Original Research Article

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

ABSTRACT

Sunset Yellow (SY) is an organic azo dye that is used extensively as a coloring agent in many industries, such as cosmetics, pharmaceuticals ,and foodstuffs. Many studies have conflicting results about the genotoxicity effect of SY. Thus, the purpose of this study was to provide additional data concerning SY genotoxicity in human lymphocytes by using chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) 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, SY caused a significant increase in CAs and SCEs frequencies at all concentrations. A total of five types of CAs were observed, such as gaps, fragments, RCF, stickiness ,and polyploidy. According to the present results, high concentrations of SY are genotoxic *in vitro* to cultured human lymphocytes. To determine its full genotoxicity potential, SY should be tested in other test systems.

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

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1. INTRODUCTION

Sunset Yellow (SY) is a yellow-orange colorant that 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 SY as a colorant [3]. According to the Joint FAO/WHO Expert Committee, the Acceptable Daily Intake (ADI) of SY for humans is 1.0 mg kg-1 body weight 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 SY 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 the 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 body weight, SY did not cause genotoxicity in mice using micronucleus gut assay [12]. In contrast, several 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 CAs and SCEs 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. Lymphocyte cultures were carried out according to Mamur et al. [18].

2.3 Chromosomal aberrations and sister-chromatid exchange assay

A 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 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 SCEs assay, slides were stained by fluorescence plus Giemsa (FPG) according to Wojcik et al. [19] Once the

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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 SCEs assay.

2.4 Statistical Analysis

The statistical analysis was performed using the procedure of Analysis of Student's t-test with Microsoft Excel version 2010. Data were expressed 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, CAs and SCEs. Table 1 presents CAs induced in human lymphocyte culture after treatment with different concentrations of SY. Comparing the negative control, the number of CAs increased significantly at all SY concentrations. The percentages of CAs 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 SCEs test. The frequency of SCEs 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 SCEs induction was less than that observed in the positive control. Mitomycin C increased the frequency of SCEs significantly (22.5± 0.2) in the positive control group versus 2.4± 0.22 in the control group, P < 0.01).

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

Dose (mg/ml)	Chromosomal aberration (CAs)					Total CAs	CAs%±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**

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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 treatment.
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).

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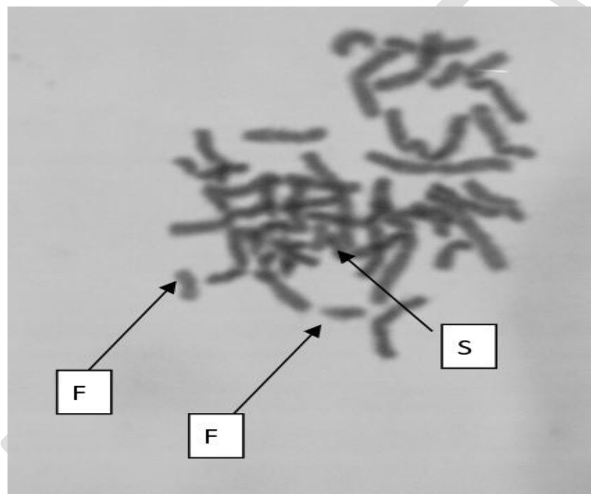

Figure 1. Different types of aberrations induced by SY in human peripheral lymphocytes. (S) sticky chromosomes; (F) chromosomal fragments.

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 SCEs
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).

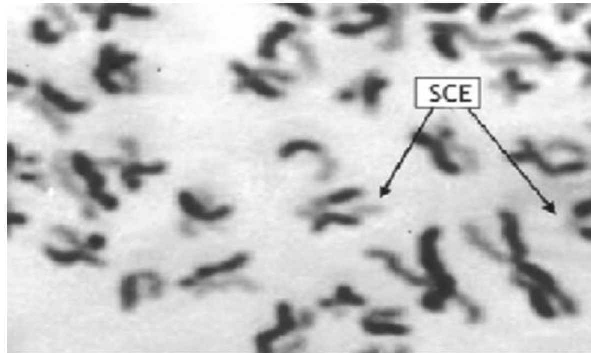

Figure 2. sister chromatid exchanges (SCEs) in human peripheral lymphocytes after treatment with SY.

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4. DISCUSSION

Sunset Yellow is an organic azo dye used in food products, cosmetics, and drugs. The main purpose of the present study was to evaluate the genotoxicity of SY using two cytogenetic parameters CAs and SCEs which are considered to be the most reliable tests for detecting the possible genotoxic effects of chemicals [20-21]. Based on the findings of the current study, SY at all concentrations increased the frequency of CAs significantly. The CAs can be used as effective biomarkers for the early detection of genetic abnormalities and predict cancer risk [22]. Changes in chromosomal structure can cause CAs when the chromosomal material is broken or exchanged [23]. Several types of CAs were induced by SY, including polyploidy, chromatic gap, fragment, FCR and stickiness. Stickiness (40.6%) were the most common type of chromosomal abnormalities. The presence of chromosome stickiness relates to highly toxic effects of a particular chemical [24]. These data confirmed the previously reported *in vitro* and *in vivo* clastogenic effects of SY. According to Ali et al. [25], 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 CAs in bone marrow and germ cells [3]. The SCEs are considered a highly reliable marker of human exposure to carcinogens and mutagens [26]. According to this study, SY significantly

increased the frequencies of SCEs 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 [27]. Our results are in positive agreement with Sayed et al. [3]. who reported an increased frequency of SCEs 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.[28] Several studies have shown that most azo dyes and their products are carcinogenic or mutagenic and can cause DNA damage [29]. Among these azo dyes are quinoline yellow [30], Tartrazine [31], and Disperse Red 1 [32].

5. CONCLUSION

There are several concerns regarding the 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 studies should be conducted in other test systems to evaluate the overall genotoxic potential of SY and to determine the mechanisms of action of this dye.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used 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.

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