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Evaluation of mutagenic potential of food dye (Apple green)

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Abstract

Food dyes are the vital constituents of food enhancing the aesthetic appeal of it by providing different colours. But synthetic dyes contained different heavy metals like lead, mercury, arsenic, copper, nickel, manganese, cobalt etc. which on the other hand are known mutagens/carcinogens. Considering the alarming mutagenic and carcinogenic potential of food dyes, the present study was planned to evaluate the mutagenic effect of apple green, the most widely used food dye, by confectioners in pista burfi, candies, cakes, ice creams, pasteries, jellies and cold drinks. The dye was purchased from local market and was of Ajanta make. This dye is a blend of sodium chloride, tartrazine and brilliant blue. Different concentrations of the dye ranging from 25 µg to 2500 µg/0.1 ml culture were prepared by using sterile double distilled water. The mutagenic effects of prepared extract were estimated employing Ames test using two tester strains TA98 and TA100 of *Salmonella typhimurium*. It was observed that the dye was moderately mutagenic at higher concentrations in *Salmonella* strain TA100 and non mutagenic in TA98.

Keywords: *Salmonella*, Ames test, mutagenicity, tatrazine, brilliant blue.

Introduction

Food dye is any substance that is added to food or drink to alter its colour, flavour and aesthetic value. Human beings have been using a variety of colour additives for a long time since 1500 BC and most of those colours were of natural origin. But since 1800 BC with advent of synthetic food dyes, many food industries started using synthetic food dyes without knowledge of their safety. Today, the synthetic food colours are being preferred to natural food colours due to their low cost and tinctorial power (Babu & Shenoliker, 1995). According to food adulteration act (FDA), eight synthetic colours namely-indigo, carmoisine, erythrosine, brilliant blue, tartrazine, fast green, sunset yellow and ponceau 4R were permitted to be added in various food items (Sharma & Goyal, 2005). These synthetic dyes have been widely encountered in a variety of eatables from both urban and rural markets (Khanna *et al.,* 1973). Generally, the permitted synthetic food colours are available in the form of blends of one or more dyes that are combined in numerous ways to produce a huge array of shades. Blends of two or more dyes can produce altogether different effects than observed with individual components. The effects may be additive, synergistic or even antagonistic (Singh *et al.,* 1988). Khanna and Das (1999) reported mentil yellow caused testicular damage in gametogenic element to arrest spermatogenesis in guinea pigs, rats and mice, whereas Tanaka (2006) reported reproductive and neurobehavioral toxicity of tartrazine in mice. Sharma *et al.* (2008) reported the toxicity of tomato red, a popular food dye blend on male Swiss albino rats. Haemotoxic effects of chocolate brown, a commonly used blend of permitted food colour on Swiss albino rats. As apple green is the most commonly used dye blend and various festival sweets of like pista burfi, coloured rasgulla, jellies, ice creams etc. Amritsar city, Punjab (India) the present study was planned to estimate the mutagenic potential of apple green by employing the standard protocol of Ames assay (Maron & Ames, 1983).

Materials and methods

Preparation of dye solution

Dye sample of apple green (in powdered form of Ajanta make) was purchased from the local market of Amritsar. This dye is a blend of sodium chloride, tartrazine and brilliant blue. The dye was dissolved in sterile double distilled water to prepare different concentrations (25, 50, 75, 100, 250, 500, 750, 1000 & 2500 µg/0.1 ml culture) which were used to estimate direct and indirect mutagenic effects of apple green.

Ames test

The Ames test was performed by following the method of Moran and Ames (1983) using two tester strains TA98 and TA100 of *Salmonella typhimurium*. To 2 ml of top agar, 0.1 ml culture (TA98/TA100) and 0.1 ml of dye solution was added for direct mutagenic potential while an additional 0.5 ml of S9 mix was added for indirect metabolic activation. Mix was spread on the minimal agar plates. After solidification, the petridishes were kept in the BOD incubator in inverted position at 37°C for 48 h. For direct mutagenicity, 20 µg of 4-nitro-o-phenylenediamine (NPD)/0.1 ml culture/plate was used as positive control for TA98 while 2.5 µg of sodium azide (SA)/0.1 ml culture/plate was used as positive control for TA100. For estimation of indirect mutagenicity, 20 µg of 2 amino fluorine (2AF)/0.1 ml culture/plate was used as positive control for both TA98 and TA100 strains. Distilled water was used as negative control. The experiment was set in triplicate and colonies were counted after 48 h of incubation.

Preparation of liver homogenate (S9)

Freshly excised livers were immediately placed in pre-weighed beakers containing approximately 1 ml of chilled 0.15 M KCl/g of wet liver. After weighing, livers were washed several times in fresh chilled KCl. Successive washings in KCl are essential to ensure a sterile preparation and to remove hemoglobin which can inhibit the activity of cytochrome P^{450} enzymes. The washed livers were transferred to sterile beakers containing chilled sterile 0.15 M KCl (3 ml/g wet liver) and were cut into small pieces with scissors and homogenized (Remi homogenizer). The homogenate was then centrifuged at 9,000 x g (8,700 rpm) for 10 min in a refrigerated C-24 Remi centrifuge. The supernatant (S9 fraction) was decanted and distributed in 2 ml cryotubes, frozen quickly in liquid nitrogen till required. The sterility of the preparation was determined by plating the S9 fraction

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(0.1 ml) on minimal agar plates containing histidine and biotin.

Table 1. Mutagenic potential of different concentrations of apple green food dye in Ames Salmonella mutagenicity test.

Conc. (µg/plate)	TA 98 (Mean ± S.E)		TA100 (Mean ± S.E)	
	without S9	with S9	without S9	with S9
Spontaneous	25.67±5.21	23.67±2.96	121±3.61	162.3±17.57
N.P.D	1350±50.48	-	-	-
Sodium azide	-	-	2322±39.98	-
2 AF	-	1580±112.4	-	2660±112.4
25	17.33±1.45	21.67±1.66	145.3±23.70	162.3±17.57
50	19.0±4.16	22.3±1.45	163.3±14.53	175.7±13.78
75	24.0±2.08	23.3±1.76	183.3±12.02	198.3±143.95
100	25.0±0.33	26.3±1.76	218.7±19.54	211.0±56.67
250	26.0±5.033	26.6±3.33	233.7±17.13	231.7±54.68
500	37.0±1.15	27.3±1.45	260.0±10.02	284.7±28.87
750	38.0±1.52	30.0±1.73	269.0±41.07	235.0±52.04
1000	40.0±1.15	34.0±0.57	307.0±9.074	253.3±24.58
2500	44.33± 2.33	55.67±2.33	339.0±17.64	319.7±49.06

Preparation of S9 mix

16.75 ml of Sterile distilled was taken in the autoclaved culture tube. To it, 25 ml of 0.2 M phosphate buffer (pH 7.4), 2 ml of 0.1 M Nicotine adenine dinucleotide phosphate (NADP), 0. 25 ml of 1 M Glucose-6-phosphate (G-6-P), 1 ml of $MgCl_2$-KCl salt solution and 5 ml of Rat liver S9 (phenobarbitone induced) were added. All the solutions (fresh and chilled) were always added in the order indicated so that S9 should be added to the buffered solution. S9 mix was maintained at 0-4^{0}C while performing the experiments. Any left over S9 mix was discarded. S9 was never refrozen.

Results

The results of mutagenic effects of apple green food dye in *in vitro* Ames *Salmonella* mutagenicity assay with and without metabolic activation are shown in Table 1. It was observed that the dye was moderately mutagenic in TA100 and non mutagenic in TA98. There was no significant increase in both strains on addition of S9 mix. However, except at concentration100 µg/plate in TA100 tester strain, the numbers of colonies were found to be increasing in dose dependent manner. The maximum number of revertants was observed at 2500 µg/plate in both the strains.

Discussion

The dye blend of apple green (Ajanta make) caused a dose dependent increase in the colonies (revertants) per plate and maximum number of colonies was obtained at 2500 µg/plate. Hence the excess intake of apple green food dye can cause various adverse effects. Many scientists have reported the adverse effects of various blends (Hansen *et al.,* 1963; Gerundo *et al.,* 1991; Fujitani*,* 1993; Mc Farlene *et al.,* 1997; Kaboglu & Aktac, 2002; Kanki *et al.,* 2003; Aktac *et al*., 2003). Metanil yellow has been shown to cause testicular damage in gametogenic elements to arrest spermatogenesis in guinea pigs, rats and mice (Khanna & Das, 1991). Moutinho *et al.* (2007) studied the mutagenic and carcinogenic effects of tartrazine (FD & C n^o 5) on the gastric mucosa of *Wistar* rats. The effects of fast green food dye on the blood of rats were studied by Ashour and Abdela (2009). In the present study, aqueous solutions of apple green was found to be moderately mutagenic in TA100 tester strain which indicated that the sample was responsible for base pair substitution type of mutations. Although the mutagenic potential was not significantly high, but it clearly indicates that continuous or prolonged exposure/consumption of apple green can pose a potential risk to human health.

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