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**Research Article**

# An *in vivo* study on *Drosophila melanogaster*, *Artemia salina*, and *Daphnia magna*: Is activated carbon used as a food additive reliable?

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## Abstract

Activated carbon, one of the most important adsorbents used in the industry, is a general definition used to describe carbon adsorbents, which have a large crystal and amorphous structure and wide internal pores. Activated carbon, which has started to be used in many fields in recent years, is observed to be preferred as an important additive in the food industry. This study aimed to investigate the reliability of the use of activated carbon as a food additive in different model organisms, such as *Drosophila melanogaster*, *Artemia salina*, and *Daphnia magna*. To this end, the organisms were kept alive in nutrient media containing activated carbon at different concentrations (0.1 $mgmL^{-1}$, 0.5 $mgmL^{-1}$, 1 $mgmL^{-1}$, 2.5 $mgmL^{-1}$, and 5 $mgmL^{-1}$), and changes that occurred in their percentage of survival were determined for 48 h. According to the data obtained, for all three organisms, it was found that in comparison with the control group, there was no decrease in survival percentages in any of the experimental groups in which activated carbon was used. On the contrary, there were increases depending on concentration. Especially in *A.salina*, the percentage of survival, which was 78 % in the control group, increased up to 87 % ($P < 0.05$). As a result of the study, it was concluded that activated carbon at the specified doses might be used reliably as a food additive. It was evaluated that these results should be supported by *in vivo* and *in vitro* studies to be conducted in different organisms.

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## 1. Introduction

The carbon element present in the structure of living things exists very rarely in nature. The carbon element is also a good absorbent due to its important properties, such as pore volume, density, abrasion resistance, hardness, and grain size (Gündüzoğlu, 2008). One of the

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most important features of activated carbon is the wide surface area and its developed pore structure (Dermanlı, 2006; Akyıldız, 2007).

The number of studies on the use of many biomass wastes such as hazelnut husk, apricot kernel, corn cob, olive kernel, and rice stalk in the production of activated carbon is quite high. In industrial applications, mostly coal and hazelnut husk are preferred as the starting material for activated carbon production (El-Hendawy, 2005).

Activated carbon is most commonly used in adsorption and colour removal processes. The removal of colour from textile wastes and especially, the purification of wastewater are of great importance in reducing environmental problems (Köseoğlu, 2005).

There are two types of activated carbon in the food industry as an application area: 1-Medical activated carbon is used as a food additive/drug and can be consumed orally, 2-Activated carbon adsorbs unwanted compounds as a purification agent and removes them from the medium. Activated carbons, which can be consumed orally, are commercially sold as E153-coded food additive (CAS no: 7440-44-0), in the form of powder or granules, to be used for colouring food and the adsorption of toxins in the body.

In the food industry, it is benefited from the high colour adsorption feature of activated carbon to remove non-enzymatic browning reaction products in sugar, syrup, and molasses production (Ozsoy, 2010; Bernal et al., 2016). There are some studies in the wine, vinegar, and alcoholic beverage industries in which activated carbon is used to remove excess grains, unwanted colour, odour elements, sediment, turbid substances, and provide aroma isolation (Quintela et al., 2013; Lisanti et al., 2017; Tubia et al., 2018). Besides these, activated carbon is nowadays added to foods such as bread, hamburger, lemonade, and ice cream.

In the current study, the possible effects of activated carbon used in foods were investigated in three different model organisms. One of them, *Drosophila melanogaster*, is an important model organism that is frequently preferred in biological studies and known as the fruit fly. *D. melanogaster*, which was first used in experimental studies by Thomas Morgan in 1911, has many advantages in terms of use in such studies (Gui and Grant, 2008). *Artemia salina* is a species widely used in ecotoxicological studies and applications worldwide (Sanchez-Fortun et al., 1995). *A. salina*, of which the commonly known name is saltwater

shrimp, is used in many scientific fields, such as ecology, physiology, ecotoxicology, aquatic ecosystem, and genetics (Nunes et al., 2006). Another organism used in the present study is *Daphnia magna* (Water Flea) freshwater zooplanktonic crustaceans and is known as water flea among the people. Water fleas, which are rich in protein and essential fatty acids, constitute the most important food source of fish and are used as a living food source by fish farmers (Demirel, 2011). The easy and economical production of its culture due to its small structure, short life, high spawning capacity, and sensitive structure to pollution, enabled water fleas to be frequently used in aquatic toxicity tests (Demirel, 2011).

The aim of this study is to determine the toxic effect of activated carbon, which is used as a nutrient in different concentrations, on survival percentages in different model organisms such as *Drosophila melanogaster*, *Artemia salina* and *Daphnia magna* and investigate the safety of its use as a food additive.

## 2. Materials and Methods

### *2.1. Material*

Activated carbon, of which effect on living things was investigated, was procured from a commercial producer. The activated carbon supplied is sold on the market in the powder form for use in foods.

The Oregon R lineage (Diptera: Drosophilidae) of *D. melanogaster* used in the experiments is a wild type (w.t.) lineage. This lineage is a homogeneous stock that has been tailored for years at the Biological Research Laboratory of Amasya University, Faculty of Science, Department of Biology. Reasons such as short life cycle (9-10 days), breeding a lot, cheap raising conditions, and easy observation of possible variations make *Drosophila* an ideal experimental organism. The eggs of *Daphnia magna*, another organism used in the study, were obtained from a commercial firm and cultured in Çorum Science and Art Center Biology Laboratory. The eggs of *Artemia salina* were obtained from a commercial company and their larvae were obtained by using the egg hatching mechanism at the Biology Laboratory of Çorum Science and Art Center.

### *2.2. Method*

#### *2.2.1. Preparation of experiment sets and substance application*

In the study, mortality rates in 3 different model organisms living in media containing chronically activated carbon were calculated. The stock solution of the activated carbon, of which effects on different living things were investigated, was prepared in 5 different concentrations (0.1 $mgmL^{-1}$, 0.5 $mgmL^{-1}$, 1 $mgmL^{-1}$, 2.5 $mgmL^{-1}$, and 5 $mgmL^{-1}$).

In the study, the Oregon (Wild Type) race was used to obtain the 3rd stage larvae of *D. melanogaster* were procured from a commercial company operating in Turkey. Twenty-five female and twenty-five male Oregon individuals were put in bottles containing nutrients. These individuals were kept in the same medium for at least one day, and their mating was provided. The individuals were then transferred to new bottles containing nutrients and were expected to lay eggs in the medium for 8 h. Then, the individuals were transferred to other bottles. An 8-h egg collection process aimed to obtain individuals in the same larval stage. The individuals who reached the 3rd larval stage after 72 ± 4 h were separated under tap water with the help of fine porous sieves. The 3rd stage larvae collected with the help of the sieve were transferred to plastic bottles containing *Drosophila* ready-made food that was wetted by adding 9 mL of the freshly prepared activated carbon concentrations to be studied. One-two spatulas (100 larvae) of larvae were placed in each application medium. The number of flies that became mature from 100 larvae (after 84-108 h), which were placed in media containing different activated carbon concentrations, was noted. All treatment groups were fed in the culture tubes placed in an oven, which was set at 25°C and had 40- 60 % relative humidity.

In the current study, *D. magna*, another organism on which the effect of activated carbon was investigated, was reproduced in the laboratory under standard living conditions. *Daphnia magna* procured from a private company serving in Turkey was used as a trial material. As the experimental organism, firstly, individuals with eggs were selected and placed in a separate medium, and after spawning was provided, the offspring were subjected to the same feeding program (algae + yeast) for five days and used in the trial. One hundred individuals with the same size and without eggs were taken from the *D. magna* stock solution and placed in media containing activated carbon at different concentrations. The number of individuals was counted for 48 h, and immobile and dead individuals were noted. No feeding was made

during the experiments. 100 mL of the test medium containing the required activated carbon was prepared in each experimental vessel, and 10 *Daphnia magna* individuals were used for each experimental vessel. The temperature was kept at 20 ± 2°C during the experiment.

The eggs of *A. salina*, which was the other organism used in the present study, were procured from a commercial company operating in Turkey. The eggs obtained were first immersed in demineralized water at + 4°C and kept waiting for 1 h. During this period, cysts that sank to the bottom of the water and remained on the surface were separated. The cysts obtained were placed in the medium that had been previously prepared by dissolving 25 g sea salt in 1 L of water. Nauplii larvae hatched from the eggs, which were exposed to powerful aeration for 24 h, were drained and used in the experiments. The obtained *Artemia* individuals were kept alive in media containing different concentrations of activated carbon, as 100 individuals in each. It was checked for 48 h, and immobile and dead individuals were counted and noted. All experiments were repeated three times.

### *2.2.2. Statistical analysis*

The statistical analysis of the data obtained from the experiments investigating the effects of the examined substances on the percentage of survival was performed using the SPSS (Statistical Package for the Social Sciences) 15.0 program.

## 3. Results

Activated carbon, which was applied in different doses to determine larval toxicity, did not reduce the survival rates of *D. melanogaster* in stage 3 larvae (Table 1). As observed in Table 1, the survival rate, which was 94 % in the negative control group, increased to 95 % in the highest treatment group of activated carbon (5 $mgmL^{-1}$) depending on the dose increase. This increase was found to be statistically insignificant ($P<0.05$). As a result of this study, it was detected that activated carbon did not show any toxic effects in *D. melanogaster* larvae at any concentration and did not increase larval mortality (Figure 1).

**Table 1.** Survival and mortality rates of *Drosophila melanogaster* larvae chronically fed with different concentrations of activated carbon after 84-108 h

| Treatment Groups | Concentration ($mgmL^{-1}$) | Number of Larvae | Mortality Rate ± SE | Survival Percentage (%) |
|---|---|---|---|---|
| **Control (Distilled Water)** | | 100 | $5.66 \pm 0.33^{a}$ | $94^{a}$ |
| **Activated Carbon ($mgmL^{-1}$)** | 0.1 | 100 | $6.66 \pm 0.66^{a}$ | $94^{a}$ |
| | 0.5 | 100 | $4.66 \pm 0.66^{b}$ | $96^{b}$ |
| | 1 | 100 | $5.0 \pm 0.15^{a}$ | $95^{a}$ |
| | 2.5 | 100 | $6.33 \pm 0.33^{a}$ | $94^{a}$ |
| | 5.0 | 100 | $5.0 \pm 0.15^{a}$ | $95^{a}$ |

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.

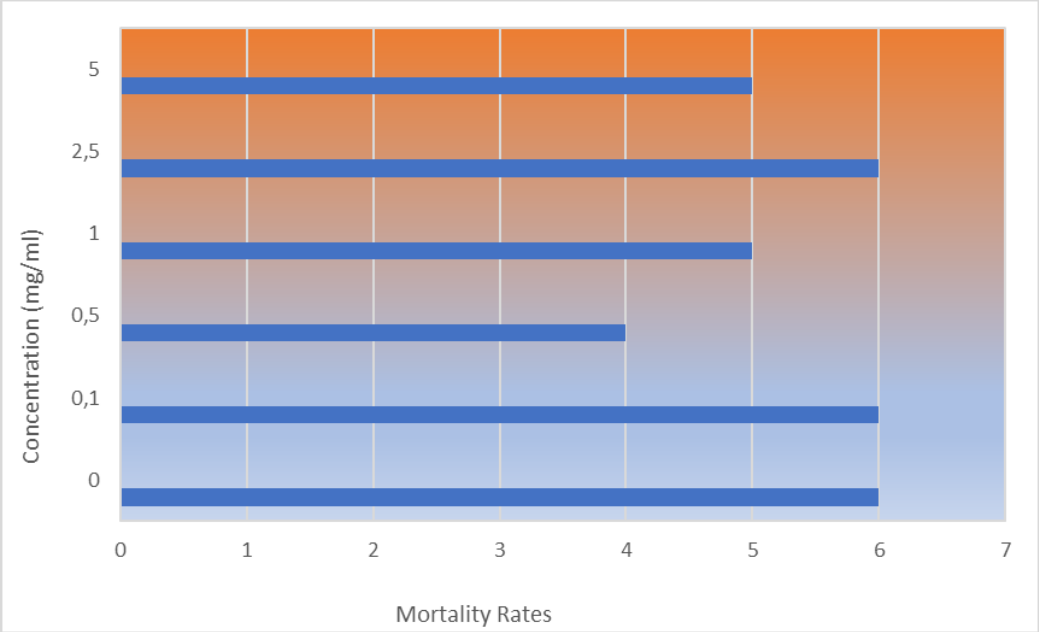


**Figure 1**. Comparison of the mortality rates of *Drosophila melanogaster* larvae raised in media containing different concentrations of activated carbon

Afterwards, in the study, 100 individuals were used for each concentration to determine the effects of activated carbon on *D. magna* (Table 2). Activated carbon, which was applied in different doses in order to determine toxicity in *D.magna* adults, did not reduce survival rates in *D. magna* individuals (Table 2 and Figure 2). As observed in Table 2, the survival rate, which was 84 % in the negative control group, increased until 92 % in the highest treatment group of activated carbon (5 $mgmL^{-1}$) depending on the dose increase. This increase was found to be statistically significant ($P<0.05$).

**Table 2.** Survival and mortality rates of *Daphnia magna* fed with different concentrations of activated carbon after 48 h

| Treatment Groups | Concentration ($mgmL^{-1}$) | Number of Adult Individuals | Mortality Rates ± SE | Survival Percentage (%) |
|---|---|---|---|---|
| **Control (Distilled Water)** | | 100 | $16.0 \pm 1.15^{a}$ | $84^{a}$ |
| **Activated Carbon ($mgmL^{-1}$)** | 0.1 | 100 | $13.66 \pm 0.33^{b}$ | $86^{a}$ |
| | 0.5 | 100 | $14.0 \pm 1.15^{b}$ | $86^{a}$ |
| | 1.0 | 100 | $12.66 \pm 0.33^{c}$ | $87^{a}$ |
| | 2.5 | 100 | $10.33 \pm 1.15^{d}$ | $90^{b}$ |
| | 5.0 | 100 | $8.33 \pm 0.33^{e}$ | $92^{c}$ |

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.

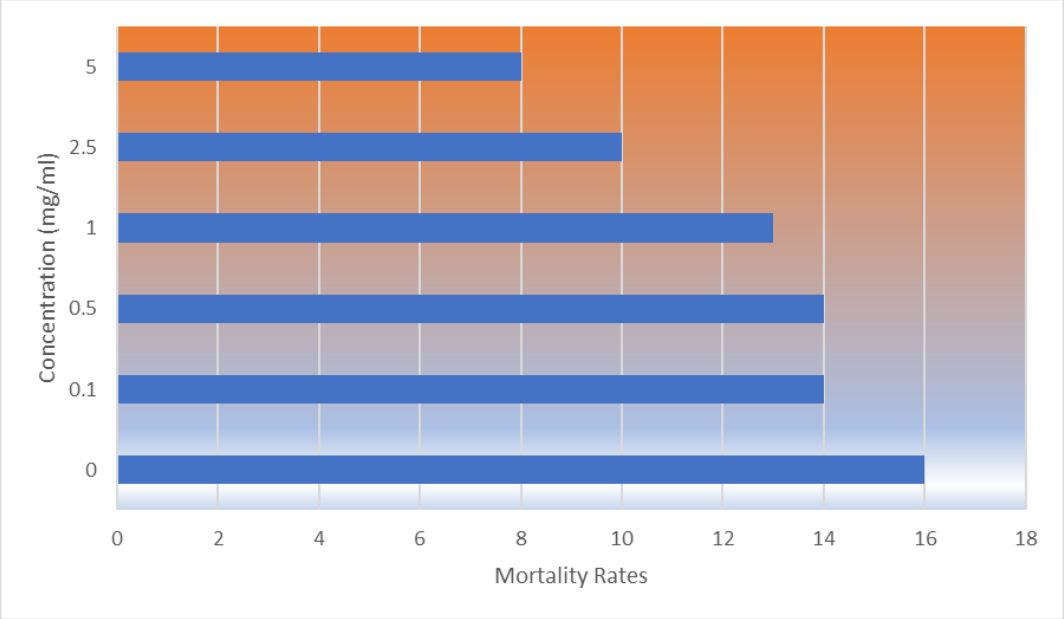


**Figure 2**. Comparison of the survival percentages of *Daphnia magna* raised in media containing different concentrations of activated carbon

The data obtained as a result of the feeding of *A. salina*, which is commonly used in ecotoxicological studies and applications, with activated carbon are presented in Table 3. In *A. salina*, 100 larvae were used for each concentration. Activated carbon, which was applied at different doses to determine toxicity in *A. salina* nauplii larvae, did not reduce survival rates in *A. salina* individuals (Table 3 and Figure 3). As observed in Table 3, the survival rate, which was 78 % in the negative control group, increased up to 87 % in the highest treatment group of activated carbon (5 $mgmL^{-1}$) depending on the dose increase. This increase was found to be statistically significant ($P<0.05$).

**Table 3.** Survival and mortality rates of *Artemia salina* (nauplii) fed with different concentrations of activated carbon after 48 h

| Treatment Groups | Concentration ($mgmL^{-1}$) | Number of Larvae | Mortality Rates ± SE | Survival percentage (%) |
|---|---|---|---|---|
| **Control (Distilled Water)** | | 100 | $22.0 \pm 0.57^{a}$ | $78^{a}$ |
| **Activated Carbon ($mgmL^{-1}$)** | 0.1 | 100 | $22.0 \pm 0.33^{a}$ | $78^{a}$ |
| | 0.5 | 100 | $22.0 \pm 0.66^{a}$ | $78^{a}$ |
| | 1.0 | 100 | $18.0 \pm 0.33^{b}$ | $82^{b}$ |
| | 2.5 | 100 | $16.33 \pm 0.88^{b}$ | $84^{b}$ |
| | 5.0 | 100 | $13.66 \pm 0.66^{c}$ | $87^{c}$ |

SE: Standard Error; Statistical evaluations of the difference between the groups were made within the group. Values shown with different letters in the same column are significant at the level of $p<0.05$.

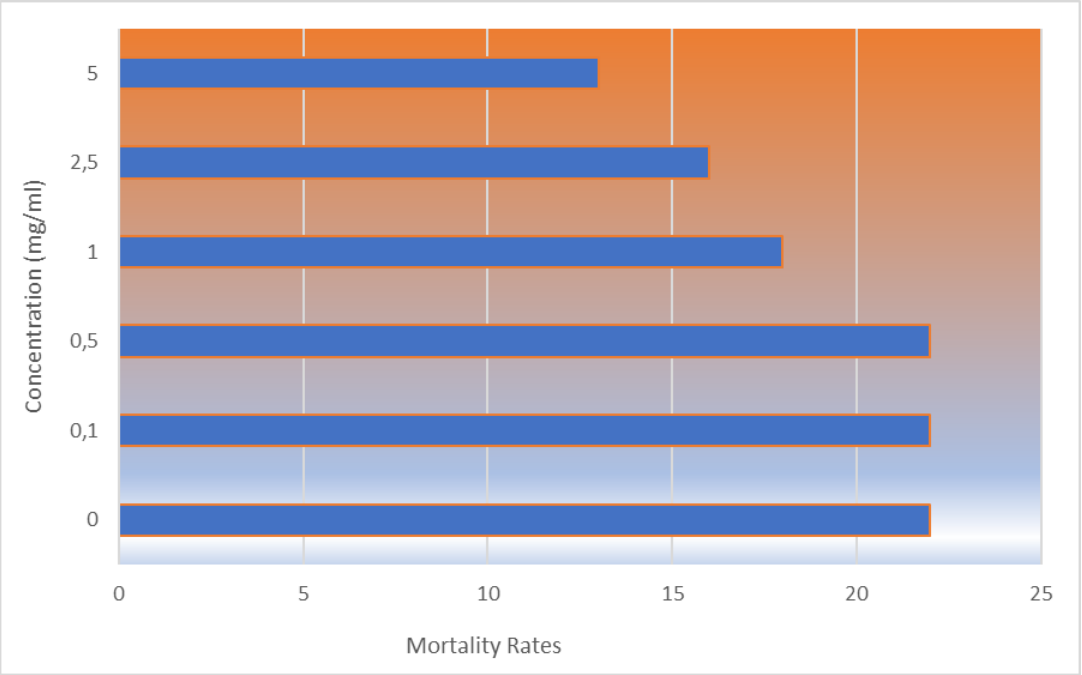


**Figure 3**. Comparison of the survival percentages of *Artemia salina* nauplii larvae raised in media containing different concentrations of activated carbon

## 4. Discussion

In the study, the effect of activated carbon on mortality rates in 3 separate organisms was investigated. When firstly, the data on *Drosophila melanogaster*, which was the organism on which the effect of activated carbon was investigated, were examined, the survival percentage was 94% in the control group, while the same or higher data were obtained in the treatment

groups (Table 1). When the data on *Daphnia magna*, which is a freshwater organism that is frequently used in toxicity studies, were examined, while the survival percentage in the control group was 84 % after 48 h, an increase occurred in survival percentages depending on the increase in concentration in the experimental groups. The highest rate was observed to be 92 % in *Daphnia* fed in the medium containing 5 $mgmL^{-1}$activated carbon (Table 2). When the effect of activated carbon on *Artemia salina*, a brine organism, was examined, the survival percentage in the control group was 78 %, while the survival percentage at 5 $mgmL^{-1}$ concentration increased to 87 % (Table 3).

When the obtained results were examined, no negative effect of activated carbon was found in 3 different organisms. It is thought that the results are significant in terms of eliminating concerns about the use of activated carbon in foods. The use of activated carbon in main consumption materials such as bread, hamburger, and lemonade, which are important nutrients, is a subject that interests all people. Therefore, it is thought that this study should be supported by other studies that will be conducted.

New application areas of activated carbon in the food industry are listed as follows: retaining volatile fatty acids in anaerobic digestion processes, increasing methane gas production performance with ammonia nitrogen rate control, purifying food additives, $CO_2$ gas control in modified atmospheric applications, new generation antimicrobial agent, removal of volatile organic compounds, recovery of aroma components, activated carbon electrochemical sensors (EDCL electrode), and efficient separation processes with electromagnetic activated carbons.

In the production of renewable energy (biofuels) from food waste, the overloading of the reactor with volatile fatty acids in anaerobic digestion processes and increased ammonia nitrogen, which is toxic to microorganisms with its high protein content, reduce the process efficiency. Therefore, activated carbon applications are used to remove these components from the reactor environment (Capson-Tojo et al., 2018).

Activated carbon is also used in the purification of additives such as monosodium glutamate (MSG), glutamic acid, and hydrolyzed vegetable proteins (HVPs), which are commonly used in the food industry (Wang et al., 2017; Kobayashi et al., 2018). Hydrocarbons, cyclic structures, and aromatics, released in food production stages such as

fermentation, cooking, evaporating, condensation, heating, and drying processes, are volatile organic compounds that generally produce bad smell-taste in foods and show toxic, mutagenic, and carcinogenic effects. Furthermore, during the cleaning and disinfection steps, chlorine compounds and other polluting gasses such as $H_2S$, NOX must be removed from the medium. Activated carbons are more economical and reusable adsorbents for removing volatile organic compounds from the medium (Olgun et al., 2017).

In a study, magnetic carbons that were produced with $FeCl_3$, $FeCl_2$, and urea-catalyzed hydrothermal reaction, in which carbohydrates (fructose, glucose, and sucrose) of expired beverages were used as carbon sources, were activated with KOH. Then, their removal with water-soluble methylene blue dye with a 404.73 $mgg^{-1}$ adsorption capacity was achieved (Liu et al., 2019). In another study, in order to increase the absorption of triazine herbicides from model food samples such as milk and rice, the separation of triazine herbicides depending on the hydrophobic interaction between triazine herbicides coated with magnetic modified activated carbon on a nonionic silicon surface and the adsorbent was achieved at a rate of 81 % in food samples (Mohd et al., 2019).

The effects of various food additives on the model organisms used in the study have been tested in different studies. Some of these studies are as follows: It was determined that various preservatives (sorbic acid, potassium sorbate, benzoic acid, sodium benzoate, potassium acetate, sodium metabisulfite, potassium metabisulfite, sodium tetraborate, sodium sulfite, and boric acid) had different effects and that among these substances, potassium acetate had the least effect and sorbic acid had the highest effect. Benzaldehyde, which is used to give flavour to foods, was revealed to be mutagenic and genotoxic (Güneş, 2016).

Activated carbon causing a colour change in the consumed foods leads to the emergence of an unusual appearance such as black toothpaste, black hamburger. In a study conducted with different food dyes, these food dyes were determined to cause a mutagenic effect. The genotoxic effect of tartrazine, one of the food dyes, was investigated by Niraj et al. (1989) with somatic mutation and recombination test in *Drosophila*. As a result of 0.06 % and 0.03 % tartrazine application, the researchers detected that tartrazine had both mutagenic and recombinogenic effects (Niraj et al., 1989).

Among food dyes (erythrosine, indigo carmine, patent blue, amaranth, and carminic acid), the use of more than 25 mg of patent blue, more than 20 mg of carminic acid, more than 6 mg of erythrosine, more than 2 mg of indigo carmine, and more than 10 mg of amaranth were determined to show a lethal effect on the insect, and benzaldehyde, which is used to give flavour to foods, was determined to be mutagenic and genotoxic (Güneş, 2016).

In another study conducted with *Daphnia magna*, the effect of monosodium glutamate (MSG), which is a highly controversial compound and found in the American diet, was investigated. This compound, which is generally used as a supplement for flavour, was reported as being considered safe (U.S. Food and Drug Administration, 2012).

In another study conducted with *Daphnia magna*, the effect of food dyes was evaluated. In the study conducted by Abe et al. in 2017, Basic Red 51 (BR51) dye was observed to be extremely toxic. An increase was detected in the respiratory levels of *D.magna* in the short term, and negative effects were detected in its reproduction in the long term. *Artemia salina*, another organism used in the study, is an organism commonly used in toxicity and ecotoxicity tests.

Activated carbon, which has started to be also used in food products in recent years, attracts attention due to the change it causes in the colour of food products. Its presence also in frequently consumed foods, such as bread, hamburgers, and lemonade, has raised doubts in consumers about its benefits and harms. Determining the genotoxic potential of preservatives used in foods is of great importance in terms of food safety, human health, and quality of life. Even if the preservatives commonly used in foods are used in amounts that do not harm the health, it should be taken into consideration that these substances may accumulate in the body over time and thus threaten human health directly or indirectly. It is necessary to show the utmost care and sensitivity in the use of food additives, to increase the level of awareness of manufacturers and consumers in terms of food safety and to strengthen the control mechanisms related to food health. In this respect, the current study is thought to be valuable in terms of eliminating doubts. Not observing any negative effects in *Drosophila melanogaster*, *Artemia salina*, and *Daphnia magna*, which are important model organisms, creates a hope about the subject that these products containing activated carbon will be consumed safely.

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