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Research Paper

The Effect of Turkish Coffee and Cadmium Acetate on *Drosophila melanogaster*

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Abstract: In the study, Cadmium (Cd), which is a environmentally polluting toxic heavy metal, was added to the diet of model organism *Drosophila melanogaster* Meigen through water and food, and it was tried to determine the effect on life-development and sex ratio by using dry commercial Turkish coffee (TK). The female and male individuals fed with the Cd acetate (0-30 ppm) and TK (2%) used also had a long life span. It was determined that the decreasing survival rate with Cd acetate may increase with the addition of TK to the food, the duration of puberty may be shortened with TK, and the sex ratio in favor of men even though there is no difference in nutrition. In addition, it was determined that the use of Cd acetate shortens the life span of adult individuals, while feeding with TK does not affect this negative effect much.

Keywords: *Drosophila melanogaster, sex ratio, developmental period, Cadmium acetate, Turkish coffee, longevity.*

Introduction

Many substances that cause pollution in nature cannot be destroyed due to the developing industry and the waste materials released accordingly. This situation brings pollution with it and affects all living things negatively. Environment, soil, water and air pollution caused by heavy metals are seen as a very important problem. These contaminating metals affect all organisms directly or indirectly through the food chain / respiration. Cd is the symbol of Cadmium, which is a dangerous and toxic heavy metal, and it is a white colored element. The atomic number is 48, the atomic weight is 112.4, and its valence is + 2. The biological half-life is on average 29 years (Asri et al., 2007; Çoşan et al., 2017). The effect of cadmium acetate [Cd (CH3COO) 2.2H20] on living things has not been fully proven, and research is ongoing (Güner & Kavlak, 2017). The organs first affected by cadmium and its components are kidneys and liver. Cd accumulating in tissues; high blood pressure, anemia, bone loss, and even lung cancer can cause important illnesses (Demir et al., 2002, Asar et al., 2004). In addition, it has been reported to cause toxicity in the urinary, cardiovascular, gastrointestinal and central nervous systems (Asri et al., 2007; Çoşan et al., 2017). The possible effect of cd acetate especially on the reproductive system is the main purpose of this study. Because Cd acetate taken indirectly with water and nutrients; It causes the reproduction, survival and development of the species in nature and the change of the sex. This situation causes species to be endangered or populations change and another process begins.

The toxic effects of chemical compounds used in industry should be thoroughly tested in rodents, rabbits or monkeys such as rats or mice. However, it is not possible to test every chemical compound. Therefore, alternative screening methods, which are cheap and fast, are used in which the possible effects of a chemical compound in mammals can be accurately defined. For this purpose, some in vivo and in vitro test systems with non-mammalian animal species are used in the studies. *Drosophila melanogaster* Meigen, one of these test systems; It is one of the organisms used in modeling metabolic disorders, neurodegenerative diseases and cancer (Bilen & Bonini, 2005; Gonzalez, 2013; Owusu-Ansah & Perrimon, 2014; Hirabayashi, 2016). However it is used, in toxicity studies (Pappus & Mishra, 2018; Amorim et al., 2019; Rand et al., 2019) and environmental impact studies (Van Straalen and Roelofs, 2005; Wiman et al., 2016; Ko et al. (2017). Based on the information obtained from model insects, inferences are made for mammalian models (Brumby and Richardson, 2005; Hariharan and Bilder, 2006; Miles et al., 2011; Gonzalez, 2013; Hirabayashi, 2016).

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Coffee is the most consumed beverage in the world and has attracted the attention of researchers due to its carcinogenic, genotoxic and antioxidant effects (Duarte et al., 1999; Abraham & Stopper, 2004; Araque et al., 2007; Arab, 2010; Trinh et al., 2010; Wang et al. 2016; Ko et al., 2017; Nagpal & Abraham, 2019). It is known that beverages such as wine, coffee and tea can reduce Cd accumulation in tissues (Winiarska-Mieczan, 2013). However, the effect of Turkish coffee (TK) against heavy metals such as Cd acetate taken with our daily diet has not been tested in terms of model organisms. Nowadays, researches on the use of natural ingredients in treatment have gained importance. Therefore, in our study; It is aimed to investigate the effect of TK, which has a high flavonoid content and antioxidant effect on heavy metal chelating, on some biological properties of *D. melanogaster* against foodborne Cd toxicity.

Materials and Methods

Wild-type (W^{1118}) adult flies of *D. melanogaster* have been cultured in Necmettin Erbakan University Research Laboratories (Turkey). *D. melanogaster* was kept in a standard nutrient medium containing mashed potatoes, agar, sucrose, dry yeast, ascorbic acid, and nipagin at 60-70% humidity, constant temperature of 25 ± 2° C and dark by Nüve cooled, ES 120.

Cd acetate was obtained from Dr. Mehmet Okan ERDAL (Meram Vocational School, Necmettin Erbakan University, Konya, Turkey). Cd acetate (0-30 ppm) was dissolved in distile water was added to standard insect nutrient, TK (added to 2% of the nutrient) were purchased commercially in October 2019, and the experiment was set up with pre-feeding experiments and previous studies (Cheng, 1980; Abraham & Graf, 1996; Nagpal & Abraham, 2019). The first stage larvae obtained from the culture were transferred to this experimental nutritents (Figure 1) by means of a fine tip brush. All experiments were carried out on four replicates and 100 larvae were used in each replicate.

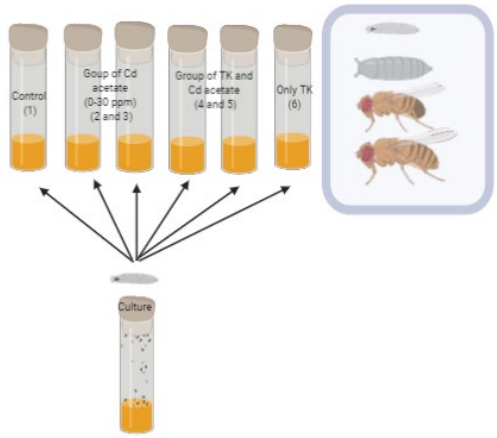

Figure 1. Experimental design with stock culture (create in BioRender.com)

Newly hatched larvae were feed with extracts until adult stage, and biological traits (survivorship, development, sex ratio, longevity) of 3rd instar larvae, puparium, female and male were investigated. In calculating the life expectancy of individuals, the rate of survival was calculated by determining the non-mature individuals. In mature individuals, they were fed the same food during their survival. Adults in the control and administration groups were checked and died at the specified time each day and recorded. The living individuals were transferred to new containers containing food (from the beginning).

The effects of Cd acetat and TK on insect survival and development were evaluated considering the percentage of individuals reaching the 3rd larval stage, pup and adult stage and the average time (days) to reach this stage. Experimental data were expressed as means ± S.E. The data were subjected to statistical analysis (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA) was followed by lest significant difference (LSD) test to determine significant differences between means. Mann Whitney U test was used to determine the changes occurring between male and female adult stages. Data on survivorship were compared by Chi-square test (Snedecor and Cochran, 1967). A values of $p<0.05$ was considered significant.

Results

Commercial TK, which was determined to cause contamination, was sterilized in powder form and added to the food. According to Table 1, by using Cd acetate and TK in nutrition;

- Feeding the insect with 30 ppm Cd acetate in the larval stage shows lethal effect (Lc_{50}); It was determined that the rate of larval survival increased from 50% to 60% with TK added to the food. In Pupal survival rates, TK; While it was not effective in those who fed low doses of Cd acetate, it was found that the insects fed with high doses of Cd acetate caused a statistically significant increase in survival rates (from 34% to 50%). In adult individuals; It has been determined that TK affects the survival rate, but even the low amount of Cd acetate allows half of 100 individuals to survive. It has been observed that the decrease in adult life repair observed in individuals fed with nutrients supplemented with Cd acetate increased with the addition of TK to the fattening medium. While the larval development time in *Drosophila* can be between 3-4 days, it has been determined that TK increases this period by 7 days and Cd acetate by 9 days. No statistically significant difference was observed in the larvae fed with the increased dose of Cd acetate, although shortening the larval development time with the two substances (Cd acetate + TK) used together. 4-5 days of pupal development can be extended with Cd acetate approximately 4 times, and adding TK to the food can decrease this period up to 5 days (from 16 days to 11 days). Although the use of cd acetate causes the insect to complete its maturation time in about 19 days, this time can be shortened by 4 days with the use of TK. Although it is statistically low in female and male ratios; firstly it can be said to be equal or in favor of men
- It has been identified with the use of Cd acetate, the life span of individuals is reduced by approximately half; male individuals live longer and adding TK to the nutrient medium does not affect this negative situation much (Figure 1, LSD Test). When the groups are evaluated among themselves; There is a statistically significant difference in the life span of females and males in the group where 10 ppm Cd acetate and TK are added to the food medium (Table 2, $p < 0.05$, Mann whitney U Test).

Table 1. Effect of Turkish coffee and Cadmium acetate in insects on survival, development and sex ratio on insect

ppm	Survival to the 3rd larval stage (%) (M* ± SE)†	Time to the 3rd larval stage (days) (M* ± SE)†	Survival to pupal stage (%) (M* ± SE)†	Time to pupal stage (day) (M* ± SE)†	Survival to adult stage (%) (M* ± SE)†	Time to adult stage (day) (M* ± SE)†	Sex ratio (%) Female /Male (M* ± SE)†
0.0[a]	98.0 ± 0.1a	3.8 ± 0.2a	97.5 ± 0.1a	4.8 ± 0.2a	96.0 ± 0.2a	7.3 ± 0.1a	25/75 ± 0.1a
2%TK	90.1 ± 0.1a	11.1 ± 0.1b	88.0 ± 0.1b	12.0 ± 0.1b	70.0 ± 0.1b	15.5 ± 0.1b	55/45 ±0.1d
10 Cd acetate	65.0 ± 0.1b	13.0 ± 0.2c	62.0 ± 0.1c	14.1 ± 0.2c	50.0 ± 0.1c	17.4 ± 0.2c	40/60±0.5bc
30 Cd acetate	50.0 ± 0.2c	13.2 ± 0.2c	34.0 ± 0.2d	16.0 ± 0.2d	22.0 ± 0.2d	19.3 ± 0.2d	45/55 ± 0.5c
10 Cd acetate +2% TK	62.5 ± 0.2b	10.0 ± 0.3b	60.0 ± 0.2c	11.1 ± 0.3b	56.0 ± 0.2c	15.8 ± 0.3b	35/65 ±0.2b
30 Cd acetate + 2% TK	60.2 ± 0.2b	13.0 ± 0.3c	59.0 ± 0.2c	15.0 ± 0.3c	48.0 ± 0.2c	19.0 ± 0.3d	48/52 ± 0.2c

*Mean of four repetitions † Values containing the same lower case in the same column are not different from each other, $p > 0,05$ (χ^2 test, LSD Test); [a]Control, **TK**: Turkish Coffee, **Cd acetate**: Cadmium acetate **M:** mean, **Standart Error:** SE

Table 2. Effect of nutrition groups created with Turkish coffee and Cadmium acetate on *Drosophila melanogaster* life span

ppm	Max. Life span/Female (N=100)	Female life span (day) (M* ± SE)†	Max. Life span/Male (N=100)	Male life span (day) (M* ± SE)†
0.0[a]	62.5	60.0 ± 0.2A	65.0	62.0 ± 0.2A
TK 2%	66.0	65.0 ± 0.8A	63.0	65.0 ± 0.8A
10 Cd acetate	42.2	40.0 ± 1.2A	48.1	45.2 ± 1.2B
30 Cd acetate	39.5	39.0 ± 1.2A	38.5	40.0 ± 1.2A
10 Cd acetate + TK 2%	43.4	39.0 ± 0.5A	43.0	41.0 ± 0.5B
30 Cd acetate + TK 2%	41.7	39.5 ± 0.5A	40.7	40.5 ± 0.5A

† Values containing the same capital letter in the same line are not different from each other, $p< 0.05$ (χ^2 test, Mann whitney U Test)

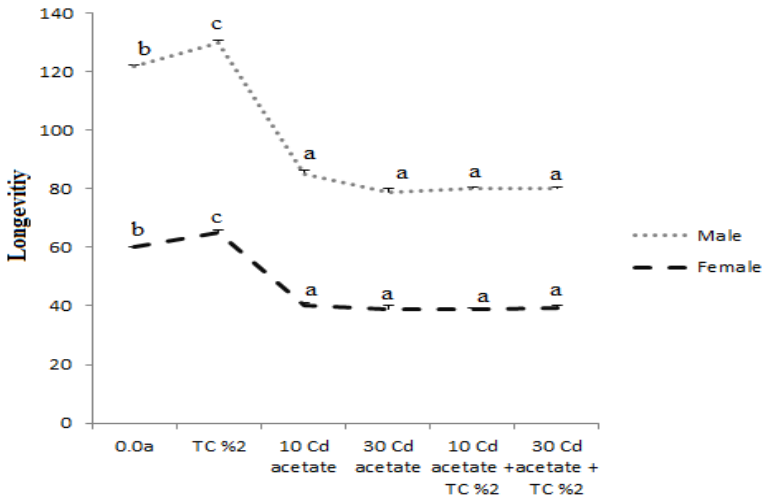

Figure 1. Effect of nutrition groups created with Turkish Coffee and Cadmium acetate on *Drosophila melanogaster* life span ($p< 0.05$; LSD Test)

Discussion

In the literature; there are studies in which the biological parameters are tested on *Drosophila* in a dirty and clean environment created using a single dose of a heavy metal (Posthuma and Van Straalen 1993; Shirley & Sibly, 1999). In these studies, individuals with and without resistance to the environment are tested. Food chain or respiratory substances affect all organisms. Environmental impact determination can be easily done in insects, but invertebrates (especially the Diptera group) that exhibit heavy metal resistance can provide metal homeostasis by forming a resistance protein (metallotionein) to heavy metals during development (Morgan et al., 2007). Metallotionein, which is important in heavy metal resistance, is found in *Drosophila* as it can be found in many creatures (Maroni & Watson 1985; Sterenborg & Roelofs, 2003; Nguyen et al., 2014). In our study, the culture of living was prepared with non-Cd water in order to eliminate resistance in living and a clean environment was created in terms of Cd acetet and generations were grown in this clean environment.

While *D. melanogaster* larvae have 98% survival rate under constant and normal conditions, this rate can decrease up to 80% during adulthood. Development period can different between 7-8 days in total, from larvae to adolescents (Güneş et al., 2017). Foods and their contents can change the rate of living (Güneş, 2016). For example, it is known that the resistant *Drosophila* individuals in the area contaminated with Cd chlorite (80 µg) increase the survival rate by 62% and the development time by

40% from 13 days (Shirley & Sibly, 1999). In the study, the rate of survival in the groups formed with 10 ppm Cd acetate (larva-adult) decreased 1.5-1.9, respectively. In order to reduce the toxicity, it was found that while the rate of 2% TK added to the food remained the same in the larva, it decreased by 1.7 in adulthood. There is tannic acid in the diet used in TK. Tanic acid is also found in beverages such as tea and fruit. Tanic acid is involved in protecting from heavy metals and reduces Cd accumulation in tissues (Graf & Würgler, 1986; Winiarska-Mieczan, 2013; Çoşan et al., 2017; Çalış et al., 2019). This information supports our work. In the study, it was determined that feeding the insect with TK slightly decreased the rate of living in pup and adolescent. In insects with 30 ppm Cd acetate and TK added to the medium; It was observed that TK decreased the 1.9% decrease in the larval stage and the 1.6 decrease in the rate of living. In the process of adulthood; It was determined that the survival rate of insects fed with Cd acetate decreased by 4.3 compared to the control group. It was determined that adding TK to the food of these insects caused the relative improvement by reducing the survival rate by half. Thus, it has been observed that TK has a positive effect on survival rate. For survival flies, a dose-related decline is responded (Nguyen et al., 2014). In experiments with Cd nitrate, it has been stated that the substance given from the larval stage is not effective, and the rate of maturation decreases depending on the concentration after adulthood (Gelegen & Yeşilada, 2000). While lethal effect was 900 ppm Cd nitrate in the same study, the same effect was observed in our study with 30 ppm Cd acetate. In our study, it was determined that adding Cd acetate to the food did not reduce the effect of Cd acetate, although the development period of the insect was extended from 7 days to 19 days with the use of Cd acetate. In fact, it was observed that the development, which extended to 13 days in the larval period, completed 13 days despite the high amount of Cd acetate and TK. In previous studies, it has been reported that 0.15% µg / ml coffee added to the food shortens the development period of the insect (Trinh et al., 2010). It is known that, in terms of Cd content, the rate of living of flies at 80 ppm can decrease significantly (Callaghan & Denny, 2002; Nguyen et al., 2014). In fact, flies for Cd chloride have been reported to have a maximum tolerance of 10 µM (Christie et al., 1985; Debec et al., 1985). In our study, it was determined that the flies tolerated a maximum of 30 ppm Cd acetate. As in other studies (Maroni & Watson 1985; Balamurugan et al., 2007), liveliness is reduced and development time is extended due to Cd acetate exposure.

Although Cd nitrate does not differ between sex ratios in flies (Yeşilada & Gelegen, 2000), feeding can affect the sex ratios. While some foods increase the ratio of females (Güneş et al., 2019), sometimes an increase in the ratio of males can be observed (Arıca et al., 2017). Even coffee added to food is known to be more effective in males (Nagpal & Abraham, 2019). Because the sex ratio in insects is important for determining the population density (Yeşim & Gülel, 2006). Factors such as the amount and type of nutrients affect the survival-breeding-egg release status of the species. This causes an increase in the population. This situation may endanger the continuation of the species. Study; Although adding Cd acetate and TK to the medium of insects does not ensure that the gender ratios are equal, it has been found to affect the male part in favor.

The lifetime that differs between species may not be the same even on different sexes of the same species. It may even differ between different nutritional and environmental conditions and populations of the same genotype (Kızılet & Uysal, 2012). Nutritional factors such as diet, regimen or intake of specific nutrients have been shown to alter animal life (Le Bourg 2001; Page et al., 2010; Altun et al., 2011). Flies can live 60-78 days under constant conditions (Yeşilada & Gelegen, 2000; Uysal & Semendöken, 2011). However, heavy metals such as Cd nitrate shorten the adult lifetime of flies (Balamurugan et al., 2007). In our study; add Cd acetate in nutrition, it has been observed that the life span of individuals is shortened by approximately half, males live longer and feeding with TK has no effect. Coffee is a substance that contains phytochemicals with effective activity against compounds such as ethyl methanesulfonate and has a known protective effect (Prakash et al., 2014). Coffee taken up to 2% of the food has been reported to have a positive effect and reduce genotoxicity and mutagenicity (Abraham & Graf, 1996; Nagpal & Abraham, 2019). It can be said that this situation supports our study. In another study, it was stated that males individuals live longer than females similar to this situation (Yeşim & Gülel, 2006). In another study with coffee; It has been reported that coffee has no effect on lifetime (approximately 60 days) without discrimination between females and males, but decaffeinated coffee reduces the life span (Trinh et al., 2010). Especially the mutations seen in males individuals have been reported to decrease significantly with coffee (Nagpal & Abraham,

2019). This situation can be shown as evidence for long-lasting male individuals to get rid of the effect more quickly, as in our study.

Conclusion

In this study; It has been determined that using TK against exposure to CD acetate has some effect on the survival rate of the insect, but not on the life span. In the following studies, it is suggested to study the relationship between female reproduction and aging, biochemical parameters and metal detoxification.

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