Original Research Article

In Vitro Effects of Sunset Yellow on Chromosomal Damage and Sister Chromatid Exchange in Human Peripheral Lymphocytes

ABSTRACT

Sunset yellow is an organic azo dye that is used extensively as a colouring agent in many industries, such as cosmetics, pharmaceuticals and foodstuffs. Many studies have conflicting results about the genotoxicity effect of Sunset Yellow. Thus, the purpose of this study was to provide additional data concerning Sunset Yellow genotoxicity in human lymphocytes by using chromosomal aberrations and sister chromatid exchanges assay. Four concentrations of Sunset Yellow (1, 5, 20 and 50 mg/ml) were used on human lymphocyte cultures. Positive and negative controls were mitomycin C and distilled water, respectively. Compared to the control, Sunset Yellow caused an significant increase in chromosome aberrations and sister chromatid exchanges frequencies at all concentrations. A total of five types of chromosome aberrations were observed, such as gaps, fragments, RCF, stickiness and polyploidy. According to the present results, high concentrations of sunset Yellow has been found to be genotoxic in vitro to cultured human lymphocytes. Sunset Yellow should be studied in other test systems to determine its full genotoxicity potential.

Keywords: DNA Damage; Cytogenetic parameters; Food dye; Genotoxicity effects.

1. INTRODUCTION

Sunset yellow (SY) is a yellow-orange colorant which belongs to synthetic azo compounds [1], and is commonly used in cosmetics, drugs and in the food industry due to its low manufacturing cost, color uniformity and stability to light, oxygen, and pH [2]. Many food products such as energy drinks, orange sodas, chips, sweets and ice cream use sunset yellow as a colorant [3]. According to Joint FAO/WHO Expert Committee, the Acceptable Daily Intake (ADI) of SY for humans is 1.0 mg kg-1 bodyweight per day [4]. Toxicological data have shown that SY produced health issues such as diarrhea, migraines, intestinal upset, skin swelling and angioedema as well as vomiting in some cases [5]. A high concentration of sunset yellow in humans can lead to a wide range of conditions, including infertility, thyroid cancer, eczema, lupus, and hyperactivity [6]. Regarding blood composition, Osman et al. [7] reported that SY inhibits cholinesterase and erythrocytes in the human blood. In addition, SY caused histopathological and physiological

changes in the liver and kidney of male rats [8]. Dietary exposure to SY has also been shown to cause histopathological changes in rats' testes and brains [9-10]. Regarding genotoxic effect of SY, Sasaki et al. [11] reported no abnormalities in the stomach cells of mice after oral administration of high doses of SY. In addition, at doses up to 2000 mg kg-1 bodyweight, SY did not cause genotoxicity in mice using micronucleus gut assay [12]. in contrast, a number of studies have reportedly suggested that azo dyes, including SY, have mutagenic as well as cytotoxic and genotoxic effects [13-17]. Because of many conflicting observations regarding the genotoxic effects of SY, additional research is needed to assess the possible adverse effects of SY. Hence, this work aimed to provide additional genotoxicity data on the effect of SY in the induction of chromosomal aberrations (CAs) and sister-chromatid exchanges (SCE) in cultured human lymphocytes.

2. MATERIALS AND METHODS

2.1 Chemicals

Sunset yellow, as well as all chemicals, were obtained from Sigma-Aldrich (Germany).

2.2 Lymphocyte cultures

Two healthy male volunteers provided peripheral blood (both non-smokers, 24-26 years old); neither subject had exposure to any known mutagenic agents or drug therapies in the previous 24 months and had not had an x-ray for at least six months. They had no recent viral infections or a history of chromosome fragility. A local ethics committee approved this study, and it was conducted according to the 1964 Helsinki Declaration on ethical standards.

2.3 Chromosomal aberrations and sister-chromatid exchange assay

Whole-blood Heparinized sample (0.2 mL) was added to 2.5 ml Chromosome Medium B containing 10 ug/ml bromodeoxyuridine. After 72 hours of incubation in the dark, cells were treated with SY (dissolved in distilled water) at concentrations of 1, 5, 20 and 50 ug/ml for 24 hours. For each experiment, a negative control (distilled water) and a positive control (0.20 mM mitomycin-C) were used. During the last two hours of incubation, colchicine (0.06 ug/mL added to the culture) was used to arrest the cell cycle at metaphase. Then the culture was centrifuged (2000 rpm for 5 min) and treated with KCl (75 mM) for 30 minutes at 37 ◦C and fixed with methanol:glacial acetic acid (3:1). Finally, in order to prepare the metaphase spreads, the concentrated cell suspension was dropped onto slides. Slides of CAs were stained for 15–20 minutes using 5 percent Giemsa stain prepared in Sorensen buffer (pH = 6.8). For the SCE assay, slides were stained by fluorescence plus Giemsa (FPG) according to Wojcik et al. [18] Once the slides had dried, a total of 100 well-distributed metaphases per donor (200 metaphases per

concentration) were examined for the CA assays and A 50 second mitosis was scored for each of the experimental concentrations in the SCE assay.

2.4 Statistical Analysis

Using Student's t-test, the results were reported as mean ± standard error (Mean ± SE). P- values < 0.05 indicate significant differences in the data.

3. RESULTS

Two parameters were used to assess the genotoxic potential of SY in cultured lymphocytes, CA and SCE. Table 1 presents CAs induced in human lymphocyte culture after treatment with different concentrations of SY. Comparing the negative control, the number of CA increased significantly at all SY concentrations. The percentages of CA were 10 ± 0.02, 18 ± 0.04, 28 ± 0.12 and 40 ± 0.07% at SY doses of 1, 5, 20 and 50 mg/ml, respectively. The positive control significantly increased the frequency of CAs (67%± 0.06 in mitomycin C group versus 2 ± 0.01 in the control group, $P < 0.01$). The potency of SY for CAs induction was less than that of the positive control. A total of five types of CAs were observed, such as gaps, fragments, RCF, stickiness and polyploidy. Stickiness (40.6%) and Fragments (21.9%) were the most common types of chromosomal abnormalities (Figure 1). Table 2 presents the results of the SCE test. The frequency of SCE increased significantly in all SY concentrations in comparison to the control. The SCEs mean values were 4.3 ± 0.2, 8.22 ± 0.4, 10.3 ± 1.1 and 14.4 ± 1.2 at SY doses of 1, 5, 20 and 50 mg/ml, respectively (Figure 2). The effect of SY on the SCE induction was less than that observed in the positive control. Mitomycin C increased the frequency of SCEs significantly (22.5± 0.2 in positive control group versus 2.4± 0.22 in the control group, $P < 0.01$).

Table 1. Percentage of mitotic index (MI) and Chromosomal aberrations induced in cultured human lymphocytes treated with different doses of SY.

Dose (mg/ml)	Chromosomal aberration (CAs)					Total CA	CA%±SE
	Gap	Fragment	RCF	Stickiness	Polyploidy		
1	4	8	0	8	0	20	10±0.02*
5	8	6	4	16	2	36	18±0.04**
20	14	12	8	18	4	56	28±0.12**
50	12	16	8	36	8	80	40±0.07**
N.C	0	0	0	4	0	4	2 ± 0.01
P.C	22	24	8	36	44	134	67±0.06**
Total abnormalities	19.8	21.9	10.4	40.6	7.3		

(%)

Totally 200 cells were scored for each treatments
NC= Negative Control.
PC= Positive Control.
RFC: Robertsonian Centric Fusion (RCF).
* Significant from the control P<0.05 (t test).
** Significant from the control P<0.01 (t test).

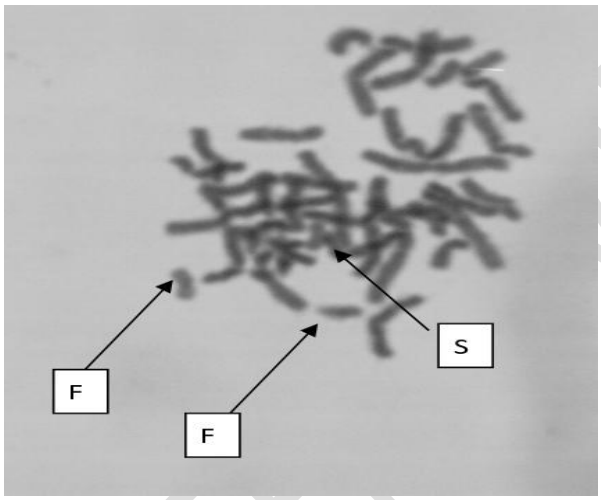

Figure 1. Different type of aberrations induced by SY in human peripheral lymphocytes. (S) sticky chromosomes; (F) chromosomal fragments.

Table 2. Frequency of sister chromatid exchanges (SCEs) in cultured human lymphocytes exposed to SY.

Dose (mg/ml)	No. of metaphases	mean ± S.E	Min-max SCE
1	100	4.3 + 0.2 *	0-6
5	100	8.2 + 0.4**	2- 10
20	100	10.3 ± 1.1 **	2-12
50	100	14.4 + 1.2**	6-12
NC	100	2.4 + 0.22	0-4
PC	100	22.5 + 0.2**	18-28

NC= Negative Control.
PC= Positive Control.
* Significant from the control P<0.05 (t test).
** Significant from the control P<0.01 (t test).

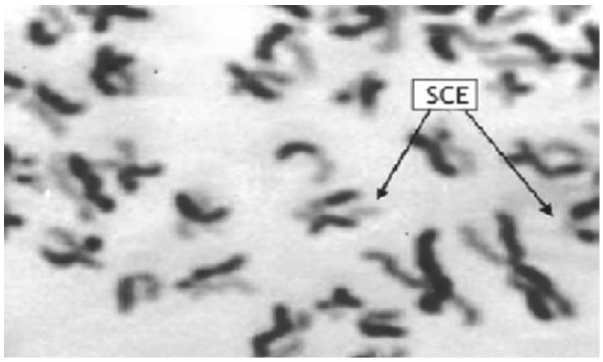

Figure 2. sister chromatid exchange (SCE) in human peripheral lymphocytes after treated with of SY.

4. DISCUSSION

Sunset yellow is organic azo dye which used in food products, cosmetics, and drug. The main purpose of the present study was to evaluate the genotoxicity of SY using tow cytogenetic parameters (CAs and SCE) which are considered to be the most reliable tests for detecting the possible genotoxic effects of chemicals [19-20]. Based on the findings of the current study, SY at all concentrations increased the frequency of CAs significantly. Chromosomal aberrations can be used as effective biomarkers for the early detection of genetic abnormalities and predict cancer risk [21]. Changes in chromosomal structure can cause CAs when the chromosomal material is broken or exchanged [22]. Several types of CAs were induced by SY, including polyploidy, chromatic gap, fragment, FCR and stickiness. Stickiness (40.6%) were the most common types of chromosomal abnormalities. The presence of chromosome stickiness relates to highly toxic effects of a particular chemical [23]. These data confirmed the previously reported in vitro and in vivo clastogenic effects of SY. According to Ali et al. [24], a mixture of SY and sodium benzoate led to structural abnormalities in rat chromosomes such as fragmentation, ring chromosome, centric fusion breakage, and chromatid breakage. In mice, SY has been reported to have genotoxic effects based on the increased frequency of CA in bone marrow and germ cells [3]. Sister chromatid exchange (SCE) is considered a highly reliable marker of human exposure to carcinogens and mutagens [25]. According to this study, SY significantly increased the frequencies of SCE induction in human lymphocytes compared to control. In fact, SCEs occur especially when both sister-chromatids of a replicating chromosome are involved in an equal interchange of DNA segments in the "S-phase" of the cell division process [26]. Our results are in positive agreement with Sayed et al. [3]. who reported an increased frequency of SCE in somatic and germ cells, indicating the genotoxic potential of SY. Azo dyes like SY are synthetic aromatic compounds with a functional azo group (-N = N-), which is the main reason for their coloring

property.[27] Several studies have shown that most azo dyes and their products are carcinogenic or mutagenic and can cause DNA damage [28]. Among this azo dyes quinoline yellow [29], Tartrazine [30], and Disperse Red 1 [31].

5. CONCLUSION

There are a number of concerns regarding the of commonly used food dyes. Based on our findings, SY can cause DNA damage and chromosomal alterations in human lymphocytes in vitro. Food additives, including SY, should be continuously observed and reevaluated whenever necessary, keeping in mind changing conditions of use and new scientific findings. Further genotoxicity studies of SY are needed, especially under in vivo conditions.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Pandir D. DNA damage in human germ cell exposed to the some food additives in vitro. Cytotechnology. 2016;68(4):725-33.
doi: 10.1007/s10616-014-9824-y.

2. Llamas NE, Garrido M, Di Nezio MS, Band BS. Second order advantage in the determination of amaranth, sunset yellow FCF and tartrazine by UV–vis and multivariate curve resolution-alternating least squares. Anal Chim Acta. 2009;655(1-2):38-42.
dol: 10.1016/j.aca.2009.10.001.

3. Sayed HM, Fouad D, Ataya FS, Hassan NH, Fahmy MA. The modifying effect of selenium and vitamins A, C, and E on the genotoxicity induced by sunset yellow in male mice. Mutat Res Genet Toxicol Environ Mutagen. 2012;744(2):145-53.
dol: 10.1016/j.mrgentox.2012.02.003.

4. Kus E, Eroglu HE. Genotoxic and cytotoxic effects of Sunset Yellow and Brilliant Blue, colorant food additives, on human blood lymphocytes. Pak J Pharm Sci. 2015; 28(1):227-30.

5. Ghoneim MM, El-Desoky HS, Zidan NM. Electro-Fenton oxidation of Sunset Yellow FCF azo-dye in aqueous solutions. Desalination. 2011;274(1-3):22-30.

dol: 10.1016/j.desal.2011.01.062.

6. Caliman FA, Apostol L, Bulgariu D, Bulgariu L, Gavrilescu M. Sorption of Acid Yellow 23 from aqueous solutions onto soil. Afinidad. 2009;66(544).

7. Osman MY, Sharaf IA, Osman HM, El-Khouly ZA, Ahmed EI. Synthetic organic food colouring agents and their degraded products: effects on human and rat cholinesterases. *Br J Biomed Sci*, 2004;61(3):128-32.

dol: 10.1080/09674845.2004.11732657.

8. Khayyat LI, Essawy AE, Sorour JM, Soffar A. Sunset Yellow and Allura Red modulate Bcl2 and COX2 expression levels and confer oxidative stress-mediated renal and hepatic toxicity in male rats. *PeerJ*, 2018;6: e5689.

dol: 10.7717/peerj.5689.

9. Mathur N, Chowdhary V, Mehta M, Krishnatrey R. Effect of sunset yellow on testis in rats. J Ecophysiol Occup Health, 2005;5(1):1-3.

10. Khiralla G, Salem S, El-Malky W. Effect of natural and synthetic food coloring agents on the balance of some hormones in rats. Int J Food Sci Nutr Eng. 2015;5(2): 88-95.

dol: 10.5923/j.food.20150502.03.

11. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res Genet Toxicol Environ Mutagen. 2002;519(1-2):103-19.

dol:10.1016/s1383-5718(02)00128-6.

12. Poul M, Jarry G, Elhkim MO, Poul JM. Lack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice. Food Chem Toxicol. 2009; 47(2): 443-8.

dol: 10.1016/j.fct.2008.11.034.

13. Biswas SJ, Khuda-Bukhsh AR. Cytotoxic and genotoxic effects of the azo-dye p-dimethylaminoazobenzene in mice: a time-course study. Mutat Res Genet Toxicol Environ Mutagen. 2005;587:1-8.

dol: 10.1016/j.mrgentox.2005.06.011.

14. Chung KT, Chen SC, Claxton LD. Review of the Salmonella typhimurium mutagenicity of benzidine, benzidine analogues, and benzidine-based dyes. Mutat Res Rev Mutat Res. 2006;612(1):58-76.

dol: 10.1016/j.mrrev.2005.08.001.

15. Tsuboy MS, Angeli JP, Mantovani MS, Knasmüller S, Umbuzeiro GA, Ribeiro LR. Genotoxic, mutagenic and cytotoxic effects of the commercial dye CI Disperse Blue 291 in the human hepatic cell line HepG2. Toxicol in Vitro. 2007;21(8): 1650-5.
dol: 10.1016/j.tiv.2007.06.020.
16. Oliveira GA, Ferraz ER, Chequer FM, Grando MD, Angeli JP, Tsuboy MS, Marcarini JC, Mantovani MS, Osugi ME, Lizier TM, Zanoni MV. Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mutat Res Genet Toxicol Environ Mutagen. 2010;703(2): 200-8.
dol: 10.1016/j.mrgentox.2010.09.001.
17. Koç K, Pandir D 2018. All aspect of toxic effect of brilliant blue and sunset yellow in Allium cepa roots. *Cytotechnology*, 70(1): 449-63.
dol: 10.1007/s10616-017-0161-9.
18. Wojcik A, Bruckmann E, Obe G. Insights into the mechanisms of sister chromatid exchange formation. Cytogenet Genome Res. 2004;104(1-4):304-9.
dol: 10.1159/000077507.
19. Mpountoukas P, Vantarakis A, Sivridis E, Lialiaris T. Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. Food Chem Toxicol. 2008;46(7):2390-3.
dol: 10.1016/j.fct.2008.03.021.
20. Zengin N, Yüzbaşıoğlu D, Ünal FA, Yılmaz S, Aksoy H. The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food Chem Toxicol. 2011;49(4):763-9.
dol: 10.1016/j.fct.2010.11.040.
21. Rossner P, Boffetta P, Ceppi M, Bonassi S, Smerhovsky Z, Landa K, Juzova D, Šrám RJ. Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environ Health Perspect. 2005;113(5):517-20.
dol: 10.1289/ehp.6925.
22. Kumari M, Mukherjee A, Chandrasekaran N. Genotoxicity of silver nanoparticles in Allium cepa. Sci Total Environ. 2009;407(19):5243-6.
dol: 10.1016/j.scitotenv.2009.06.024.
23. Türkoğlu Ş. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res Genet Toxicol Environ Mutagen. 2007;626(1-2):4-14.
dol: 10.1016/j.mrgentox.2006.07.006.

24. Ali MY, Hassan GM, Hassan AM, Mohamed ZA, Ramadan MF. In vivo genotoxicity assessment of sunset yellow and sodium benzoate in female rats. Drug Chem Toxicol. 2020;43(5):504-13.
dol: 10.1080/01480545.2018.1510416.
25. Norppa H, Bonassi S, Hansteen IL, Hagmar L, Strömberg U, Rössner P, Boffetta P, Lindholm C, Gundy S, Lazutka J, Cebulska-Wasilewska A. Chromosomal aberrations and SCEs as biomarkers of cancer risk. Mutat Res Fundam Mol Mech Mutagen. 2006;600(1-2):37-45.
dol: 10.1016/j.mrfmmm.2006.05.030.
26. Johnson MD, Schilz J, Djordjevic MV, Rice JR, Shields PG. Evaluation of in vitro assays for assessing the toxicity of cigarette smoke and smokeless tobacco. Cancer Epidemiol Biomark Prev. 2009;18(12):3263-304.
dol: 10.1158/1055-9965.EPI-09-0965.
27. Barragán BE, Costa C, Marquez MC. Biodegradation of azo dyes by bacteria inoculated on solid media. Dyes Pigm. 2007;75(1):73-81.
dol: 10.1016/j.dyepig.2006.05.014.
28. Chequer FM, Lizier TM, de Felício R, Zanoni MV, Debonsi HM, Lopes NP, Marcos R, de Oliveira DP. Analyses of the genotoxic and mutagenic potential of the products formed after the biotransformation of the azo dye Disperse Red 1. Toxicol in Vitro. 2011;25(8):2054-63.
dol: 10.1016/j.tiv.2011.05.033.
29. Chequer FM, de Paula Venâncio V, de Souza Prado MR, Lizier TM, Zanoni MV, Burbano RR, Bianchi ML, Antunes LM. The cosmetic dye quinoline yellow causes DNA damage in vitro. Mutat Res Genet Toxicol Environ Mutagen. 2015;777:54-61.
dol: 10.1016/j.mrgentox.2014.11.003.
30. Khayyat L, Essawy A, Sorour J, Soffar A. Tartrazine induces structural and functional aberrations and genotoxic effects in vivo. PeerJ. 2018;5:e3041.
dol: 10.7717/peerj.3041.
31. Fernandes FH, Botasso-Nasciutti MO, Svio AL, Souza LD, Fernandes-Cal JR, Cardoso FF, Fontes MR, Albuquerque AF, Munari CC, Kummrow F, Umbuzeiro GD. In Vivo genotoxicity of a commercial CI Disperse Red 1 dye. Environ Mol Mutagen. 2018;59(9):822-8.
dol: 10.1002/em.22226.