EXPERIMENT ON THE GENETIC TOXICITY OF TARTRAZINE YELLOW AND BEHAVIORAL EFFECTS ON *Drosophila melanogaster*

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Tartrazine (E 102) is an azo dye used as a foodstuff additive and in various human drugs, The hazard characterization of tartrazine is toxicological findings in laboratory animals confirming the initial hazard since the last assessment carried out by FAO/WHO Expert Committee on Food Additives (JECFA) in 1964 (Elhakim *et al*., 2007). Tartrazine dye is used in many colored foods and drinks products, aspirin, vitamins and other substances. Mutagenic and toxicity of tartrazine action were determined on *E. coli.* Ishidate *et al*. (1984) detected that the tartrazine has mutagenic potential effect, and can induce chromosomal aberrations in Chinese hamster, has activity to clastogenic, DNA damaged in mice (Sasaki *et al*., 2002). Tennant (2009) observed the mutagenicity of tartrazine and has high ability to induce cancer in cells or tissues of multicellular animals in genetic change, and carcinogenesis. For genotoxicity in somatic mutation and recombination in *D. melanogaster*, five combinants at 25 mM concentration were evaluated (Sarikaya and Cakn, 2005).

Drosophila melanogaster was used as a simple model for studying genetics in research laboratories; many factors make the assay in *D. melanogaster* advantageous and is capable of promutations and procarcinogens enzymatic activity. Fruit fly has four pairs of chromosomes and 14,000 genes, and be suitable for this kind of studies which private with the harmful effects of mutagenic dyes for the human and environment (Tantiado, 2012) and as a model system in olfaction (Fiala *et al*., 2002). Scherer *et al.* (2003) showed that larval *Drosophila* learning paradigm will use in synaptic physiology analysis by link behavioral levels.

Genetic and cellular basis for learning and memory were understood by *D. melanogaster* (Silva *et al*., 2015). Scott *et al*. (2001) determined the patterns of expression of gene families in *Drosophila* that encoded to both odorant and gustatory receptors. Kulig *et al*. (1996) reported that the intellectual abilities related to memory and learning capacity are important to adapting to changes in the environment; concern has been raised regarding the need to include measures of learning in evaluating the health influences of drugs and chemicals. Gerber and. Stocker (2007) presented the larval learning by olfactory and visual stimuli and Khurana *et al.* (2009) trained larvae of *Drosophila* to

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avoid odors associated with electric shock and applied to learning mutants.

MATERIALS AND METHODS

Drosophila flies

Population of *Drosophila* was catchted from a natural population of Drosophila at the Faculty of Agriculture Farm, Tanta, Egypt. Corn flour media was used in this experiment. Five males and five females were placed into each vial, where the tartrazine concentrations were test (Tartrazine was obtained from Sigma Aldrich).

Larvae

Chromosome rearrangements were screened for chromosome squashing. Inversions were identified according to the standard chromosomal map of Lindsley and Grell (1968). For behavior study, wild-type aged 5 days after egg lying was used. We removed a spoonful of food medium from a food vial, collected the number of larvae in distilled water, before each experiment. Petri-dishes of 90-mm inner diameter were used, filled with 1% agarose (electrophoresis grade; Roth, Karlsruhe, Germany). As Oder, *n*-amyl acetate (AM; CAS: 628-63-7; Merck, Darmstadt, Germany) were used, diluted 1:50 in paraffin oil (Merck, Darmstadt, Germany).

For learning

Odor containers were prepared: 10 µl of odor substance was filled into custom-made Teflon containers (5-mm inner diameter with a lid perforated with seven 0.5-mm diameter holes). Before the experiment started, Petri dishes were covered with modified lids perforated in the center by 15 holes of 1-mm diameter to improve aeration, each group of larvae trained AM+/EM, larvae were transferred to a test Petri dish that, as specified for each experiment, did or did not contain a reinforce and given the choice between the two trained odors. Larvae were counted after 3 min, and a preference score calculated as:

$$\text{PREF COUNTED} = \frac{(\#\text{AM} - \#\text{no AM})}{\#\text{Total}}$$

In this equation, # indicates the number of larvae on the respective half of the dish. PREF values are constrained between 1 and −1 with positive values indicating a preference for AM and negative values indicating a preference for no AM or (EM).

Thirty larvae were placed on a Petri dish filled with pure agarose (PUR) or agarose containing Tart. Larvae were given the choice between an odor-filled and an empty Teflon container; as odor. Placed the larva in the center of the petri dish, closed the lid of plat and the position of the larva was noted every 20 sec for 5 min, positions were defined as (in the middle of the assay plate), “AM” or “EM”. Noted that, larvae that moved onto the cover of the plate or onto the odorant containers were discarded.

Statistics analysis

Chi-square statistic was used to measuring the sexual ratio between males and females. One-sample sign test,

Kruskal-Wallis test, nonparametric statistics. (The one-sample sign-test uses a web-based statistic tool provided on http://www.fon.hum.uva.nl/Service/Statistics/Sign_test.html).

RESULTS AND DISCUSSION

The results introduce toxicity and mutagenicity effects of tartrazine yellow 5- E102 as a chemical dye which used in many foods, color wool and silk, and the impact on the behavior of *Drosophila* due to learning.

When studying the harmful effect of tartrazine concentration on male and female of *Drosophila* in the first generation, as shown in Fig. (1) it observed adverse effect from the beginning of 2.5% tartrazine and reached its maximum at 10%, at 7.5% the value of $\chi 2 = 6.0$ No flies in vail 3, so progeny could not be carried over a second, third and fifth generations as in Figs (2 and 3), Chi-square values between (0.61-6.0) for sexual ratio.

In the 3rd generation data in Fig. (2) concluded that increase the number of females than males in the same vial and the ratio between them was disrupted, no progeny in vial 5 in addition to vial 3 in 2.5% and 5.0% concentrations of tartrazine. The flies did not show also in vials 3, 4 and 5 in 7.5% tartrazine concentration. After a week in 10% tartrazine, it was observed that all of the adult flies were dead. They did not lay eggs, and there were no progeny, as it noted in all the first three tables.

Results in Fig. (3) revealed that no flies in vial 3 in the fifth generation beginning the lower concentration of tartrazine, what happened also in vial 1 in the second concentration, then attached to vial 5 and vials 2 and 4 there were no flies in the two higher concentrations.

Data in Table (1) presented that in the fifth generation no inversions on the second chromosome in tartrazine concentrations, but inversions were appeared in the control. Same result in inversion *3R(Mo)* at the third chromosome, *3L(M)* inversion did not show at all, but inversions *3L(P)* and *3R(C)* deleted from third concentration of tartrazine.

Follow by discussing the data which collected and recorded for the *Drosophila melanogaster* with the varying concentrations of tartrazine for five generations. This result compatible with El-Keredy (2014) on monosodium glutamate (MSG) in *3R(Mo)* inversion in the fifth generation and quinine in the 10th generation.

Mutagenesis due to processes that result in genetic change and carcinogenesis in cells or tissues of multicellular animals and there are strongly correlated between the ability of mutations and cancer, (Tennant, 2014). Sodium nitrite, potassium nitrite, sodium nitrate and potassium nitrate were ranked according to their genotoxic and toxic effects by Sarikaya and Cakn (2005) and determined that positive correlation between total mutations and the number of wings having mutation with taking into mind the differ-

ence between inversion and wing examination. Harmful effects on human and *D. melanogaster* with different tartrazine concentrations, amount, se ratio and distortion rate of *Drosophila* (Yanzhi *et al*., 2012). High concentrations of tartrazine had certain genetic toxicity on *Paramisgurus dabryanus* (Yan *et al*., 2008), 1% and 5% concentration of laboratory dyes were increased the rate of mutations on eyes color, color of the body and wing shapes of *D. melanogaster* (Tantiado, 2012).

Figure (4) show the cytological part in this study, translated the influence of tartrazine yellow on inversion frequencies compared to the control starting from lower concentration of tartazine in the fifth generation, Inversions *2 L (Cy), 2 R (Ns)* in the second chromosome and *3R (MO)* inversion in the third chromosome were disappear as the result of this effect, small number of inversions *3L (P)* and *3R (C)* which did not exceed the ratio 4% in the third chromosome were appeared in the first and second concentrations of tartrzaine.

Behavioral data suggest that the ability of larval *Drosophila* to learning was less as a result of the effects of tartrzaine concentrations in the fifth generation (Fig. 5)

Drosophila larvae were tested for olfactory preference for their choice between n-amyl acetate (AM) and empty container (EM). The same result was obtained by Schleyer *et al*. (2015) on 5 mM quinine and 4 M sodium chloride, 0.2 g/l of quinine (El-Keredy, 2014) and 5 mM quinine (El-Keredy *et al*., 2012), but the results on monosodium glutamate (MSG) were difference in (El-Keredy, 2014). High concentrations of salt reported the same result (Niewalda *et al*., 2008; Russell *et al*., 2011). Addition to this result, (high concentration salt or quinine considered a bad signal which delivered *via* a different set of aminergic neurons and sends to many) and 'odor'' reward that (AC) adenylyl cyclase (*rut* gene, CG9533) activated cAMP levels, protein kinase A (PKA) and phosphorylation of protein substrates (Schleyer *et al*., 2013). There are similarities among the behavioral effects of systemically toxic agents (Gerber and O'Shaughnessy, 1986).

The olfactory of the fruit fly has emerged as an excellent model for studying the principles and mechanisms of information processing in neuronal circuits (Liang and Liqun, 2010). On the other hand without behavior influences of associative memories when tested in the sugar (Schleyer *et al*., 2015).

SUMMARY

The aimed of this study is to know harmful effects of tartrazine (yellow 5-E102) on chromosomes and behavior in a natural Egyptian population of *Drosophila melanogaster* from Tanta, Egypt. Five concentrations of tartrazine (1, 2.5, 5, 7.5 and 10%) previously were used for five generations. Each generation was allowed to reproduce for 12 days under tartrazine exposure. Additionally, tartrazine effects (toxicity) on a long term of the male and

female lethal flies were detected, and it had an impact on the ratio between male and female (sexual ratio). Chi-square statistic at 0.05 level of significance showed that there are significant difference on the sexual ratio between males and females ($\chi 2 = 6.0$) at the 5% Tart., concentration in the fifth generation.

Inversions *3L(P)* and *3R(C)* were detected only after treatment with tartrazine concentrations in fifth generations at the Cytological part in this study.

The dose-effect- behavioral functions (learning) for tartrazine concentrations determined that high dose reduced insects ability to learn which affects in his behavior.

Results of the study showed that tartrazine concentrations increased the rate of toxicity, mutations, genotoxicity, disruption of sex ratio and the ability to learn was lost.

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Table (1): Effects of Tartrazine on the chromosome inversion frequencies of *D. melanogaster* in the 5th generation.

Inversion	Tartrazine (E102) %								Control	
	1%		2.5%		5%		10%			
Chromosome I	N	%	N	%	N	%	N	%	N	%
2 L (Cy)	-	-	-	-	-	-	-	-	5	10.0
2 R (Ns)	-	-	-	-	-	-	-	-	4	8.0
Chromosome III										
3 L (P)	2	4	1	2	-	-	-	-	15	30
3 L (M)	-	-	-	-	-	-	-	-	-	-
3 R (MO)	-	-	-	-	-	-	-	-	11	22
3 R (C)	1	2	1	2					14	28
Total No. of Chromosome examined	50		50		50		50		50	
	100				100				50	

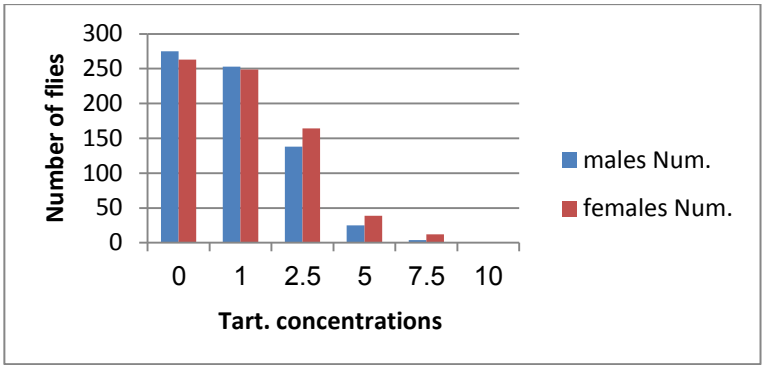

Fig. (1): *Drosophila melanogaster* progeny counts in the first generation, males and females were counted separately.

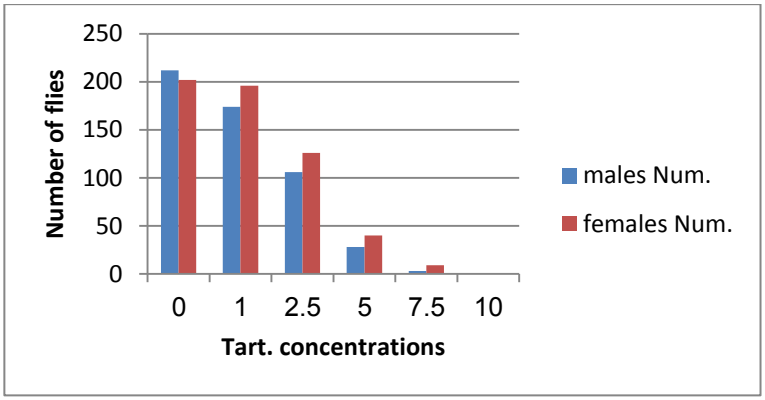

Fig. (2): Progeny counts in the third generations of *Drosophila melanogaster*, males and females were counted separately. The flies were scored after breeding about for two weeks.

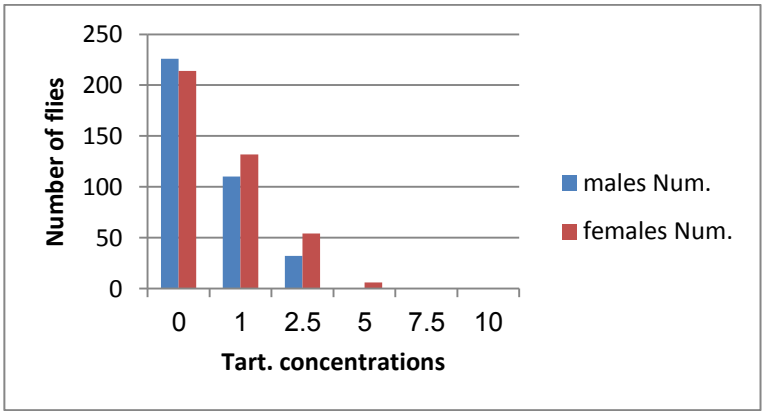

Fig. (3): Progeny counts in the fifth generations of *Drosophila melanogaster*, males and females were counted separately. The flies were scored after breeding about for two weeks

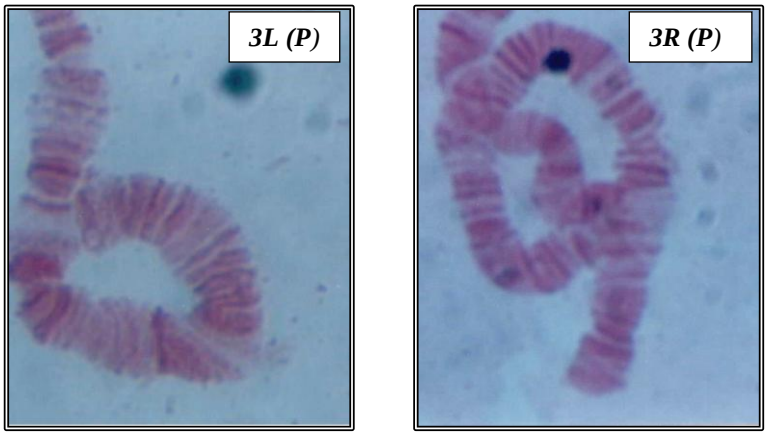

Fig. (4): Microphotographs of chromosomal inversions.

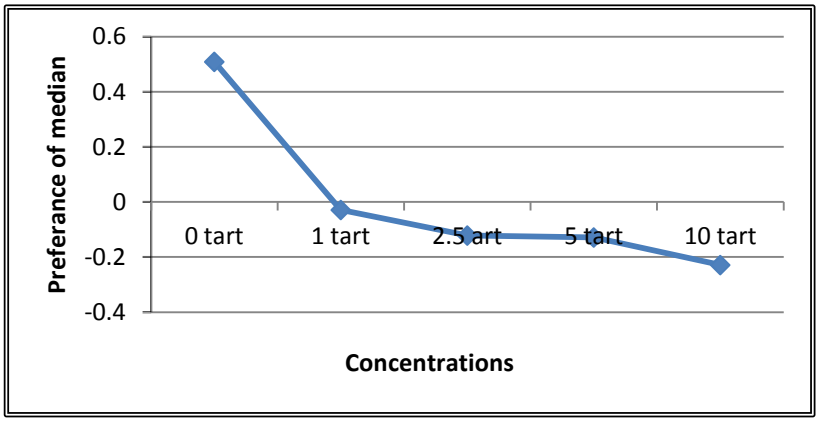

Fig. (5): Median of preference curve for tart. concentrations in fifth generation on larval *D. melanogaster*.