ROLE OF BIOASSAYS FOR EVALUATION OF MUTAGENIC AND GENOTOXIC EFFECTS OF SYNTHETIC FOOD DYES

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ABSRACT

In recent years, synthetic food dyes are used to develop the aesthetic value of food items. The indiscriminate use of synthetically prepared food colours is perceived to pose serious health problems. Considering this, present work was embodied to evaluate the mutagenicity and genotoxicity of three most commonly used synthetic food dyes viz., blue, orange and pink employing Ames *Salmonella* incorporation assay and *Allium sativum* root chromosomal aberration assay (*Al*SRCAA), respectively. During Ames test in TA98 strain, pink and blue dyes did not induce any effect while orange food dye induced moderately mutagenic effect. In TA100 starin of *S. typhimurium,* all three dyes induced moderate to high mutagenic effect. During *Al*SRCAA, all the dyes have shown genotoxic effect by inducing physiological as well as clastogenic aberrations. The physiological aberrations included c-mitosis, abnormal anaphases, abnormal metaphases, delayed anaphases, vagrants and stickiness while clastogenic aberrations chromosomal included chromatin bridges, chromosomal breaks and chromatin rings. Among all the dyes, orange food dye has shown the maximum mutagenic and genotoxic effect in both bioassays.

Key words: food colors, physiological aberrations, clastogenic aberration, mutagenicity, Ames test, *Allium sativum* chromosomal aberration assay.

Food dyes are added to various food commodities to enhance their appearance (Kaur et al. ; 2012). Synthetic food dyes are extensively used in foodstuffs in order to improve their aesthetic value. Although the use of these colorants dates back to 5000 BC, their use in processed, packed and fun foods, has been increasing since 19th century (Kumar and Shrivastva ; 2013). Synthetic food dyes can be used in number of domestic food commodities and industrial preparations for many reasons, viz., to correct natural variations in food color, high tinctorial power and huge array of shades (Atri et al. ; 2014). However, use of these synthetic food dyes is one of the most controversial issues for the food industry from health point of view. Some food dyes have been suspected of being toxic and have various genotoxic effects (Kaur et al. ; 2012; Zanoni et al. ; 2013; Atri et al. ; 2014). Apart from this, these dyes can cause diseases like low blood pressure, reduced platelet aggregation, coughing and hyperactivity (Atri et al. ; 2014). The foremost reason for use of synthetic food dyes is that they are available as different blends. Blends can provide different shades to the food items which attract the consumers, especially, the children. Moreover, the blends (combination of dyes) show different effects like additives, synergistic, potentiating or even antagonistic as compared to the original dyes (Atri et al. ; 2014). There are several reports on the toxicity of various blends. Sharma et al. (2008) evaluated the toxicity of tomato red, a commonly used food dye blend. Divya and Devika (2012) reported the progressive effects of kesri

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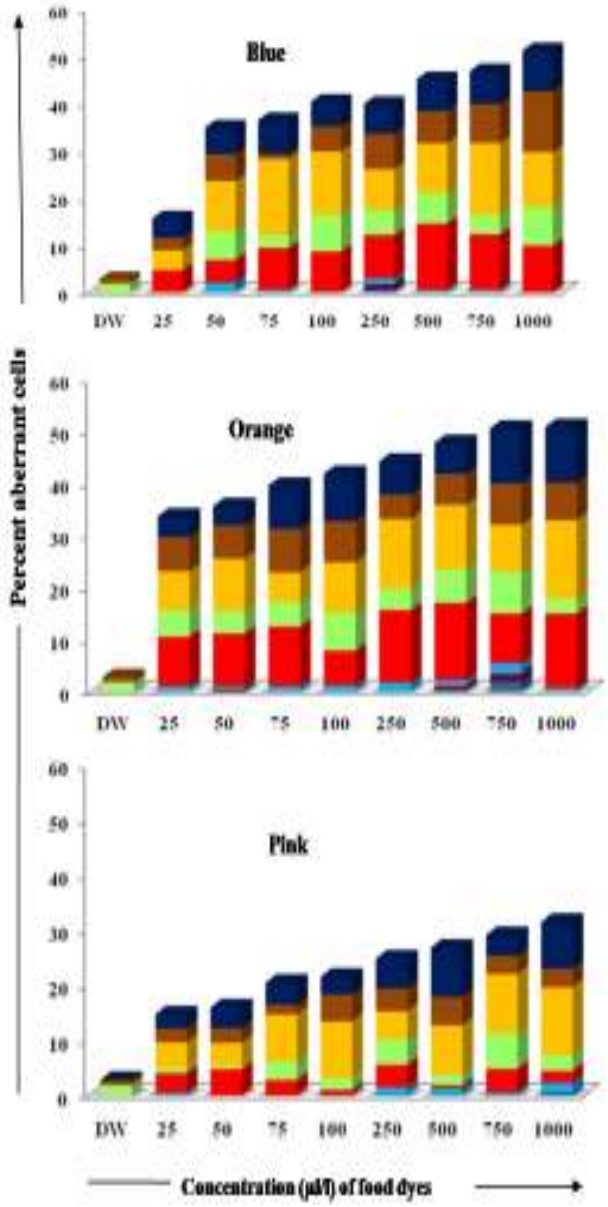
powder on different hematological and serological parameters in female Wistar rats. Considering this, present study was planned to evaluate the mutagenic and genotoxic effects of food dyes viz., blue, orange and pink using Ames test and *Allium sativum* root chromosomal aberration assy.

Fig. 1. Spectrum of different types of aberrations induced under the treatment of some of food dye solutions



MATERIAL AND METHODS

Chemical and Make

Various chemicals used for the present study were glacial acetic acid (Thomas & baker); ethanol (Changshu Yangyuen Chemical, China); orcein stain (Spectrochem); hydrochloric acid (Qualigens). All the chemicals used for Ames test were of Qualigen make.

Preparation of food dye solutions

Different concentrations (25, 50, 75, 100, 250, 500, 750, 1000 µl/l) of dye sample of blue, pink and orange were made with the double distilled water. These concentrations were used to estimate the genotoxic effects of the dyes**.**

Ames *Salmonella* incorporation assay

2 ml of soft agar was mixed with 0.1 ml culture of TA98/TA100 tester strains of *S. typhimurium* along with 0.1 ml of food dye extract for estimation of direct mutagenicity while to 2 ml soft agar, 0.1 ml culture of TA98/TA100, 0.1 ml of food dye extract and 0.5 ml of S9 mix were mixed for indirect mutagenicity. The prepared mixture was poured and spread on the minimal agar plates, kept in the B.O.D incubator at 37^{o} C for 48 h. For direct mutagenicity, 20 µg/0.1 ml culture of 4-nitro-o-phenylenediamine (NPD) and 2.5 µg/0.1 ml culture of sodium azide (SA) was used as positive control for TA98 and TA100 tester strains, respectively For estimation of indirect mutagenicity, 20 µg/0.1 ml culture of 2 amino fluorine (2AF) was used as positive control for both the strains. Distilled water was used as negative control during estimation of both direct and indirect mutagenicity. The steps for Ames test were followed as per the protocol given by Moran and Ames (1983).

Treatment	Concentration	Number of revertant colonies			
		TA98 (Mean ± S.E.)		TA100 (Mean ± S.E.)	
		wot S9	wt S9	wot S9	wt S9
Spontaneous	-	22.6±1.45	28.67±03.28	121.00±00.57	181.60±01.66
NC		25.67±5.21	23.67±2.96	121±3.61	162.3±17.57
NPD (µg/0.1 ml)	20	1350±50.48	-	-	-
SA (µg/0.1 ml)	2.5	-	-	2322±39.98	-
2AF (µg/0.1 ml)	20	-	1580±112.4	-	2660±112.40
Blue (µl/0.1 ml)	25	27.66±2.40	34.33±2.40	244.33±7.84	155.66±2.90
	50	32.66±3.28	35.33±4.26	269.33±4.63	170.33±1.76
	75	36.66±2.96	36.33±7.06	282.33±5.78	181.66±4.05
	100	35.33±3.84	47.66±5.70	287.66±3.28	197.66±2.40
	250	40.33±4.67	45.66±0.88	302.66±2.02	211.66±10.98
	500	43.66±4.33	49.66±0.66	305.66±7.69	221.33±8.46
	750	48.33±5.81	53.33±1.85	310.33±9.53	228.66±9.07
	1000	57.66±5.46	77.33±6.64	322.33±3.76	237.66±4.70
Orange (µl/0.1 ml)	25	17.33±2.02	23.66±1.76	216±2.31	157.66±3.18
	50	20.33±2.02	23.66±2.33	221.66±2.90	162.33±3.53
	75	30.66±2.02	22.33±1.76	236.33±2.90	172.0±3.60
	100	45±1.15	33.66±1.76	240v±4.04	181.0±4.62
	250	47.33±0.88	44±1.73	260±5.20	184.33±2.90
	500	61.33±1.45	57±2.08	247.66±5.54	193.66±1.76
	750	54±1.73	62±1.73	283±3.79	196.66±2.96
	1000	81.33±1.45	83.66±1.76	332.66±2.60	207.66±3.53
Pink (µl/0.1 ml)	25	20.33±4.05	16.66±2.85	164.66±2.18	124±4.04
	50	23.33±4.48	23.66±1.76	168.66±7.54	130±4.58
	75	26.33±4.48	24.33±3.18	178.66±6.99	174.33±5.24
	100	29.33±2.85	31±1.52	185.59±5.37	184.33±3.53
	250	30±4.04	34.66±0.88	193.60±4.81	196.33±5.93
	500	33.66±4.81	50±2.64	221.66±8.01	214.33±8.11
	750	34.33±5.70	40±5.78	231.66±10.42	229.66±7.54
	1000	35±4.51	64.66±2.60	240.66±6.07	239±8.33

Table 1: Mutagenic effect of different synthetic food dyes in Ames mutagenicity Assay

NC: Negative control; NPD: 4-nitro-o-phenylenediamine; SA: Sodium azide; 2AF: 2 Amino fluorine; wot: without; wt: with.

***Allium sativum* root chromosomal aberration assay**

The old roots and hard scales of the garlic heads were removed and cloves were suspended in test tubes containing distilled water. The test tubes containing denuded garlic cloves were placed in germinator at 25 ± 2^{o} C. After 24 - 36 h, garlic roots of approx. 0.5 – 1.0 cm were treated with different concentrations of synthetic food dyes. After 3 h treatment, the roots were washed under tap water and fixed in Farmer's fluid (glacial acetic acid: ethyl alcohol :: 3 : 1) for 24-48 h and then replaced by 70%

ethanol and stored at 7°C. The fixed root tips were hydrolyzed in 1 N HCl at 60° C for 8-10 min and transferred to a mixture of aceto-orcein and 1 N HCl in ratio of 9 :1 contained in a watch glass for half an hour. The watch glass was warmed at regular intervals of 5 min. Single root tip transferred to glass slide and squashed in a drop of 45 % glacial acetic acid. The slides were scored under microscope (Model: CH20i : Make: Olympus) for different type of chromosomal aberrations.

Table 2: Genotoxic effects of some of the food dyes in *Allium sativum* root chromosomal aberration assay .

Treatment	Conc. (µl/L)	TDC	TAC (PA+CA)	Physiological aberrations (PA)								Clastogenic aberrations (CA)			TAC
				Vg	Cm	St	Da	Aa	Am	TPA (%)	Cb	Ck	Cr	TCA (%)	TPA+TCA (%)
NC		210	6	-	-	4	-	2	-	2.85	-	-	-	-	2.85
Blue	25	190	30	-	9		8	5	8	15.78	-	-	-	-	15.78*
	50	245	86	5	12	15	26	14	14	35.10	-	-	-	-	35.10***
	75	180	66	1	16	5	29	2	13	36.66	-	-	-	-	36.66***
	100	207	84	-	18	16	28	11	11	40.57	-	-	-	-	40.57***
	250	209	88	-	17	14	20	22	15	42.10	-	-	-	-	42.10***
	500	205	93	1	29	13	22	14	14	45.36	-	-	-	-	45.36***
	750	172	69	2	16	9	15	13	11	38.37	1	2		1.74	40.11***
	1000	170	80	1	20	7	26	14	12	47.05	-	-	-	-	47.05***
Orange	25	198	68	2	19	10	15	13	8	34.34	-	1	-	0.50	34.84***
	50	187	66	1	19	8	19	12	7	38.50	-	-	-	-	42.07***
	75	171	76	3	24	7	23	8	11	46.19	-	-	-	-	46.19***
	100	191	76	2	22	9	11	16	16	39.26	-	-	-	-	39.26***
	250	164	69	2	11	12	16	13	15	42.07	-	-	-	-	42.07***
	500	201	99	4	30	13	25	12	12	47.26	1	1	1	1.49	48.75***
	750	199	85	4	19	16	18	16	21	39.20	4	2	1	3.51	42.71***
	1000	139	71	1	20	4	21	10	15	51.10	-	-	-	-	51.10***
Pink	25	195	29	1	7	2	9	5	5	14.87	-	-	-	-	14.87*
	50	187	30	-	9	1	8	5	7	16.04	-	-	-	-	16.04*
	75	176	36	-	5	6	15	3	7	20.45	-	-	-	-	20.45**
	100	185	40	-	2	4	19	9	6	21.62	-	-	-	-	21.62**
	250	195	47	3	8	9	10	8	9	24.10	-	-	-	-	24.10***
	500	185	54	1	8	12	20	6	7	29.18	-	-	-	-	29.18***
	750	156	42	2	1	3	14	8	14	26.92	-	-	-	-	26.92***
	1000	177	56	3	4	5	22	6	15	31.07	1	-	-	0.56	31.63***

TPA: Total physiological aberrations; Cb: Chromatin bridges; Ck: Chromosomal breaks; Cr: Chromatin ring; TCA: total clastogenic aberration; TDC: Total dividing cells; TAC: Total aberrant cells; Vg: Vagrants; Cm: c-mitosis; St: stickiness; Da: delayed anaphase; Aa: abnormal anaphase; Am: abnormal metaphase. *significant at p≤0.05, ** significant at p≤0.01, * significant at p≤0.001 as compared to control.**

RESULTS AND DISCUSSION

The result of the mutagenic response of three food dye viz., blue, orange and pink is presented in Table 1. The order of the induction of revertant colonies at the maximum doses i.e. 1000 µl/l for food dyes was observed as.

i. TA98 without S9 mix: orange (81.33) > blue (57.66) > pink (35.00).

ii. TA98 with S9 mix: orange (83.66) > blue (77.33) > pink (64.66).

iii. TA100 without S9 mix: orange (332.66) > blue (322.33) > pink (240.66).

iv. TA100 with S9 mix: pink (239) > blue (237.66) > orange (207.66).

The genotoxic effect of the blue, pink and orange food dyes is presented in the Table 2. Spectrum of physiological aberrations contained vagrant, c-mitosis, stickiness, delayed anaphase, abnormal anaphase and abnormal metaphase and clastogenic aberrations viz., chromatin bridge/s and chromosomal break/s (Fig. 1). In case of the root tip cells treated with synthetic food dyes, the incidence of physiological aberrations was found to be in the range of 14.87 – 51.10 % whereas clastogenic aberrations was 0.56 – 3.51 %. The order of the genotoxic effect of food dyes was orange > blue > pink. It was observed that delayed anaphases dominated among physiological aberrations whereas for orange food dye c-mitosis dominated. Among the clastogenic aberrations, frequency of chromosomal break was found to be the maximum for all the food dyes. Blue food dye have shown 47.05 % physiological aberrations and 1.74 % clastogenic aberrations, pink food dye induced 31.63 % of physiological aberrations 0.56 %) clastogenic aberrations and orange food dye induced 51 % of physiological aberrations and 3.51 % clastogenic aberrations at (1000 µl/l). The values of the chromosomal aberrations of the treated cells with the different concentrations of food dyes were significantly different at three values of $P<0.05$, $P<0.01$, $P<0.001$ as compared to control.

In the present study, all the food dyes have induced the mutagenic and genotoxic effects in both bioassays. During Ames test, the revertant colonies induced were observed to be in the range of 16.66 - 83.66 for TA98 and 124 - 332 for TA100 tester strain of *Salmonella typhimurium.* The mutagenic responses in TA100 tester strains of *Salmonella typhimurium* is an indicator of occurrence of base pair substitution type of mutations while that of TA98 for frame shift type of mutations (Atri et al. *;* 2014).

It was observed that the frequency of physiological aberrations were much higher than the clastogenic aberrations in root tip cells of *Allium sativum.* Among the physiological aberrations, c-mitosis dominated during treatment with orange dye whereas delayed anaphase dominated during the treatments with other food dyes. The results of the genotoxic effects of the synthetic food dyes are in agreement with earlier studies (Roychaoudhry and Giri ; 2003; Bhattacharjee ; 2014). There number of reports on the genotoxic effects of synthetic food dyes (Dixit and Goyal ; 2013; Gomes et al. ; 2013; Bhattacharjee, 2014). Roychaoudhry and Giri (2003) studied the effects of four food dyes like fast green FCF, indigo carmine, orange G, tartrazine and metanil yellow, employing chromosomal aberration assay in *Allium cepa*. They observed that all the 4 dyes were found to be genotoxic and induced the mitotic aberrations in the root tip cells of *Allium cepa*.

Gomes et al. (2013) assessed the genotoxic effects of the three food dyes viz., sunset yellow, bordeaux red and tartrazine yellow on the cellular cycle using *Allium cepa* test system. All the dyes were reported to be cytotoxic to the cells of the test system used. Likewise, Bhattacharjee (2014) evaluated the mitodepressive effects of sunset yellow FCF in root tips of *Allium sativum* root tip cells. The author observed that sunset yellow induced an inhibitory effect on cell division of *Allium sativum*. Dixit and Goyal (2013) estimated

the reproductive toxicity caused by indigo carmine on Swiss albino mice. Gomes et al. (2013) evaluated the cytotoxic effects of the food dyes viz., sunset yellow, bordeaux red, and tartrazine yellow on the cellular cycle of *Allium cepa* L.

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