Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes

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Abstract: The synthetic dyes over fifty are used in many areas including the food industry around the world. Sunset Yellow FCF and Brilliant Blue FCF are used as colorant food additives in many food products. The present study investigated the genotoxic and cytotoxic effects of Sunset Yellow and Brilliant Blue. Genotoxic and cytotoxic activities of the food additives were evaluated in lymphocyte cell cultures using mitotic index, replication index and micronucleus assay. Mitotic index frequencies and replication index values were decreased and micronucleus frequency was increased with increasing concentrations of Sunset Yellow and Brilliant Blue. The changes in mitotic index and micronucleus are statistically significant ($p<0.05$). The results show that the Sunset Yellow and Brilliant Blue can have cytotoxic and genotoxic potential. It care must be taken when using these materials as a food additive.

Keywords: Sunset yellow, brilliant blue, genotoxic, cytotoxic.

INTRODUCTION

The synthetic dyes over fifty are used in many areas including the food industry around the world. The use of synthetic dyes and types vary in each country and are determine by the regulations (Özgür and Koyuncu, 2002).

Sunset Yellow FCF, is an azo dye widely used all over the world, known by different names such as FD&C Yellow 6, Orange Yellow S and C.I. 15985 (Committee on Food Chemicals Codex, 2003). The E number of Sunset Yellow is E110 (Wood *et al*., 2004). The Sunset Yellow is used as a colorant food additive in many food products such as apricot jam, custard powders, citrus marmalade, orange sodas, sweets, energy drinks, squashes, margarine, marzipan, chips, packet soups, ice creams. It is usually used in chocolates and caramel to obtain the brown color in conjunction with amaranth dye. The EFSA acceptable daily intake (ADI) for sunset yellow is 1.0 mg/kg bodyweight per day (EFSA, 2009). It can induce an allergic reaction (Middleton *et al.*, 2003). Also, it can cause many health problems such as diarrhea, migraines, gastric upset, swelling of the skin, nettle rash and vomiting (Schultz-Ehrenburg and Gilde, 1987).

Brilliant Blue FCF, is a colorant food additive widely used all over the world, known by different names such as C.I. 42090 and FD&C Blue 1. The E number of Brilliant Blue is E133. The Brilliant Blue is made of aromatic hydrocarbons from petroleum (Mohammad and Bassam, 2005). It is a food additive in many foods such as canned peas, dairy products, drinks, packet soups, sweets, icings and ice cream. The EFSA acceptable daily intake (ADI) for Brilliant Blue is 6.0 mg/kg bodyweight per day (EFSA, 2010).

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In the present work, Sunset Yellow and Brilliant Blue were assessed for genotoxic effect (the micronucleus frequency) and cytotoxicity (mitotic and replication indexes) in the human peripheral lymphocyte cultures.

MATERIALS AND METHODS

Chemicals

Sunset Yellow (NarmaCol, India) (fig. 1A), Brilliant Blue (NarmaCol, India) (fig. 1B), peripheral blood karyotyping medium (Biological Industries, Israel), colchicine (Sigma, Germany), cytochalasin-B (Merck, Germany) and 5-bromo-2'-deoxyuridine (BrdU) (Sigma, Germany) were used in human lymphocyte cultures. The culture medium includes the phytohemagglutinin, L-glutamine, serum and antibiotic.

Human peripheral lymphocyte cultures

After getting approval from Yozgat Government Hospital, 0.4mL of the blood samples, collected from in good health ten persons, and 5mL of the culture medium were added the culture tubes. Later, 10, 20, 30 and 40 mg/mL of aquatic extracts of Sunset Yellow and Brilliant Blue were added the tubes. However, the extracts of Sunset Yellow and Brilliant Blue were not added to the tubes of control groups. The cultures were stored at 37℃ for 72 h for incubation. 5µg/mL of Cytochalasin B was added for micronucleus and 10µg/mL of BrdU was added for replication index. At 70h, 0.1mL of colchicine was mixed to each culture tube. At 72h, the lymphocyte cultures were harvested and stained according to Fenech and Morley (1985).

The proportions of metaphase of 1000 cells of ten person were evaluated for the calculation of mitotic index. Replication index was estimated by using RI = (1 x M1 +

2 x M2 + 3 x M3)/500. M values are the number of cells of the first (M1), second (M2) and third (M3) metaphases (Holland *et al*., 2002). The proportions of micronucleus of 500 cells were evaluated for the calculation of micronucleus.

STATISTICAL ANALYSIS

Statistical analyzes were performed using SPSS 10.0 program. The analysis of variance (ANOVA) were used for the statistical analysis of the micronucleus, mitotic index and replication index results of ten persons. The differences with the control group was tested by LSD test at $p<0.05$ of significance level.

RESULTS

The results of mitotic index are given in table 1. The mitotic index values of extracts of Sunset Yellow were between 1.56±0.63 and 1.03±0.49. Likewise the mitotic index values of extracts of Brilliant Blue were between 1.47±0.58 and 1.00±0.47. According to the mitotic index results, statistically significant reductions were found at 30 and 40mg/mL ($p<0.05$).

Replication index results are parallel to mitotic index results (fig. 2). Depending on the concentration increasing extract, replication index values decreased. The changes in replication index reflecting the genotoxic and cytotoxic effects not statistically significant ($p>0.05$). The replication index rates of control and Sunset Yellow extracts (10, 20, 30 and 40mg/mL) were 1.245, 1.245, 1.224, 1.198, and 1.186, respectively. The replication index rates of the control and Brilliant Blue extracts (10, 20, 30 and 40 mg/mL) were 1.247, 1.241, 1.229, 1.217, and 1.208, respectively. The least replication index values were 40mg/mL concentration for both the Sunset Yellow and Brilliant Blue. These results show that mitotic and replication indexes values of the Sunset Yellow and Brilliant Blue were lower than in controls. This demonstrates that many cells are alive the first cell division. These cells then enter the cell death such as necrosis or apoptosis.

Micronucleus rates are shown in figs. 3 and 4. When micronucleus formation was analyzed after treatment with the extracts of the Sunset Yellow, significant increases in the micronucleus rates were detected 20, 30 and 40 mg/mL of the concentrations ($p<0.05$). Especially, the micronucleus rates (1.12 and 1.18%) of the 30 and 40 mg/mL extracts of the Sunset Yellow were approximately two times higher than the control (0.60%). When compared with the untreated group, other concentration (10mg/mL) did not affect the rate of micronucleus ($p>0.05$). Also, when micronucleus formation was analysed after treatment with the extracts of the Brilliant Blue, significant increases in the micronucleus rates were detected 30 and 40 mg/mL of the concentrations ($p<0.05$). The micronucleus rates (1.22 and 1.30%) of the 30 and 40 mg/mL extracts of the Brilliant Blue were approximately two times higher than the control (0.68%). When compared with the untreated group, other concentrations (10 and 20 mg/mL) did not affect the rate of micronucleus ($p>0.05$).

DISCUSSION

The genotoxic and cytotoxic potentials of Sunset Yellow and Brilliant Blue were investigated in cultured human lymphocytes at this study. The lymphocyte culture is the best material for the determination of genotoxic and cytotoxic effects. Mitotic and replication indexes calculate to the proliferation status of a cell group. The number of cells in metaphase stage determines to the mitotic index ratio. The reduction in mitotic index refers to decrease in cell proliferation kinetics or increase in cell death (Rojas, 1993). The decreases of mitotic and replication indexes rates of the Sunset Yellow and Brilliant Blue could be resulted from controlled cell division. It indicates that reduction in mitotic index could be considered as controlled cell division in the cell proliferation kinetics (Öcal and Eroğlu, 2012). The decrease of mitotic index and replication index with age is possible by the combined effects of many factors including (i) the cumulative effects of the mutations in genes involving DNA repair, chromosome separation and cell division

Table 1: Mitotic index rates of sunset yellow and brilliant blue in human lymphocyte cultures

Food Coloring	Concentrations (mg/mL)	Total counted cells	Total number: dividing cells	Mean ±SDs (%)
Sunset Yellow	Control	10 000	175	1.75±0.70
	10	10 000	156	1.56±0.63
	20	10 000	140	1.40±0.70
	30	10 000	114	1.14±0.55*
	40	10 000	103	1.03±0.49*
Brilliant Blue	Control	10 000	168	1.68±0.64
	10	10 000	147	1.47±0.58
	20	10 000	134	1.34±0.53
	30	10 000	109	1.09±0.50*
	40	10 000	100	1.00±0.47*

* $p<0.05$ (significantly different from control), SDs: Standard Deviation

control points and (ii) the genotoxic and cytotoxic effects occurred by exposure to environmental or occupational genotoxic agents, irregular or inadequate nutrition and unhealthy lifestyle factors (Fenech and Bonassi, 2011). Walker defined that the rates of cell proliferation and mitotic index will decrease with advancing age (Walker, 1952).

The interactions between the genotoxic or cytotoxic agents and DNA can be resulted in the induction of micronuclei. The micronucleus assay is a valuable method to determine of potential carcinogen agents. Our results showed induce in the micronucleus rates (figs. 3 and 4), suggesting the interactions between DNA high concentrations of food colorings, that may result to determined genotoxicity and cytotoxicity. The micronucleus frequency arises during cell division either from chromosomes that are lagging in anaphase or from chromosome fragments (Fenech and Morley, 1985) with the effect of many factors including toxic agents, alcohol consumption, smoking and viral infections (Seitz *et al*., 1998). The blood samples taken from persons who non-smokers and do not consume alcohol. They have not been any viral infection and exposed to X-ray and gamma-ray recently.

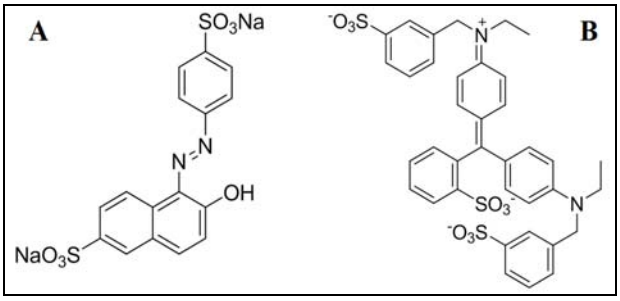

Fig. 1: Chemical structure of the colorant food additives. A- Sunset Yellow, B- Brilliant Blue

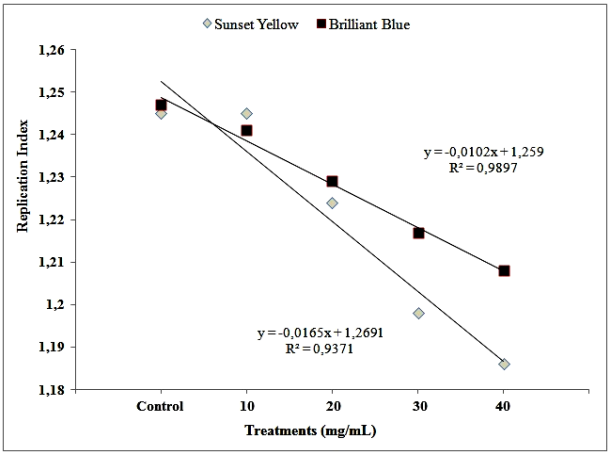

Fig. 2: Replication index changes in the lymphocytes, according to the control and treatment with different concentrations of the Sunset Yellow and Brilliant Blue.

CONCLUSIONS

As summary, the Sunset Yellow and Brilliant Blue, colorant food additives, induced micronucleus frequency and decreased mitotic and replication indexes rates in human peripheral lymphocyte cultures. It can be concluded that the Sunset Yellow and Brilliant Blue show considerable clastogenic, cytotoxic and genotoxic effects as observed *in vitro* lymphocyte cultures. The results show that the Sunset Yellow and Brilliant Blue can have cytotoxic and genotoxic potential. It care must be taken when using these materials as a food additive.

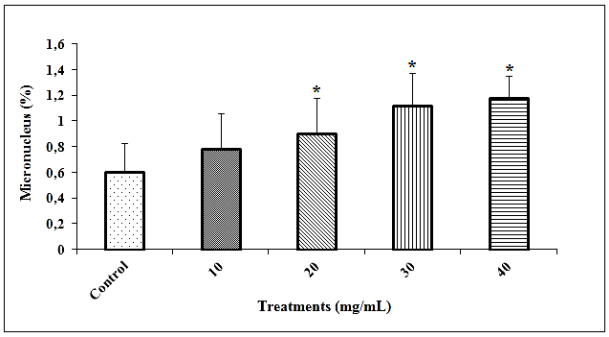

Fig. 3: Percentage change in lymphocyte micronuclei, according to the control and treatment with different concentrations of the Sunset Yellow.

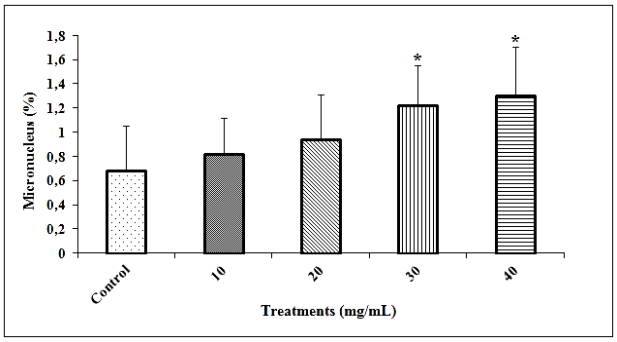

Fig. 4: Percentage change in lymphocyte micronuclei, according to the control and treatment with different concentrations of the Brilliant Blue.

ACKNOWLEDGEMENTS

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