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Effect of Developmental Toxicity in *Drosophila melanogaster* on Exposure to Different Food Dyes (Brilliant Blue and Sunset Yellow)

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Abstract: ***Drosophila melanogaster are model organisms commonly used to understand biological phenomena due to their small size, short lifespan and short generation time. Drosophila flies are used as model organisms for examining fundamentally important problems in biology, especially developmental biology. Food additives are substances that are added to food to modify its visual appearance, taste, texture, processing or shelf life. The increased exposure of these food dyes may lead to high neurological effects like Developmental delay, reduced locomotor activity, morphological anamolis, paralysis and it may even alter the content of neurotransmitters. This study reveals that on exposure to food dyes, larvae and pre-adult stages are prone to developmental toxicity.***

Keywords: *Drosophila melanogaster*, Brilliant Blue, Sunset Yellow, Developmental toxicity, life-history traits

1.Introduction

Drosophilae are dipterous holometabolous insects that have four stages of development. The adult fruit fly emerges from the operculum of the paparium and the female fly becomes receptive within 8 to 12 hours of emergence. It then begins mating with a male fly for 30 minutes, collects and stores the sperms from the male fly to use later for laying eggs. They can lay upto 2000 eggs over the course of their lifetime. The average life span of *Drosophila* is normally around 30-40 days in habitable environment (Bainton., et al., 2000).

The advantageous characteristics of this animal model that has contributed to its success include its genetic pliability, invariant and fully described developmental program, well characterized genome, ease of maintenance, short and fertile life cycle and small body size (Kaletta., *et al*., 2006).

Drosophila melanogaster has emerged as a tractable model organism for studying a number of biological phenomena including feeding behaviors under various conditions. Considered as an herbivore, *Drosophila* flies primarily thrive on vegetative matter decomposed by microbes (Carson., *et al.,* 1971). At times, *Drosophila* larvae may encounter nutritional stress that could be transient or chronic and therefore must learn to adapt to it for survival. Remarkably, this species have evolved to adapt to ephemeral as well as persistent nutrition stress (Kolss., *et al*., 2009). An earlier report suggested that larval malnutrition affect foraging behavior in *Drosophila* (Vijayendravarma., *et al*., 2012).

Drosophila larvae being the major feeding stages of flies' life cycle, have a numerically simple brain, may be 10 million fewer neurons compared to man and possess correspondingly moderate behavioral complexity. These features together with the general potential of the *Drosophila* for transgenic manipulation, (Sokolowski, 2001) make them an attractive study case when trained to achieve a circuit-level understanding of the behavior, in particular with regard to chemosensory processing and odour-tasting learning.

The method encompasses treating the entire metamorphosis period, i.e., from the egg through the larval stages to pupa formation, by incorporating the test chemical into the medium.

D. melanogaster is used to screen for reproductive fitness and developmental toxicity. Compared with other non-mammalian models, *D. melanogaster* has many similarities with the mammalian reproductive system, including putative sex hormones and conserved proteins involved in genitourinary development. Furthermore, *D. melanogaster* would present significant advantages in time efficiency and cost-effectiveness compared to mammalian models.

Developmental toxicity was evaluated based on the number of days taken for development and number of adults eclosed after treating with the drugs in pre-adult stage. Adult flies were systematically examined under a binocular microscope for external morphological anomalies. Data from treated flies can be compared with those from concurrent untreated flies using statistical tests. The external development of flies eliminates the complications of maternal–placenta–foetal interactions seen in mammalian studies.

Brilliant Blue FCF is synthetic dye produced by the condensation of 2-formylbenzenesulfonic acid and the appropriate aniline followed by oxidation (Allaire SE., *et al*., 2009). It can be combined with tartrazine to produce various shades of green. Like many other color additives, the primary use of Blue is to correct or enhance natural coloring or to give colorless compounds a vivid hue.

Brilliant Blue FCF is extensively used as a water tracer agent (Flury M., *et al*., 1994).The dye is poorly absorbed from the gastrointestinal tract and 95% of the ingested dye can be found in the feces. When applied to the tongue or shaved skin, Brilliant Blue FCF can be absorbed directly

into the bloodstream (Lucová., *et al*., 2013). Due to its non-toxic properties, Brilliant Blue FCF has been used as a biological stain. When dissolved in an acidic medium, this dye has been used to stain cell walls, bacteria, and fungal cells. The dye does not inhibit the growth of any of these species (Chau HW., *et al*., 2011).

Figure 1: Brilliant Blue FCF with chemical structure

Sunset Yellow is used in foods and condoms, cosmetics, and drugs. Sunset Yellow FCF is used as an orange or yellowish-orange dye. Late 1970s under the advocacy of Benjamin Feingold claimed Sunset Yellow FCF causes food intolerance and ADHD-like behavior in children but there is no scientific evidence to support these broad claims (Tomaska LD., *et al*., 2013).

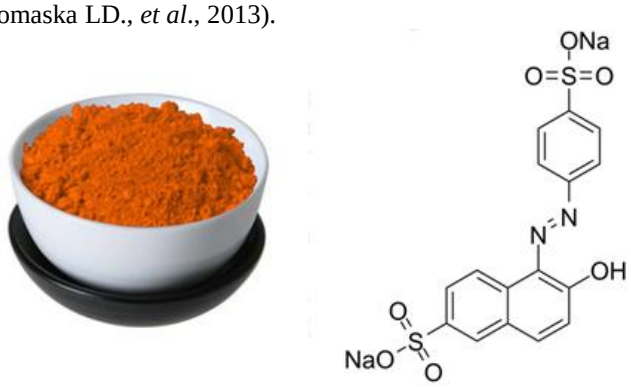


Figure 2: Sunset Yellow FCF with chemical structure

2.Review of Literature

Among the variety of species used for research on mechanisms of nutrient selection, one of the most promising animal models is the fruit fly (*Drosophila melanogaster*) owing to the abundance of protocols available for studying its neurogenetics, behavior ecology and evolution.

Indeed, a recent research effort has been devoted to examining mechanisms of nutritional regulation in adult fruit flies (Amrein and Thorne, 2005; Burke and Waddell, 2011; Fujita and Tanimura, 2011; Lee., *et al*., 2008; Stafford., *et al*., 2012).
The ability to choose "the greater of two goods" is advantageous for animal survival. To do so, animals must assess and rank the values of their choice options. Primate studies have made significant progress in elucidating the neural basis of "goods-based decisions" (Glimcher*et al*., 2009; Padoa-Schioppa, 2011).

In particular, activities of some neurons in the orbital frontal cortex have been shown in correlate with values of different food options (Tremblay and Schultz, 1999; Padoa-Scioppa and Assad, 2006, 2008; Padoa-Schioppa, 2013).

3.Materials and Methods

3.1 Fly stock-

Flies belonging to the Oregon R wild-type strain of *Drosophila melanogaster* were used in the experiments. The flies were maintained at a constant temperature of 25°C±1°C in an uncrowded condition on a standard medium composed of maize flour, agar, dried yeast, and propionic acid (Standard *Drosophila* media). The flies were kept in the dark, except during the transfer onto fresh medium (usually twice a week). The humidity of the experimental chamber was 40–60% with 12:12 hour light and dark periods, and the female flies used in this experiment were virgins.

The test flies were cultured in wheat cream agar medium along with different concentrations of the Food dyes at room temperature (28-32ºc).

3.2 Rate of Development-

The virgin females and unmated males were collected and maintained separately for 5 days in order to age and then transferred to wheat cream agar medium containing Food dyes (Brilliant Blue and Sunset Yellow) along with control. The Food dyes were added to wheat cream agar medium in different concentration (i.e., 10%, 20% & 30% and 0.5mg/ml, 1mg/ml & 1.5mg/ml respectively). The control cultures were raised on the wheat cream agar media without addition of Food dyes.

Figure 3: Culture bottles and vials with *Drosophila melanogaster*

Flies were allowed to lay eggs on the medium containing food dyes in two different doses alongside the control and the number of eggs laid was recorded. Dilute yeast was added to the same set of vials for the eggs to hatch, complete larval and pupal development and for the eclosion of adults. The number of adult eclosed was recorded from the treated (all three doses) and untreated (control) vials. Simultaneously, the same sets of vials were assessed for the developmental time. The same set of vials was further recorded for larval and pupal development and subsequently the same set of vials were accorded for the emergence of the adult flies. The number of adult's enclosed from pupa was counted from the treated (i.e., 10%, 20% & 30% and

0.5mg/ml, 1mg/ml & 1.5mg/ml) and untreated (control) vials. In addition to developmental time (recorded in number of days).

Figure 4: Vials with *D.melanogaster* on supplementation of Sunset Yellow and Brilliant Blue

4.Result

The fly stocks of *Drosophila melanogaster* (oregon k) wild type, were grown on wheat cream agar medium supplemented with varied doses of Food dyes (Brilliant Blue and Sunset Yellow) viz 10%, 20% & 30% and 0.5mg/ml, 1mg/ml & 1.5 mg/ml respectively along with control. The Mean viability for the same is presented in Table 1 and 2.

Table 1: Mean ± S.E of Rate of Development of *Drosophila melanogaster* on supplementation of Brilliant Blue

Dyes→	Brilliant Blue			
Conc↓	Egg	Larvae	Pupae	Adult
Control	49.22±0.73	46.15±0.71	38.48±0.64	27.33±0.54
10%	48.53±1.25	44.80±1.20	38.40±1.11	26.73±0.93
20%	50.06±1.27	47.73±1.24	39.20±1.12	28.93±0.96
30%	47.73±1.24	44.60±1.19	36.53±1.08	25.00±0.89
ANOVA	F=0.94 df=3 p<0.005	F=2.91 df=3 p<0.005	F=1.26 df=3 p<0.005	F=2.61 df=3 p<0.005

In Brilliant Blue, the results reveal that the differences are insignificant with respect to control and low dose in case of *Drosophila melanogaster* (oregon k) and for the hatchability, pupation and adult eclosion, while the differences are significant between mid-dose and high dose for the said parameters.

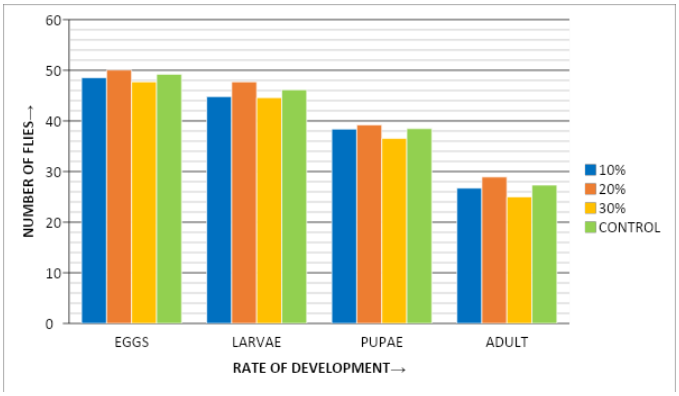
Table 2: Mean ± S.E of Rate of Development of *Drosophila melanogaster* on supplementation of Sunset Yellow

Dyes→	Sunset Yellow			
Conc↓	Egg	Larvae	Pupae	Adult
Control	60.55±0.81	53.22±0.76	49.22±0.73	46.15±0.71
0.5	66.86±1.46	53.73±1.31	48.53±1.25	44.80±1.20
1.0	58.93±1.37	54.40±1.32	50.06±1.27	47.73±1.24
1.5	54.40±1.32	50.06±1.27	47.73±1.24	44.60±1.19
ANOVA	F=26.81 df=3 p<0.005	F=3.67 df=3 p<0.005	F=0.94 df=3 p<0.005	F=2.06 df=3 p<0.005

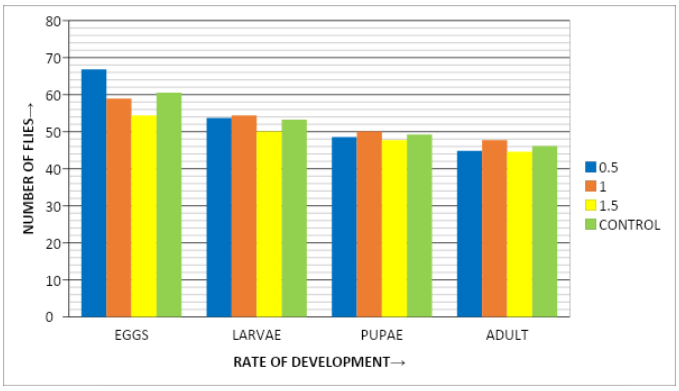
In Sunset Yellow, the results reveal that the differences are insignificant with respect to control and low dose in case of *Drosophila melanogaster* (oregon k) for the hatchability, pupation and adult eclosion, while the differences are significantly increased at high dose when compared to low dose and mid dose for the said parameters.

4.1 Developmental time in *D.melanogaster*

The mean Developmental time from egg to adult emergence in *Drosophila melanogaster* (oregon k) for all doses of Brilliant Blue and Sunset Yellow a Food dyes viz 10%, 20% & 30% and 0.5mg/ml, 1mg/ml & 1.5 mg/ml respectively along with control were depicted in the Graph 1 and Graph 2.



Graph 1: Rate of Development of *Drosophila melanogaster* on supplementation of Brilliant Blue



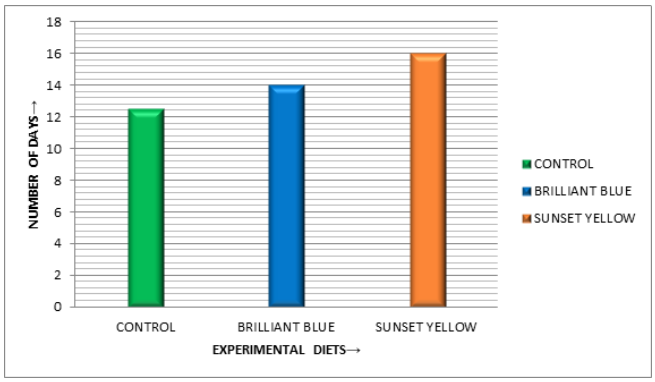
Graph 2: Rate of Development of *Drosophila melanogaster* on supplementation of Sunset Yellow

In *Drosophila melanogaster* (oregon k) the mean developmental time for control is 12±0.119 days and at low dose is 12.36±0.11 days thus result shows insignificant difference between control and low dose. While the mean developmental time at mid dose is 14.86±0.15 days and at high dose is 16.11±0.11 days thereby the time taken for the development was more compared to low dose and control.

In *Drosophila melanogaster*, the mean development time at low dose is 13.9±0.12 days and at mid dose is 12.26±0.13 days which is less than at high dose is 16.5±0.15 days and control is 14.06±0.15 days.

The mean developmental time from egg to adult emergence is significantly increased in all the doses of Brilliant Blue and Sunset Yellow food dyes viz 10%, 20% & 30% and 0.5mg/ml, 1mg/ml & 1.5mg/ml respectively in *Drosophila melanogaster* (oregon k) when compared to the control. There are increased values in the mean development time in high dose and mid dose, but the differences are insignificant at low dose when compared with control in *Drosophila melanogaster* (oregon k).

The developmental time was prolonged by two to three days on exposure to all three doses of Brilliant Blue and Sunset Yellow in *Drosophila melanogaster* (oregon k) which was statistically significant at high doses when compared to control. There is linear relationship between the increase in the developmental time and experimental doses of Brilliant Blue and Sunset Yellow. (Graph 3)



Graph 3: Developmental time of *D.melanogaster* on supplementation of Brilliant Blue and Sunset Yellow with control

5.Discussion and Conclusion

In the present study, the effects of two different food dyes, which are commonly used in the food industry as a food additive, were investigated on the developmental stages and behavior of *D. melanogaster*. We can conclude that the reason for the survival of application groups for a shorter period than the control group and the lower survival rate of larvae belonging to the groups than the control group is due to the toxic effects of high concentrations of the food dyes.

The sophisticated genetics, relatively simple anatomy, and remarkable molecular similarity between mammals and invertebrate models like *Drosophila melanogaster* provide useful insights into the developmental and behavioral traits. The developmental rate of *Drosophila* is a function of numerous metabolic and developmental processes (Church and Robertson, 1966). During development, body tissues constantly require a specific quantity and proportion of nutrients in order to attain optimal growth and performance (Bauerfeind., *et al*., 2005).

The present study reveals that *Drosophila melanogaster* supplemented with different Food dyes (Brilliant Blue and Sunset Yellow) showed significant response to different concentrations (i.e., 10%, 20% & 30% and 0.5mg/ml, 1mg/ml & 1.5mg/ml respectively).

Viability (survival values) is one of the adaptive traits of any population and determines the rate of increase or decrease of population in an environment. Therefore, it is one of the fitness parameters, which could be used to analyze the toxicity of any drug or chemical. Any change in viability reflects the somatic effect induced by them (Luning, 1966) provided the analysis is made in a uniform environment. Environmental factors which would affect the viability mainly include such as temperature, food, space and population density (Andrewantha and Birch, 1954). In the present experiment, temperature and space were uniform for both control and treated batches. Same number of eggs were allotted to vials, same strain of flies was used in the experiment, thus leaving the food medium supplemented with Food dyes (Brilliant Blue and Sunset Yellow).

It was noticed that Food dyes (Brilliant Blue and Sunset Yellow) has significant effect on the viability of *Drosophila* flies. A significant decline in viability among all different doses of Food dyes (Brilliant Blue and Sunset Yellow) was recorded. The *Drosophila melanogaster* exposed to different doses of Food dyes led to reduction in viability with increased doses. The number of offspring that successfully developed from the egg to adulthood was assessed to confirm developmental toxicity. Interestingly, the study also showed that flies reared continuously on media supplemented with different doses of Food dyes show a dose-dependent reduction in hatchability, pupation and adult eclosion in *Drosophila melanogaster* in all the doses of Food dyes, when compared to the control.

Bonnier (1960) has demonstrated that change in the rate of development is due to the compound effects of the genotype and environment. The *Drosophila* flies used in the present study originated from the Oregon K strain which was inbred for several generations. A genotypic change is not expected in this inbred culture. Therefore, perhaps the changed environment in the form of antidepressant drug in the medium must have affected the rate of development. Luning (1966) is of the opinion that cytotoxic effects of certain drugs cause the toxicity to *Drosophila* flies. In the present study the cytotoxic effect of Food dyes could have affected developmental time, which in most of the cases resulted in the enhancement of developmental rate. A significant lengthening of development time is evident in all the doses of the Food dyes tested.

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