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Research Note

Toxic effects of chronic feeding with food azo dyes on *Drosophila melanogaster* Oregon R

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Abstract. Artificial azo dyes are widely used as coloring agents for foodstuffs, drugs, and cosmetics. In this study, the toxic effects of four different synthetic food dyes (Ponceau 4R, Sunset Yellow, Amaranth, Tartrazine) on 72 ± 4h larvae of Oregon (R) wild type of *Drosophila melanogaster* were investigated. In addition, depending on chronic feeding, the life span of the flies was determined. For this purpose, six different concentrations of food dyes (5, 10, 15, 20, 25, and 30 mg mL^{-1}) were selected by preliminary studies to determine LD_{100} values. The effects of the food dyes on longevity were studied separately in female and male populations. It was determined that the maximum mean life span of the female and male *D. melanogaster* populations decreased with increasing concentrations of food dyes. Based on the results obtained from the larval mortality and life span experiments, the order of toxicity for food dyes was: Tartrazine > Amaranth > Sunset Yellow ≥ Ponceau 4R.

1. Introduction

Parallel to the growing world population, people's supply and demand are increasingly. Nowadays, it is very easy for people to have access to food, thanks to the food additives in daily shopping [1]. Color is one of the important features evaluated by food users. A wide range of food dyes are used to make food products more aesthetically appealing to consumers and restore their original appearance lost during the manufacturing process [2]. Today's food dyes are grouped into two main groups: natural and synthetic [3]. Synthetic food dyes are comprised of azo, triphenylmethane, fluorescein, and sulphonated indigo dyes. Azo dyes, as one of these subgroups, consist of a diazotized amine group containing one or more azo bonds (R-N=N-R) bonded with an amine or a phenol. These dyes are widely used in textile, food, plastic, paper, cosmetics, and pharmaceutical industries [4]; also, they constitute about 65% of the commercial paint market [5].

It is very important to investigate the adverse effects of synthetic products used so much on human beings, especially on the living things. There are many studies related to this issue in the literature. Studies conducted with various azo dyes have found that some metabolites of these dyes may produce genotoxic effects in humans and animals [6,7]. In addition, a histopathological study with rats has shown that these dyes cause toxic effects on the liver and kidney tissues [8]. Chung [9] emphasized that many azo dyes, their reduced products, and their associated aromatic amines are carcinogenic and allergenic. Epidemiological and toxicological studies on experimental animals, such as mice, rats and bacteria, however, show that azo

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stains may be genotoxic and carcinogenic under certain conditions [10-13].

Ponceau 4R, Sunset Yellow, Amaranth, and Tartrazine azo dyes are used most commonly. In this study, the effects of these four different azo dyes on larval mortality and longevity in *Drosophila melanogaster* were investigated at different concentrations.

2. Materials and methods

The flies used in the experiments were Oregon R wild type (w.t.) strain of *Drosophila melanogaster* Meigen (Diptera; Drosophilidae). This stock has been maintained for many years in the Genetics Laboratory at the Department of Biology of the Atatürk University in Erzurum and is, therefore, highly inbred with little genetic variation.

2.1. Experiments of larval mortality

Ten pairs of adult *D. melanogaster* (10 ♀♀ × ♂♂) were placed into culture bottles. Eggs were collected during 4 h periods in culture bottles containing standard medium. After 72 h of treatment, the larvae were washed and selected. Four food dyes were prepared at 5, 10, 15, 20, 25, and 30 mg mL^{-1} separately. For chronic feeding, small culture vials were prepared with 1.5 g dry instant *Drosophila* medium (Carolina Biological Supply Company Burlington, NC) and 5 mL of the respective test solutions. A hundred larvae were placed on this medium. The larvae were fed with different concentrations of the food azo dyes. Feeding ended with pupation of the surviving larvae. The developmental stages were followed daily. The experiments were repeated three times.

2.2. Experiments of longevity

Longevity experiments were conducted separately on female and male populations. First of all, it is ensured that individuals are of the same age and have not matched in terms of food dyes of the single variable parameter. For this purpose, 100 individuals from the pups developed from the larvae grown in SDM (Standart *Drosophila* Medium) were immediately collected and separated as male-female. The individuals obtained here were transferred to culture bottles containing Amaranth, Ponceau 4R, Sunset Yellow, and Tartrazine food stains at different concentrations for applications after being fasted for 2 hours in empty culture flasks. Individuals fed acutely for 2 hours were transferred to new bottles containing SDM, 25 females and 25 males, and then observations started. All applications were carried out in thermal cabinets with suitable temperature, humidity, and pH. Transfers were made to the freshly-grown culture bottles two days a week until the last individual, and the death rates were recorded. All applications were repeated 3 times, and the results were compared statistically with Duncan's one-way ANOVA. Differences between the control group and the treatment groups were assessed at $P < 0.01$.

3. Results

All food dyes used in our study, in a concentration-depended manner, increased the larval mortality and decreased the number of adult individuals in all the application groups (Table 1 (a)-(d)). However, the values of surviving and matured individuals, 96 ± 1.15 per the control group on larvae, which were fed only with SDM were determined in the lowest and highest concentrations (5-30 mg mL^{-1}) for Ponceau 4R $35 \pm$ 2.88-86 ± 1.15 (Table 1(a)), for Sunset Yellow 32 ± 1.15-82 ± 1.15 (Table 1(b)), and for Amaranth 18 ± 3.46-80 ± 2.88 (Table 1(c)), respectively. Only the first three application groups (5, 10, and 15 mg mL^{-1}) in Tartrazine, development of larvae, and adult individuals were observed. The rate of survival was identified as 52 ± 2.31 in the lower application group (5 mg mL^{-1} as this value 10 ± 1.15 in 15 mg mL^{-1} application group) (Table 1(d)). Based on (Table 1(a-d)), the results for the food dyes show a positive correlation between increasing dose and toxic effects. There was a statistically significant difference between the control and experimental groups at 0.01 level. Considering the occupancy rates of the larvae survived, the sort of food dyes toxicity was: Tartrazine > Amaranth > Sunset Yellow > Ponceau 4R.

In the second part of our experiment, we studied the effect of food dyes on the longevity of mature individuals. Analyses were separately performed on male and female populations. In our study, we found that the maximum life span of the control group was 98 days for females and 91 days for males. In addition, these values of the female application groups were respectively as follows: 53-87 days for Ponceau 4R (Table 2(a)), 46-74 days for Sunset Yellow (Table 2(b)), and 39-74 days for Amaranth (Table 2(c)) in the lowest and highest concentrations (5-30 mg mL^{-1}). It was found that the maximum life span of male application groups was 42-74 days for Ponceau 4R (Table 2(a)), 42-77 days for Sunset Yellow (Table (2b)), and 35-67 days for Amaranth (Table 2(c)).

It was determined that the mean life span was 66.42 ± 1.89 days for ♀♀ and 62.04 ± 1.65 days for ♂♂ in the control group. In the female application groups, these values belonging to the lowest and highest concentrations (5-30 mg mL^{-1}) were 58.43 ± 1.95-28 ± 1.48 days for Ponceau 4R (Table 2(a)), 51.69 ± 1.79-24.01 ± 1.41 days for Sunset Yellow (Table 2(b)), and 50.41 ± 1.97-22.50 ± 1.17 days for Amaranth (Table 2(c)). Moreover, it was observed that the mean life span was 42.76 ± 1.91-21.66 ± 1.06 days for Ponceau 4R (Table 2(a)), 46.91 ± 1.93-19.12 ± 1.27

Table 1. Rate of mortality of larvae fed with different concentrations of food dyes.

Concentrations (mg mL^{-1})	$\sum$ Larva	Application groups 1 2 3	Mean ± S.E	p	Concentrations (mg mL^{-1})	$\sum$ Larva	Application groups 1 2 3	Mean ± S.E	p
(a) Ponceau 4R, LD_{100} : 35 mg mL^{-1}					**(b) Sunset Yellow, LD_{100} : 35 mg mL^{-1}**				
Control	100	96 94 98	96 ± 1.15		Control	100	96 94 98	96 ± 1.15	
5	100	88 86 84	86 ± 1.15		5	100	84 82 80	82 ± 1.15	
10	100	80 78 76	78 ± 1.15		10	100	80 75 70	75 ± 2.88	
15	100	72 68 64	68 ± 2.31	<0.01*	15	100	63 61 59	61 ± 1.15	<0.01*
20	100	60 57 54	57 ± 1.73	<0.01*	20	100	58 54 50	54 ± 2.31	<0.01*
25	100	58 50 42	50 ± 4.61	<0.01*	25	100	47 43 39	43 ± 2.31	<0.01*
30	100	40 35 30	35 ± 2.88	<0.01*	30	100	34 32 30	32 ± 1.15	<0.01*
(c) Amaranth, LD_{100} : 30 mg mL^{-1}					**(d) Tartrazine, LD_{100} : 15 mg mL^{-1}**				
		1 2 3	Mean± S.E	p			1 2 3	Mean± S.E	p
Control	100	96 94 98	96 ± 1.15		Control	100	96 94 98	96 ± 1.15	
5	100	85 80 75	80 ± 2.88		5	100	48 52 56	52 ± 2.31	<0.01*
10	100	74 76 72	74 ± 1.15		10	100	30 35 40	35 ± 2.88	<0.01*
15	100	54 62 58	58 ± 2.31	<0.01*	15	100	8 10 12	10 ± 1.15	<0.01*
20	100	42 52 47	47 ± 2.88	<0.01*	20	100	– – –	–	
25	100	26 30 28	28 ± 1.15	<0.01*	25	100	– – –	–	
30	100	18 24 12	18 ± 3.46	<0.01*	30	100	– – –	–	

*The mean difference is significant at 0.01 level.

days for Sunset Yellow (Table 2(b)), and 45.20 ± 1.81-15.14 ± 0.96 days for Amaranth (Table 2(c)) in the male applications groups.

As a result of Tartrazine, maximum mean life span belonging to the lowest and highest concentrations (2.5-15 mg mL^{-1}) was 39-70 days in the female application groups (Table 2(d)) and 39-74 days in the male application groups (Table 2(d)). Additionally, it was determined that mean life span for ♀♀ is 47.39 ± 1.84-17.40 ± 1.01 days and for ♂♂ is 46.20 ± 1.87-15.97 ± 1.05 days.

4. Discussion

In our study, the internal and external factors that could affect longevity (maternal age, food type, photoperiod, etc.) were kept constant. Therefore, we can state that the deviations in the longevity of the same genotype and the same-age flies are caused by the effects of different food dyes.

The genotoxic effects of Tartrazine, which was one of the food dyes, on *Drosophila* were examined using the Somatic Mutation and Recombination Test (SMART), and it was observed that it stimulated recombinogencity [14]. In another study carried out by Tripathy et al. [15], the effects of Rhodamine and Amaranth on *D. melanogaster* were examined, showing that Rhodamine is genotoxic for both the somatic and reproduction cells, although Amaranth has no such effect. In a study carried out by Macioszek and Kononowicz [16], it has been stated that Black PN and Quinoline Yellow are mutagenic and clastogenic for both lymphocyte cells of humans and *Vicia faba* stem meristem cells. Also, in another study conducted by Sasaki et al. [17], the genotoxicity of food dyes, such as Amaranth, was investigated at this time, and it was observed that DNA damage occurred on the colon, bladder, stomach, and gastrointestinal organs even at the lowest dose. Mpountoukas et al. [18] investigated the potential genotoxic, cytotoxic and cytostatic in human peripheral blood cells for Amaranth *in vitro*. According to the results, it was identified that food dyes have a toxic effect on human lymphocyte cells, increase the Sister Chromatid Exchange (SCE) 1.7 times as to control group, and show effect of binding on DNA directly. Similar results were found in a study that was carried out by Shimada et al. [19]. DNA damage was done in rat colon after applying 10 mg kg^{-1} of azo food dyes. It appeared that the DNA damage was induced on pregnant female and male rats fed with Amaranth orally [20].

In another study performed by Stevenson et al. [21] to identify the hyperactivity causes in children, based on the dyes, it was found that food dyes in-

Table 2. The longevity of male and female populations of *D. melanogaster* and the probability levels between groups.

Experiment groups	N	Female population				Male population			
		Max.life span	Mean life span ± SE	Std. dev.	p	Max. life span	Mean life span ± SE	Std. dev.	p
(a) Ponceau 4R									
Control	100	98	66.42 ± 1.89	18.98		91	62.04 ± 1.65	16.46	
5 mg mL^{-1}	100	87	58.43 ± 1.95	19.52		74	42.76 ± 1.91	19.11	3-4*
10 mg mL^{-1}	100	74	50.43 ± 1.87	18.68	3-4*	67	35.91 ± 1.71	17.05	3-5*
15 mg mL^{-1}	100	67	44.09 ± 1.77	17.77	4-5*	63	33.48 ± 1.52	15.22	4-5*
20 mg mL^{-1}	100	67	40 ± 1.83	18.33	6-7*	60	30.26 ± 1.48	14.76	4-6*
25 mg mL^{-1}	100	60	33 ± 1.72	17.22		56	28.66 ± 1.35	13.51	5-6*
30 mg mL^{-1}	100	53	28 ± 1.48	14.79		42	21.66 ± 1.06	10.55	
(b) Sunset Yellow									
Control	100	98	66.42 ±1.89	18.98		91	62.04 ± 1.65	16.45	
5 mg mL^{-1}	100	74	51.69 ± 1.79	17.91		77	46.91 ± 1.93	19.31	
10 mg mL^{-1}	100	74	45.27 ± 1.99	19.90	4-5*	74	38.23 ± 1.68	16.80	4-5*
15 mg mL^{-1}	100	60	38.07 ± 1.75	17.49	5-6*	63	31.02 ± 1.78	17.83	4-6*
20 mg mL^{-1}	100	60	33.61 ± 1.69	16.91	6-7*	56	25.86 ± 1.43	14.32	5-6*
25 mg mL^{-1}	100	53	29.48 ± 1.48	14.75		56	26.08 ± 1.49	14.90	
30 mg mL^{-1}	100	46	24.01 ± 1.41	14.06		42	19.12 ± 1.27	12.72	
(c) Amaranth									
Control	100	98	66.42 ± 1.89	18.98		91	62.04±1.65	16.45	
5 mg mL^{-1}	100	74	50.41 ± 1.97	19.67		67	45.20 ± 1.81	18.09	
10 mg mL^{-1}	100	67	43.10 ± 1.72	17.24	3-4*	56	35.62 ± 1.61	16.07	
15 mg mL^{-1}	100	60	39.51 ± 1.55	15.47	4-5*	53	27.68 ± 1.46	14.61	4-5*
20 mg mL^{-1}	100	60	34.92 ± 1.59	15.94	5-6*	46	23.82 ± 1.31	13.11	5-6*
25 mg mL^{-1}	100	53	30.69 ± 1.39	13.98		46	21.09 ± 1.27	12.68	
30 mg mL^{-1}	100	39	22.50 ± 1.17	11.65		35	15.14 ± 0.96	9.61	
(d) Tartrazine									
Control	100	98	66.42 ± 1.89	18.98		91	62.04 ± 1.65	16.45	
2.5 mg mL^{-1}	100	70	47.39 ± 1.84	18.40		74	46.20 ± 1.87	18.72	
5 mg mL^{-1}	100	63	42.24 ± 1.64	16.37	2-3*	63	41.08 ± 1.75	17.49	2-3*
7.5 mg mL^{-1}	100	60	39.28 ± 1.59	15.93	3-4*	63	38.52 ± 1.73	17.32	3-4*
10 mg mL^{-1}	100	53	32.48 ± 1.42	14.16		56	33.55 ± 1.76	17.60	4-5*
12.5 mg mL^{-1}	100	46	24.02 ± 1.13	11.33		42	23.06 ± 1.16	11.55	
15 mg mL^{-1}	100	39	17.40 ± 1.01	10.08		39	15.97 ± 1.05	10.51	

N: Total number of individuals; Max.: Maximum; SE: Standard error;

p: Probability levels between groups; *: The mean difference is not significant at 0.01 level.

hibit the histamine-N-methyltransferase enzyme, and so the histamine that passed to brain and its increased amount increased the hyperactivity. In another study, the amounts of GSH (gamma glutamyl-cyteineyl-glycine) and MDA (malondialdehit) produced by lipid peroxidation, antioxidant enzyme activity (CAt, GPx), and oxidative stress parameters were identified in the CHO (Chinese Hamster Ovary) cells into which the Tartrazine and New Coccin were applied. To discover the toxic dose of food dyes, the Calcein AM cell viability testing technique was used, and it was identified that tartrazine and new coccin decrease cell viability. It was observed that GSHs in-cell level was greatly reduced; on the contrary, the level of MDA significantly increased in CHO cells that were exposed to Tartrazine [22].

In the literature search that we carried out, no publication was found regarding the effects of food azo

dyes on longevity. Therefore, we cannot compare the results of our longevity experiments with any results of literature reviews directly. However, we can state that food azo dyes have mutagenic and clastogenic effects (chromozomal aberrations) on various living beings, and that it can cause a longevity decline. Since usage of high-dose food azo dye can cause serious health threats to humans, we believe that both manufacturing companies and consumers should be informed about food additives.

5. Conclusion

In this study, the effects of four different azo dyes used in food industry on the mortality and longevity of the Oregon R wild larvae of *D. melanogaster* were examined. To this end, results of the different concentrations of the selected food dyes were compared with those of the control group. As a result of our study, a decrease in the number of maturing larvae was observed in comparison with the control group. In addition, it was determined that high doses of food dyes shorten the longevity of *D. melanogaster*. Based on the results obtained by the larval mortality and life span experiments, the order of toxicity for food dyes was Tartrazine $>$ Amaranth $>$ $SunsetYellow \geq$ Ponceau 4R.

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