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Cytotoxic and Mito depressive potential of Sunset Yellow FCF on *Chara vulgaris* (n=28)

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ABSTRACT

The food industry is one of the fastest growing economic sectors in the world, generating significant profits. Because the colour of our food visually arouses our desire, manufacturers add synthetic food colours to a variety of foods to make them more alluring. Synthetic food colours, being chemical in nature, are expected to be detrimental to humans. Children are more likely to be fascinated by foods with bright colour additives, putting them at high risk. Sunset Yellow FCF, a widely used yellow synthetic food colour, has been investigated for its cytotoxic and mito depressive potential in this study. Sunset yellow's cytotoxic effects were tested in *Chara vulgaris* (n=28) using mitotic index, nuclear, and chromosomal assays. The mitotic index decreased dramatically with increase in the concentration and time of treatment, indicating that Sunset yellow was mitodepressive in nature. A wide spectrum of nuclear and chromosomal abnormalities also have been discovered, with the incidence of abnormalities increasing as the Sunset Yellow concentrations and treatment period rose. These findings clearly indicate that the Sunset Yellow has cytotoxic and mito depressive potential. Utmost caution must be taken while using Sunset yellow as a food additive.

Key words : *Sunset Yellow FCF, Mitotic index (MI), Chromosomal aberrations, Mitodepression, Cytotoxicity*

Introduction

The colour of our food plays a significant role by visually stimulating our appetite.

The food industry is one of the fastest growing economic sectors in the world, generating high competitiveness among producers, who are in search of meeting new consumer demands. For that, they try to produce attractive food products. Colouring is mostly used in the food industry to enhance aesthetic appeal as consumers often value visual appearance, while judging taste of a food. There is a strong association of colour that go with certain food item (Burrows, 2009). The food colours are added to our food items in modern food industry to cater consumers especially the children.To make food items more attractive a number of natural and synthetic food colours are used. Being stable, cheap and easily available synthetic food colours have occupied a leading position in all food additives now –a- days. The most widely used artificial food colours are azo dyes, which are made from petroleum and coal tar, raising concerns about their potential for harm. Further the long-term effects of consuming these colours are unknown. Government has also imposed a ban on the use of some synthetic food colours owing to their detrimental impact. It has been reported that almost all azo dyes have carcinogenic potential and can induce genetic disorders by modifying DNA and may be fatal (Lau *et al.*, 2006; Chequer *et al.*, 2011). FSSAI (Food safety and standard authority of India) has set some rules

and regulations for the use of synthetic food colours in various food items. In India their are only eight synthetic food colours which are legalized to use and their ADI is also preset by FDA as well.

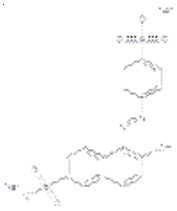
List of endorsed synthetic food colours in India

As per the Food Safety and Standard Act, 2006, the only eight synthetic food colours are endorsed for use in India.

In spite of different laws and regulations, permitted colours have raised concern since they are being used in food items in excess of their statutory limits. Sunset yellow is one of the widely used permitted food colour (Vladislavic, 2018). It is chiefly consumed in sweetmeats, beverages and fast foods (Rao *et al.*, 2008). Excessive amounts of many azo dyes including sunset yellow in food can provoke allergies, anxiety, migraines, asthma, diarrhoea, eczema, abdominal pain, headache, nausea, eye, and skin rashes and swelling as well as cancer (Senthilkumar *et al.*, 2013; Oliveira *et al.*, 2013; Ding *et al.*, 2019). Different scientists have studied the cytotoxicity and genotoxicity of many azo days, but Sunset yellow cytotoxicity is still a thrust area of research. So the present work is an attempt to find out the mitodepressive nature and cytotoxic potentiality of Sunset yellow FCF by choosing *Chara vulgaris* as test plant.

Materials and Methods

Sunset yellow is also known as Orange Yellow S, C.I. Food Yellow 3, FD and C Yellow 6). It is a disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. Its molecular formula is $C_{16}H_{10}N_2Na_2O_7S_2$. This dye is a synthetic azo dye, that is utilised in a variety of manufacturing processes like food, pharmaceutical, cosmetic, textile, and leather sectors (Elbanna *et al.*, 2017). The acceptable daily intake (ADI) of Sunset Yellow was fixed at 0–2 mg/kg b.w. by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2011). The European Food Safety Authority (EFSA) preset the ADI between 1 and 2.5 mg/kg b.w./day for Sunset yellow (EFSA, 2014).



For the current study Sunset yellow was procured from Roha dye chem., Mumbai. One gram of Sunset yellow was dissolved in 1000 ml of distilled water to get the stock solution. Different concentrations of dye like 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm were prepared by diluting the stock solution with distilled water. In the assessment of the cytotoxic and genotoxic impact of various chemical compounds, plant bioassays have been deemed a sensitive, less expensive and non-demanding test material (Giri, 1991; Iganci *et al.*, 2006). They are now recognised as a useful bio-indicator that can be easily linked to the outcomes of human testing systems. For the current study *Chara vulgaris* (n=28) was chosen as biological system. The plant body of *Chara vulgaris* is gametophyte and gametes (*n* chromosomes) are generated via mitotic division in the reproductive structures (Wood *et al.*, 1964). Mitosis occurs frequently in actively proliferating antheridial cells, making them the best material for cytological research in charophytes. For the study the plant was cautiously washed in tap water before being identified using Wood *et al.* (1964) monograph and iconograph. Antheridial filaments were separated for cytological study. The green tips with prominent reproductive structures of *C. vulgaris* were collected and treated with different concentrations of dye like 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm for 2 hours, 4 hours, and 6 hours. These were fixed in Carnoy's fluid (1:3 acetic acid: alcohol) for 24 hours before being transferred

List of endorsed synthetic food colours in India

Sr. No.	Colour	Synthetic food colour	Alternative name	Colour Index (C.I.) number	Chemical Class
1	Red	Ponceau 4R	Food Red -7	16255	Azo
		Carmoisine	Acid red 14	17420	Azo
		Erythrosine	Red No. 3	45430	Xanthene
2	Yellow	Tartrazine	Food Yellow 4	19140	Pyrazolone
		Sunset Yellow FCF	Food yellow 3	15985	Azo
3	Blue	Indigo Carmine	Blue No.2	73015	Indigoid
		Brilliant Blue FCF	Acid blue 9	42090	Triarylmethane
4	Green	Fast Green FCF	Food Green 3	42053	Triarylmethane

Molecular structure of Sunset yellow

to 70% alcohol for storage. The plants with fertile tips were immersed in double distilled water as a control. Iron –alum acetocarmine technique was used to prepare spermatogenous filament smearing (Godward, 1948). The prominent aberrations were photographed by using Olympus BX51 microscope.

Mitotic index was calculated by using with following formula -

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

A statistical analysis was also done to assess SD and SE.

Results and Discussion

Table 1 summarizes the cytological impact of different concentrations of Sunset yellow on mitotic index and chromosome behaviour in *Chara vulgaris*. The frequency of cellular division can be measured by taking mitotic index as a parameter (Marcano *et al.,* 2004). One of the major effect of the Sunset yellow was its mitodepressive influence on the rate of mitotic division. A concentration-dependent decrease tendency in mitotic index has been observed in all the concentrations of Sunset yellow with an increase in the exposure time.

An aqueous solution of 100 ppm and 250 ppm showed a stimulatory effect on mitotic activity of *Chara vulgaris* as compared to control. Although the mitotic index in these concentrations were more than control, but it was showing a decreasing trend. The rest of the concentrations 500 ppm, 750 ppm and 1000 ppm showed an inhibitory effect on mitotic activity. When the treatment of 1000 ppm was given for 6 hour,it showed maximum reduction in the value of mitotic index as compared to control .In the current study it is clearly indicated that exposure of *Chara vulgaris* to Sunset Yellow for short duration increased the % of mitotic index, whereas longer exposure treatments were mitodepressive in nature. The same pattern was studied by Bhattacharjee (2014), where Sunset yellow was proved to be mitodepressive in *Allium sativum*. Sarkar *et al.* (2013) also notice a dose dependent decrease in mitotic index in *Allium cepa* with an increase in concentration of a yellow food colour Kesar yellow. Tripathy *et al.* (2015) exhibits a rough trend of M.I. and M.D. (mitotic depression) with increasing concentration, when treated the *Allium cepa* root tips with different concentrations of Orange Red. The mito depressive effect of Sunset yellow was also observed in root meristems of *Foeniculum vulgare* and *Trigonella foenum-graecum (*Kumar *et al.*, 2011, 2018). According to Kus *et al.*(2015), Sunset Yellow FCF and Brilliant Blue FCF were able to modify mitotic index and produce micronucleus in human blood lymphocytes, resulting in genotoxicity and cytotoxicity. Hidalgo *et al.,* (1989) discovered that inhibiting cell cycle enzymes, such as DNA polymerase, which is required for DNA replication, may induce an antimitotic impact, similar to colchicine's method of action. The blocking of the G1 phase, and hence the inhibition of deoxyribonucleic acid (DNA) synthesis, was explained as the mechanism by which mitodepressive chemicals inhibit the cell cycle (El-Ghamery *et al.*, 2000). It's also been linked to a blockage of the cell cycle's G2 phase, which prevents cells from starting mitosis (Sudhakar *et al.,* 2001) and blockage of the synthesis of nucleoproteins (Mercykutly *et al.,* 1980). According to Christopher *et al.* (1988), the slowing of cell progression through mitosis could be attributable to the obstruction of the commencement of prophase, the arrest of one or more mitotic phases or the slowing of cell progression through mitosis. Mitotic index is mainly carried out to examine the quantify differences in cell division when an environmental parameter is changed (Darbelley *et al.,* 1989; Driss-Ecole *et al.,* 1994).

Beside a drastic decrease in the mitotic index, Sunset yellow also induced a variety of nuclear and chromosomal abnormalities that were dosage and exposure duration dependent. Our findings are in line with those of (Dwivedi *et al.*, 2015), who investigated the genotoxicity of Sunset yellow in *Brassica campestris* root meristematic cells and (Khan *et al.*, 2020) who investigated the genotoxicity of Carmiosine and Metanil yellow on *Allium cepa* root meristem. Only higher concentrations of Sunset yellow like 750 ppm and 1000 ppm of Sunset yellow were able to induce the binucleate cells and dumbbell shaped nuclei. The percentage of both the aberrations were increasing with the increasing concentration of the dye (Table 1). Binucleate cells were also noticed by Prajitha *et al.,* (2016) in *Allium cepa* root tips by Orange Red food dye. Two different mechanisms, cell to cell fusion and abnormal mitosis have been reported in the formation of binucleated cells. (Hu *et al.*, 2009; Hu and Ceresa, 2009; Nakanishi *et al.*, 2007; Zeng *et al.*, 2009). The examination of metaphasic chromosome is an significant and extensively used method to assess the

mutagenic capacity of a given agent (Nayak *et al.*, 1986). The most frequent type of chromosomal aberrations that were examined at metaphase were stickiness of chromosomes, chromosome scattering, grouping, chromosome condensation and chromatid separation. The stickiness of chromosome showed its minimum value at 100 ppm treatment, which reaches to its highest value at 1000 ppm (Table 1). Stickiness of chromosomes also has been observed with cytogenetic effect of some food dyes (Sarkar *et al.*, 2013; Khan *et al.*,2020). In a *in vitro* study done by Algarni (2021) stickiness of chromosomes was found to be most common type of chromosomal aberration induced by Sunset yellow in human lymphocyte culture. Chromosome stickiness indicates the highly toxic effects of a particular chemical (Türkoglu, 2007). Somashekhar (1987) suggested that sticky meta – anaphase cells resulted due to joining of chromosomal arms in majority of cells. Stickiness may leads to the cell death also (Kaur *et al.*, 2019). Free anaphase separation and chromosomal segment inversion have also been linked to sticky chromosomes (Gömürgen, 2005). All the concentrations of Sunset yellow were able to induce scattering (Fig. 1 A) except 100 ppm and showed a decreasing trend with increasing concentration. A permitted food dye Brilliant Blue FCF was also able to induce scattering in *Allium cepa* (Yadav *et al.*, 2004). In addition, the scattering of chromosome was also reported by Kumar *et al.*, (2015) in root meristem of Barley by some food preservatives . Grouping of chromosomes (Fig. 1 B) and chromosome condensation (Fig. 1C) were seen only in 500 ppm, 750ppm and 1000 ppm. of dye. Grouping of chromosome showed an increasing trend, while chromosome condensation showed a decreasing trend with an increase in concentration. Only higher concentration of Sunset yellow (750 ppm and 1000 ppm) were able to induce chromatid separation (Table 1).

At anaphase only two chromosomal aberrations ,disturbed polarity (Fig. 1 D) and laggards has been observed by higher concentrations of dye and both showed a dose dependent enhancement in the percentage. Multipolar anaphases was a dominating cytotoxic effect of food colour Kesar yellow on meristematic cells of root tips in *Allium cepa* (Sarkar *et al.*, 2013).

Chromosomal structural aberrations are regarded to be a good marker of genotoxicity. Sunset yellow was classified as a food colour having cytotoxic and genotoxic potential based on the present results. The current findings are in accordance with those of many other researchers who have demonstrated the cytotoxic and genotoxic characteristics of Sunset yellow using a variety of animal assays, including human and confirmed its threat to human health (Sayed *et al.*, 2012; Haveri *et al.*, 2018; Algarni,

Table 1. Assessment of cytotoxic and mitodepressive potential of Sunset Yellow FCF on *Chara vulgaris* (n=28)

Duration of treatment	Conc. (in ppm)	Mitotic Index %	SD +/-	SE	Nuclear Aberr. %		Metaphasic Aberr. %				Anaphasic Aberr. %			Total Aberr. %
					Bn	Dn	Cs	Sm	St'm'	Cc	Gm	Dp	Lag	
2h	Control	43.4	2.4	0.76	-	-	-	-	-	-	-	-	-	00
	100	45.1	1.3	0.42	-	-	-	-	-	-	-	-	-	00
	250	44.1	0.7	0.25	-	-	-	-	-	-	-	-	-	00
	500	42.1	2.0	0.63	-	-	-	-	2.8	-	-	-	-	2.8
	750	39.4	4.0	1.2	-	0.7	2.7	-	3.0	-	2.0	2.2	-	10.6
	1000	37.7	3.5	1.1	0.4	0.8	2.4	1.0	4.9	1.6	4.0	3.5	2.2	20.8
4h	Control	43.4	2.4	0.76	-	-	-	-	-	-	-	-	-	00
	100	44.5	1.0	0.34	-	-	-	-	-	-	-	-	-	00
	250	43.7	2.1	0.67	-	-	2.9	-	-	-	-	-	-	00
	500	40.9	1.4	0.46	-	-	1.1	-	2.9	-	-	-	-	2.9
	750	39.1	3.4	1.0	0.3	0.9		-	3.5	1.8	2.9	2.8	-	15.1
	1000	36.1	3.7	1.1	0.5	1.0	2.7	-	5.8	1.5	4.5	3.6	2.7	22.3
6h	Control	43.4	2.4	0.76	-	-	-	-	-	-	-	-	-	00
	100	44.2	1.7	0.56	-	-	-	-	1.0	-	-	-	-	1.0
	250	43.1	1.2	0.39	-	-	-	-	-	-	-	-	-	00
	500	40.0	2.4	0.76	-	-	1.0	1.1	3.5	2.2	1.1	1.3	1.3	10.5
	750	38.9	2.3	0.75	0.7	1.0	3.0	1.0	3.8	1.9	3.7	2.9	1.4	19.4
	1000	34.5	3.3	1.0	0.6	1.3	2.9	1.0	5.9	1.3	4.9	3.9	2.9	24.7

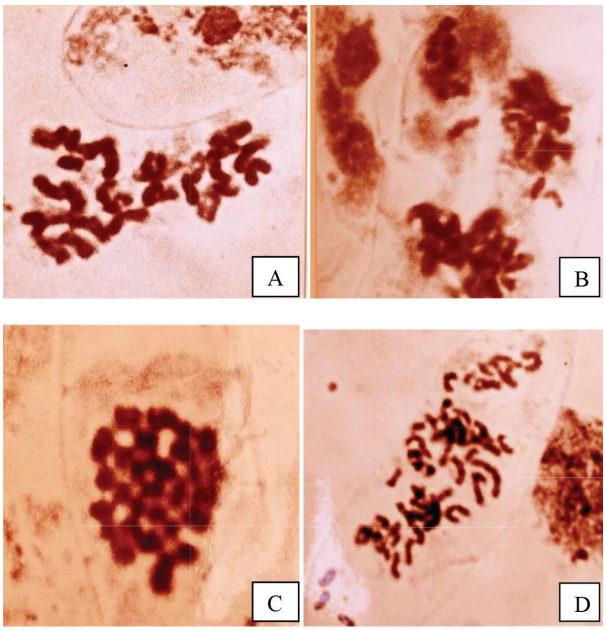


Fig. 1. A. Chromosome scattering (750 ppm, 6 hr.)
B. Grouping at metaphase (1000 ppm, 2 hr.)
C. Condensation of chromosome (1000 ppm, 4 hr.)
D. Disturbed Polarity (500 ppm, 6 hr.)

2021). The current evaluation should be regarded as introductory step to assess the toxicity of this dye. Further research is required to establish the genotoxicity and cytotoxicity of this commonly used food dye.

Conclusion

The dose-dependent cytotoxic and mito depressive potential of Sunset yellow has been determined in the current study. The above findings will help to create awareness among general public about the potential health risks of Sunset yellow, which is a frequently used synthetic food dye in various foods items including fast food, frozen foods, bakery items etc. The author strongly recommend a monitory check on excessive use of Sunset yellow in order to protect public health from serious adverse effects produced by this food colour.

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