyes on mitotic chromosomal entity in root meristems of Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.)

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Abstract : Synthetic colouring dyes are applied in diverse fields including food industry, wherein they are destined to enhance the aesthetic value of food items. Attempts are made to make food items conspicuous by application of food dyes. But their elevated application may incite various health concerns.The present cytological work is designated to investigate the effects of food dyes on the cellular level by analysing its responses on the meristematic root tip cells of Cluster bean (Cyamopsis tetragonoloba (L.) Taub.).Freshly germinated root tips were treated with graded concentrations (viz. 0.5%, 1%, 1.5% and 2%) of Orange G and Brilliant blue dyes along with a control set(roots dipped in distilled water) for duration of three hours each.Roots were then fixed in carnoy's fixative and preserved in 90% alcohol. Cytological monitoring displayed that both the food dyes were increasingly mitoinhibitory and chromotoxic. Various chromosomal anomalies were encountered included scattering, stickiness, forward movement etc. But an important abnormality of concern was the formation of micronuclei, which are the indicators of genomic loss. The decline in mitotic index and subsequent increment in frequency of chromosomal aberration was higher in the case of Brilliant blue as compared to Orange G, which reflects higher genotoxicity of Brilliant blue compared to Orange G on somatic cellular complement.

Key Words : Cluster bean, Brilliant blue, Orange G, Mitotic Index (MI) and Total Abnormality Percentage (TAB %).

Introduction

Fluorescent blend of colours have intrigued humans since ancient times. Natural indigenous colours have played a pivotal role in traditional and cultural practices and played pioneering role in civilizations. Colours like that of the yellow of turmeric, blue of indigo, red of saffron, orange of henna etc. have made human beings associated with the essence of these splendid colours. These magnificent colours have transformed the food products as well. Food industry has witnessed massive reformation and achieved new benchmarks to accomplish the increasing food demands of the world in the past few decades. In this regard, measures have been adopted to improve appearance of food commodities, which led into the invasion of artificial colours in the food industry. Since then credulous attempts have been implemented to enhance the colour and texture of food products by the assistance of food dyes. Dyes are basically the colouring reagents used to amplify the aesthetic quotient of food products. As per subscribed norms of international research and the recommendations of the Codex Committee on Food Additives and Contaminants (CCFAC), intake of dye is under the control of ADI (acceptable daily intake) (1). Most of the artificial colours are water soluble and

considered acidic (2) which are frequently used in preparation of jams, jellies, soft drink colouring, ice-cream, marmalades, pastries etc.

Lower quantities of these dyes are consumable and quint essential in the economic interests of the food industry. However their unconscientiously exceeding supplementation depreciates food quality and entails potential toxicity to the foodc ausing severe health ramifications. Dyes contain varying heavy metals concentration causing a threat of human genetic disorders (3, 4). They deteriorate the nutritional quotient and disturb the safety standards of various foodstuffs. Serious questions arise on health front also due to inclinement towards dyes as their influence may consequent into low blood pressure, reduced platelet aggregation, coughing and hyperactivity (5). Dyes are also suspected of causing cancer (6-7).These chemicals are bound to have inevitable repercussions on the trophic chain as well, which may alter the dynamics of ecosystem equilibrium.

Henceforth, a self-introspection is required prior before inducing these kinds of amendments in the ingredients profile of edible products. Many previous works have been focussed on genotoxic effects of food dyes in their works (8-11). Tripathi and Rao (12)compiled the clastogenic properties of Orange red. But researchers need to acquaint the world on this topic andhence ample amount of knowledge is required for producing a consensus. This forms the underlining foundation of the present study. The objective of this study is to plan out cytogenetical work and draw valuable cognizance in relation to food additives for human welfare.

Cluster bean or Guar(*Cyamopsis tetragonoloba*(L.)Taub.)belonging to family Fabaceae is a multipurpose self pollinated leguminous crop plant of semi arid conditions. It is used as green manure and replenishes impoverished soil. It is highly anticipated for economic interests due to its rich galactomannan and gum yielding properties. Guar gum is found to be effective in osteoarthritis, transdermal drug delivery systems (13) and for preparing anticancer medicine in the treatment of colorectal cancer (14).It has been selected for pursuing the present cytological work for its small chromosome constitution i.e. 2n = 14 and efficient chromosomal separation.

The two dyes selected for the present assessments are Orange G and Brilliant blue. Orange G is a commonly used colouring azo dye (1-Phenylazo-2-naphthol-6, 8-disulfonic acid disodium). Brilliant blue is Triaryl methane colouring reagent often used in baked goods, beverages, candies, cereals, gums, drugs etc. The effect of these two dyes was cytologically examined on the root meristems of guar. Plant bioassay is a relevant technique for evaluating genotoxicity and cytotoxicity of mutagenic compounds. It will offer a convenient approach to evaluate the effects of food dyes on all the living system which are articulately interdependent, where plants occupy a cardinal importance intricating all other organisms together.

Material and Methods

Procurement of seeds

Fresh seeds of Cluster bean (*Cyamopsiste tragonoloba* variety RGC-1038) was procured from regional station of NBPGR i.e. CAZRI, Rajasthan. Thoroughly surface sterilized seeds were water soaked for few hours and then placed in wet Whatman's Filter paper 2 on petriplates and kept under optimum temperature at pre-requisite humid conditions in the seed germinator for root sprouting.

Seed treatment

These freshly germinated seeds were then separated into various sets and treated with graded dosesof Orange G and Brilliant blue(*viz.* 0.5%, 1%, 1.5% and2% respectively)by dipping in respective solution for three hours. Along with this, a control set was also maintained by dipping the seeds in distilled water.

Root Fixation

Post treatment, the roots were washed under running water thoroughly. Then the roots were fixed in carnoy's fixative (3:1 Alcohol and Glacial acetic acid) in respectively labelled bottle. Next day roots were preserved in90% alcohol for future study.

Slide preparation

Roots were hydrolysed in1 N HCl at 50 ± 1°C in the water bath. Hydrolysing the roots is critical for softening and maceration of root tips which allows convenience in squash preparation. After thoroughly washing in distilled water, the roots were stained in 2% acetocarmine stain for chromosomal visualization. The slides were observed under Olympus microscope at 40X resolution and the photomicrographs were obtained through Nikon Phase Contrast Research microscope (Nikon Eclipse, E200, Japan) *via* PCTV software.

Statistical analysis was also employed for generating accuracy of data. Obtained data was subjected to statistical software SPSS 16.0 through One way Analysis of Variance (ANOVA). A Pair wise comparison of means was made using Duncan's multiple range test (DMRT) at $p<0.05$ significance level. Graphical representation of the data was achieved by employing Sigma Plot 10.0 software. For each respective dose, five slides were prepared and ten microscopic views from each of the slide were recorded. Mitotic Index (MI) and Total Abnormality Percentage (TAB%) were computed for each of the slide prepared.

Mitotic index (MI) = (Total number of dividing cells/Total number of observed cells)*100

Results and Discussion

Cytological study in *Cyamopsis tetragonoloba* deciphered that chromosomal complement in the plant is 2n=14.Two important criteria i.e. MI and TAB% were calculated. Mitotic index is an indicator of cell proliferation biomarker which measures the proportion of cells in mitotic phase of the cell cycle (15). Data of comparative account of MI and its graphical representation in response to dyes has been documented in Table 1 and Figure 1, respectively. The study revealed that MI retarded consistently with every increasing concentration. The calculated MI in case of control was 11.88 ± 0.31^{a}. Recorded MI in case of Orange G decreased from 11.49 ± 0.26^{ab} to7.48 ± 0.26^{d}, whereas it declined from $11.22 \pm 0.46^{a\ to}$ 6.48 ± 0.22^{d} in response to increasing concentration of Brilliant blue. The decline in MI is slightly greater in the case of Brilliant blue as compared to the case of Orange G. The decline in MI in response to Brilliant blue and Orange G implicates the mitotically retarding behaviour of food dyes. This result is in positive agreement with previous cytological reports on food dyes (16-18). The depression in MI infers an overall negative interaction of these "artificial colours" on growth and cellular responses of living entities. Perhaps the sequential cues of the cell cycle were disturbed due to the dyes, consequenting into large influence in the form of decline in MI. It might be due to delay or retardation in the metaphasic equatorial alignment and subsequent blockage in its transformation into anaphasic polar association.

Table 1: Comparative account of MI and TAB% in response to Food Dyes in root meristems of Cluster bean (*Cyamopsis tetragonoloba* (L.)Taub.)

Treatment	Concentration (%)	MI (Mean ± SE)	TAB % (Mean ± SE)
Control	-	11.88 ± 0.31^{a}	-
Orange G	0.5%	11.49 ± 0.26^{ab}	0.54 ± 0.16^{c}
	1%	10.53 ± 0.26^{b}	1.24 ± 0.43^{bc}
	1.5%	9.26 ± 0.43^{c}	2.32 ± 0.34^{b}
	2%	7.48 ± 0.26^{d}	4.31 ± 0.40^{a}
Brilliant Blue	0.5%	11.22 ± 0.46^{a}	1.10 ± 0.34^{d}
	1%	9.75 ± 0.30^{b}	2.27 ± 0.32^{c}
	1.5%	8.03 ± 0.30^{c}	3.90 ± 0.46^{b}
	2%	6.48 ± 0.22^{d}	5.68 ± 0.20^{a}

Abbreviations: MI- Mitotic Index, TAB %- Total Abnormality Percentage
Means followed by lowercase letter are statistically significant at $p < 0.05$ in DMRT

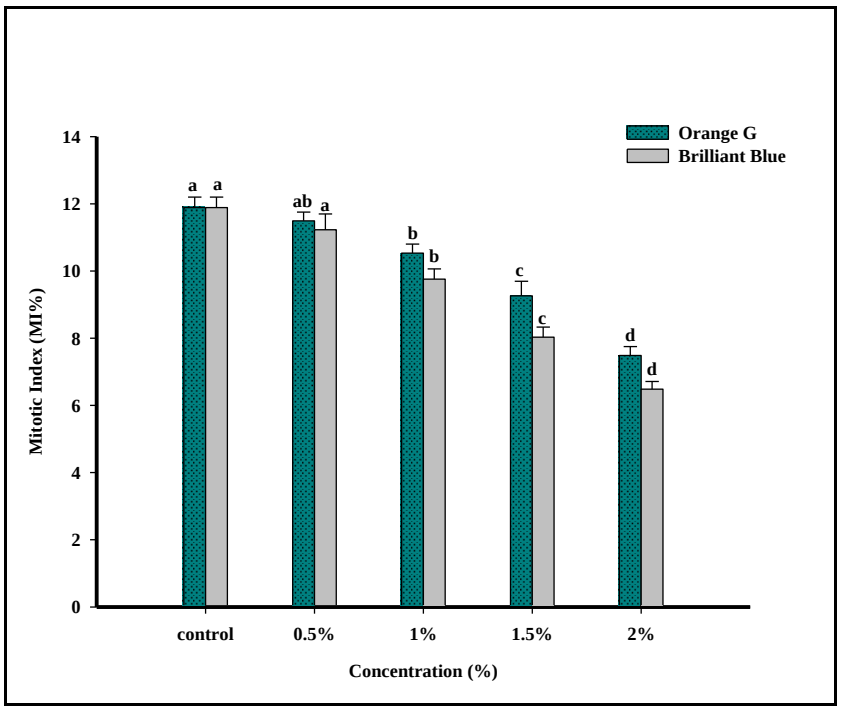

Figure 1: Effect of Food dyes on MI in root meristems of Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.)

The decrease in mitotic index could be due to blocking of G2 preventing the cell from entering mitosis (19) or it may be achieved by the inhibition of DNA synthesis at S phase (20). Inhibition of DNA synthesis might be caused by the decreasing ATP level, which is essential for the functioning of the energy production centre (21-22). Orange G is reported to produce negative impact in mutagenic and DNA assays (23Park et al. (24) stated that colouring combinations of Brilliant blue FCF had negative effects on the central nervous system of humans. Brilliant blue also interacts with anti hyperlipedemic drugs uptakes (such as atorvastatin, simvastatin, and rosuvastatin) (25). It is reported to cause chromosomal aberrations (26-27).

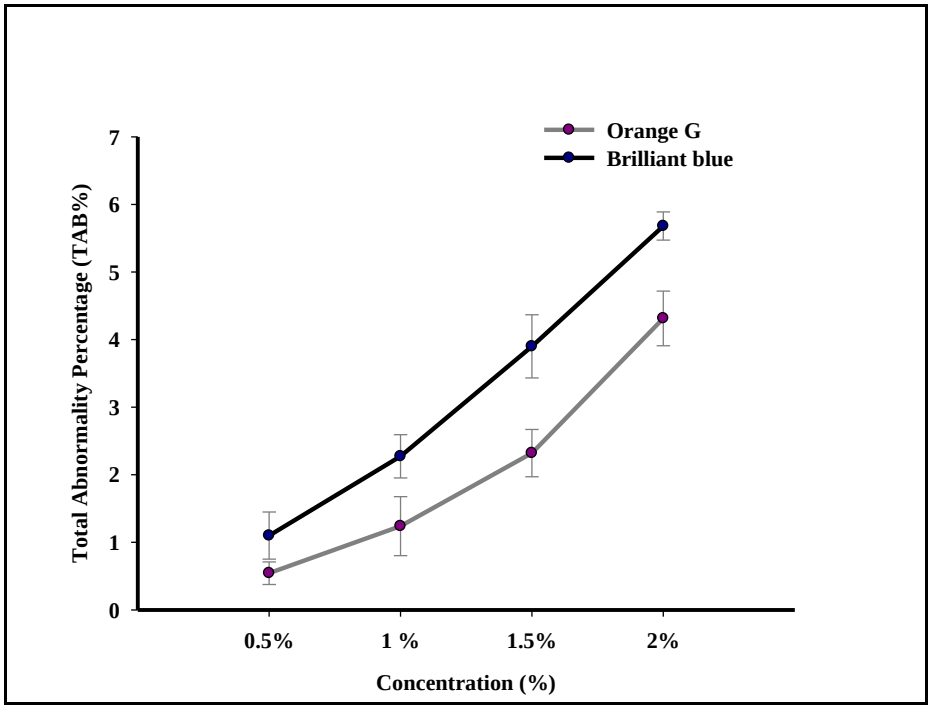

Figure 2: Food dyes induced mitotic aberrations (TAB%) in root meristems of Clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.)

Cytological investigation displayed that control set was perfectly normal with all 14 chromosomes appropriately aligned at the equator in metaphase and properly sequenced 14:14 poleward migration at anaphase. Structural identification revealed that chromosomes in *Cyamopsis tetragonoloba* were severely affected and disturbed due to the apparent distortions induced by the dyes on chromosomal entity. This was reciprocated in the form of prevalent chromosomal abnormality in the treated sets of Brilliant blue and Orange G, which was observed to increase subsequently with every increasing concentration**.** Data of TAB% has been compiled in Table 1 whereas Figure 2 depicts the TAB % trend in response to the dyes. TAB% increased from 0.54 ± 0.16^{c} at 0.5% to 4.31 ± 0.40^{a} at 2% in the case of Orange G whereas in Brilliant blue it ascended from 1.10 ± 0.34^{d} at 0.5 % to 5.68 ± 0.20^{a} at 2%.Percent frequency of the individual chromosomal abnormalities is documented in Table 2.

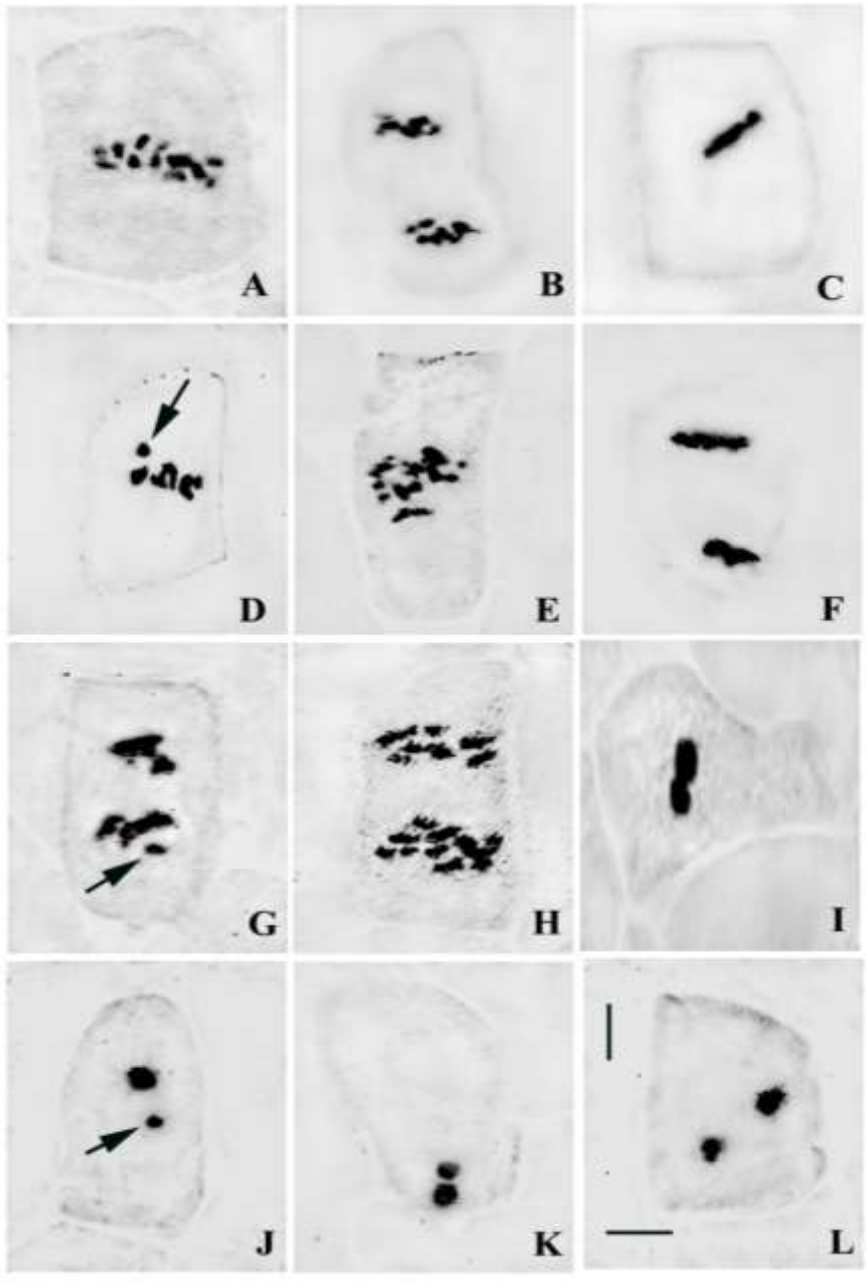

Figure 3: Cytological Plate

Legend of figures - 3(A) Normal metaphase; **3(B)** Normal anaphase; **3(C)** Sticky unoriented metaphase; **3(D)** Precocious movement; **3(E)** Scattering at metaphase; **3(F)** Sticky unoriented anaphase; **3(G)** forward movement at anaphase, **3(H)**Scatteringat anaphase, **3(I)** nuclear budding; **3(J)** micronuclei formation; **3(K)** micronuclei at one end; **3(J)** binucleate cell[**Scale Bar**: Length = 5.80 µm; Width = 5.35 µ

Table 2: Comparative account of chromosomal aberrations induced by Food Dyes in root meristems of Guar (*Cyamopsis tetragonoloba* (L.) Taub.)

Treatment	Concentration	Metaphasic Abnormalities (%) (Means ± SE)				Anaphasic Abnormalities (%) (Means ± SE)			Mn	Oth (Means ± SE)
		Sc	St	Pr	Un	St	Sc	Un		
Control	-	-	-	-	-	-	-	-	-	-
Orange G	**0.5%**	0.09±0.09^{b}	0.09±0.09^{a}	0.09±0.09^{b}	0.09±0.09^{a}	0.08±0.08^{a}	-	0.09±0.09^{a}	-	-
	1%	0.08±0.08^{b}	0.17±0.08^{a}	0.17±0.08^{ab}	0.17±0.08^{a}	0.18±0.09^{ab}	0.25±0.14^{bc}	0.08±0.09^{a}	-	0.08±0.08^{b}
	1.5%	0.27±0.15^{b}	0.18±0.09^{a}	0.36±0.08^{ab}	0.27±0.15^{ab}	0.18±0.09^{ab}	0.46±0.09^{ab}	0.27 ±0.15^{a}	0.18 ± 0.09^{a}	0.18 ± 0.09^{b}
	2%	0.64±0.09^{a}	0.27±0.00^{a}	0.45±0.09^{a}	0.45±0.16^{a}	0.45± 0.15^{a}	0.82± 0.16^{a}	0.45 ± 0.09^{a}	0.27 ± 0.15^{a}	0.45 ± 0.09^{a}
Brilliant blue	**0.5%**	0.09±0.09^{b}	0.27±0.16^{b}	0.09±0.09^{b}	0.09±0.09^{b}	0.17±0.08^{b}	-	0.08 ± 0.08^{a}	0.18 ± 0.09^{b}	0.09 ± 0.09^{b}
	1%	0.17±0.08^{b}	0.45±0.08^{ab}	0.27±0.01^{ab}	0.17±0.08^{b}	0.44±0.16^{ab}	0.17±0.08^{ab}	0.09 ± 0.09^{a}	0.17 ± 0.08^{b}	0.08 ± 0.08^{b}
	1.5%	0.34±0.07^{ab}	0.52±0.14^{ab}	0.36±0.18^{ab}	0.54±0.16^{a}	0.53±0.16^{ab}	0.35 ±0.09^{a}	0.27 ± 0.15^{a}	0.35± 0.09^{b}	0.53± 0.16^{ab}
	2%	0.49±0.99^{a}	0.90±0.18^{a}	0.60±0.17^{a}	0.59±0.01^{a}	0.69±0.08^{a}	0.55± 0.18^{a}	0.28 ± 0.15^{a}	0.80 ± 0.11^{a}	0.65 ± 0.22^{a}

Abbreviation- Sc- Scattering**, St-**Stickiness **, Pr** – Precocious movement**, Un –**Unorientation**, Mn-** Micronuclei formation**, Oth –**Others**.**
(Mean followed by lowercase letters are statistically significant at p<0.05).

Figure3 represents the cytological plate. Figure 3(A) and 3(B) are normal stages of mitosis. However, several chromosomal abnormality were encountered in treated sets including stickiness, scattering, precocious movement, bridges, micronuclei formation etc. Scattering(shown in Figure 3E and 3H),was the predominant abnormality in case of Orange G which ranged from 0.09% at 0.5% concentration to 0.64% at 2% concentration. Stickiness followed by micronuclei formation was the major abnormality in the case of Brilliant blue. Stickiness of metaphase (Figure 3C) and anaphase (Figure 3F) ascended from 0.27% to 0.90%and 0.17% to 0.69%, respectively from lower to higher concentration.

Subsequent increase in chromosomal abnormality displays the sequential negative interaction of food dyes on the chromosomal components**.** Dyes imparted a marked influence on the morphological characteristics of chromosomes which are the "DNA residing fragments". Most of these colorants bind directly to the DNA and cause both structural and numerical anomalies (28-29).Yahagi et al. (30) also confirmed mutagenic or carcinogenic action of azo dyes and suggested that, these effects may involve modifications of DNA. These chemicals plausibly affect the bio-molecular integrity of cellular constituents. The alterations in the chromosomal movements, their structural morphology and orientation are the affects dictated by the dyes. These synthetic dyes impart adverse effect on spindles which are the driving forces during cell cycle captivating or mobilizing the chromosomal locomotion. Swaroop et al. (31) have also asserted the spindle poisoning role of dye Orange red.

Structural expedition of chromosomes provided intimation that dyes have a high impact negative influence on the chromosomal integrity. Stickiness might have resulted from the defective functioning of nonhistone protein involved in the chromosomal organization, which are needed for chromosomal separation and segregation (32) or due to the entanglement of inter-chromosomal chromatin fibers that leads to sub chromatid connection between chromosomes (33). Prophase with micronuclei (Figure 3J) was seen in response to the genotoxic action of food dyes. Micronuclei may result due to acentric fragments or lagging chromosomes that fail to incorporate in to either of the daughter nuclei during telophase of mitotic cells (34). Formation of micronuclei is a very typical feature that reflects a neugenecity which implicates chromosomal loss. Any defects in cellular responses towards the DNA repair system are the prime factors which lead to micronuclei formation (35).Few of the cells showed nuclear budding (Figure 3I) and binucleate condition (Figure 3L) also. Nuclear buds are also called as "nuclear protrusions" or "blebs"**(**36**).** These nuclear buds transform into micronuclei at interphase. Thus it calls for awakening since food dyes are consumed by human beings and dyes could incite deterring effects on their genome as well. Report of micronuclei in response of dyes has also been documented by Swaroop *et al.*(31).

Normal metaphase plate aligns chromatids at the cellular median but the deflection of chromatids from equator and their dispersion in the entire cell leads to scattering. It can be attributed to wandering action of spindle fibres, which disperse the chromatids all over the cell. Precocious movement of metaphase and forward movement of anaphase are an outcome of a synchronized chromosomal drifting. Precocious movement of metaphasic chromosomes can also be attributed to early terminalisation of chromosomes (37).

Occurrence and origin of micronuclei needs to be analysed sincerely because it is the outcome of "chromosomal abstraction". Its formation confirms that food dyes act as an instigator for chromosomal loss and henceforth should be used in least possible concentration. Plant assay proved to be a significant tool for anatomizing the amplitude of adversity the dyes have on biological entities. It is envisioned to have paramount effects on living beings as well because these dyes are components of food ingredients.

Conclusion

Cytological influences evidenced by retarded MI% and aberrant chromosomal entity are the authentic proof of the cytotoxicity of the food dyes. Since the percent decline in MI and increment in TAB% was higher in the case of Brilliant blue, it can be concluded that Brilliant blue is more chromotoxic as compared to Orange G. Food alterations with dyes surely stimulate the appetite but proper standardization and validation of these dyes is quintessential to mitigate the vulnerabilities associated with them. Present world needs to devise such revolutions which could be efficiently progressive yet sustainable for mankind.

References

1. Bessonov, V.V., A.D. Malinkin, O.I. Perederyaev, M.N. Bogachuk, S.V. Volovich and Y.V. Medvedev., Development of methods for determining acrylamide in food products by gasliquid chromatography, Voprosy Pitaniia, 2011, 80(4), 79-83.
2. NIS., Permitted Artificial Colors Persian. 4th Edition. Food and Drug Administration, Tehran,2002, pp-147.
3. Stintizing F.C., TrichterbornJ. and CarleR., Characterization of anthocyanin-betalain mixtures for food colouring by chromatic and HPLC-DAD-MS analyses, Food Chemistry,2006, 94, 296-309.
4. Vishweseran B. and KrishnamoorthyG., Oxidative stress by tartrazine in the testis of Wistar rats. IOSR J. Pharm. Biol. Sci., 2012, 2(3), 44-49.
5. Atri R., SinghA., MathurN. and VermaA., Utilization of Microbial Bioassays for Screening the Possible Toxicity in Regularly Used Food Dyes. J. Chem. Biol. Physical Sci.,2014, 4, 1248-1257.
6. Tsuda S., MurakamiM., MatsusakaN., KanoK., TaniguchiK. and SasakiY.F., DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol. Sci.,2001, 61, 92- 99.
7. Das A. and MukherjeeA., Genotoxicity testing of the food colours, amaranth and tartrazine, International Journal of Human Genetics, 2004, 4(4), 277-280.
8. Kaur M., AroraS. and KatnoriaJ.K., Estimation of mutagenic potential of tartrazine using Ames test, Biospectra,2012, 7, 123-126.
9. Zanoni T.B., Lizier T.M., AssisM.D.D., ZanoniM.V.B. and de Oliveira D.P., CYP-450 isoenzymes catalyze the generation of hazardous aromatic amines after reaction with the azo dye Sudan III, Food Chem. Toxicol., 2013, 57, 217-226.
10. Dixit A. and GoyalR.P., Evaluation of reproductive toxicity caused by indigo carmine on male Swiss albino mice, Pharmacology, 2013, 1, 218 – 224.
11. Gomes K.M.S., OliveiraM.V.G.A.D., CarvalhoF.R.D.S., Menezes C.C. and PeronA.P., Citotoxicity of food dyes sunset yellow (E-110), bordeaux red (E-123), and tatrazine yellow (E-102) on *Allium cepa* L. root meristematic cells. Food Sci. Technol.,2013, 33, 218-223.
12. Tripathi S.K., and RaoD. A.,Mitotic aberrations induced by orange red (a food additive dye) as a potential genotoxicant on root tip cells of onion (*Allium cepa* L.),Int. Food Res. J.,2015, 22(1), 383-392.
13. Murthy S.N., HiremathS.R.R. and Paranjothy K.L.K., Evaluation of carboxymethyl guar films for the formulation of transdermal therapeutic systems, International Journal of Pharmaceutics, 2004, 272, 11-18.
14. Shyale S., ChowdaryK., KrishnaiahY. and BhatN. K., Pharmacokinetic evaluation and studies on the clinical efficacy of guar gum-based oral drug delivery systems of albendazole and albendazole-β-cyclodextrin for colon targetting in human volunteers,Drug Development Research, 2006, 67, 154-165.
15. Abdelmigid H.M. and Abdelrahman H.M.H., Phyto-genotoxicity Assessment of Sewage Water for Agriculture Applications. Res. J. Pharm. Biol. Chem. Sci.,2013, 4, 511-529.
16. Bhattacharjee M., Evaluation of mitodepressive effect of Sunset Yellow using *Allium sativum* assay. Int. J. Sci. Environ.,2014, 3, 1120-1130.
17. Kumar G. and SrivastavaN.,Genotoxic effects of two commonly used food additives of boric acid and sunset yellow in root meristems of *Trigonella foenum-graecum*,Iranian J. Environ Health Sci. Eng,2011, 8(4), 361-366.
18. Dwivedi K. and KumarG., Genetic Damage Induced by a Food Coloring Dye (Sunset Yellow) on Meristematic Cells of *Brassica campestris* L., Journal of Environmental and Public Health, Volume 2015, Article ID 319727, 5 pages
19. Van't Hof J., The action of IAA and Kinetin on the mitotic cycle of proliferative and stationary phase excised root meristem, Experimental Cell Res.,1968, 51, 167-176.
20. Sudhakar R., Ninge GowdaK.N. and VenuG., Mitotic abnormalities induced by silk dyeing industry effluents in the cell of *Allium cepa*, Cytologia,2001, 66: 235-239.
21. Epel D., The effect of carbon mono oxide inhibition of ATP level and the date mitosis in Sea Urchin egg, J. Cell Biol.,1963,17, 315-319.
22. Jain A.K. and Sarbhoy R.K., Cytogenetical studies on the effect of some chlorinated pesticides. III. Concluding Remarks,Cytologia,1988, 53: 427-436.
23. Giri A.K., Food dyes in India: mutagenic and clastogenic potentials- A review, Proceedings of Indian National Science Academy B57,1991,183-198.

24. Park M., ParkH.R., KimS.J., KimM.S., KongK.H., KimH.S. et al., Risk assessment for the combinational effects of food color additives: neural progenitor cells and hippocampal neurogenesis, Journal of Toxicology and Environmental Health. Part A: Current Issues,2009, 72, 1412–1423.
25. Vijayaraj S., BagyalakshmiJ. and RaviT.K., Interaction Study of Certain Dyes like Ferric oxide Red, Brilliant Blue and Soy Food Stuffs with Statins and Its Influence on Protein Binding and Intrinsic Association Constant 'K' of Statins, Int. J. Pharma. Sci. Drug Res.,2010, 2(4), 302-304.
26. Ishidate T., SenooA.*et al.*, An electron microscopic study of the pancreas and parotid gland of rats with experimental acute pancreatitis, Tohoku J. Experimental Medicine,1974, 113(3), 213-23.
27. Hayashi M., M. Matsui, et al., Genotoxicity evaluation datasheet of food additives by the MHW (1980-1998), Environ. Mutagenesis Res.,2000 22: 27-44.
28. Hamdy A., MekkawyA.A., MassoudA. and El-zawahryM, Mutagenic effects of the food colour erythrosine in rats, Problems of Forensic Sciences,2000, 43, 184-191.
29. MpountoukasP., PantazakiA., KostareliE., Christodoulou P., KareliD., PoliliouS., MourelatosC., LambropoulouV. and LialiarisT., Cytogenetic evaluation and DNA interaction studies of the food colourants amaranth, erythrosine and tartrazine, Food and Chemical Toxicology,2010, 25, 37-43.
30. Yahagi T., DegawaM., SeinoY., MatsushinnaT., NagaoM. and HashimotoY., Mutagenecity of carcinogenic azo dyes and their derivatives, Cancer Letter,1988, 1, 19-96.
31. Swaroop V. R., RoyD. D. and Vijayakumar T., Genotoxicity of synthetic food colourants, J. Food Sci. Eng.,2011, 1, 128-134.
32. Gaulden M.E., Hypothesis: Some mutagens directly alter specific chromosomal proteins to produce chromosome stickiness, Mutagenesis,1987, 2, 357-365.
33. Klasterka I., NatarajanA.T.and RamelC., An interpretation of the origin of subchromatid aberrations and chromosome stickiness on a category of chromatid aberration, Hereditas,1976, 83, 153-162.
34. Krishna G. and Hayashi M., In vivo rodent micronucleus assay: protocol, conduct and data interpretation,Mutation Research,2000, 455, 155–166.
35. Stopper H. and MullerS.O., Micronuclei as a biological endpoint for genotoxicity: A minire view, Toxicology In Vitro,1997,11, 661–667.
36. Zaharopoulos P., WongJ. and WenJ.W., Nuclear protrusions in cells from cytologic specimens. Mechanisms of formation, Acta Cytologica,1998,42, 317–329.
37. Permjit K. and GroverI.S, 1985, Cytological effect of some organophosphorus pesticides II Meiotic effect, Cytologia, 55, 199-211.

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