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Research Article

Survival Rate and Lifespan in *Drosophila melanogaster* Feeding with White Tea

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Abstract

White tea is a special tea made from the bud and young leaves of some varieties of *Camellia sinensis* L. Kuntze plant. In this study, it was aimed to determine the effects of white tea on larval mortality in *Drosophila melanogaster* and the lifespan. The effect of white tea on the lifespan was studied separately in female and male populations of *D. melanogaster*. An average of 100 individuals for each group was collected from non-mated male and female flies at the same age (1-3 days). Then, these individuals were fed for 2 hours in culture tubes containing white tea at different concentrations (0.5; 1.0; 1.5 and 2.0 mL/100mL medium) and water extracts as the control group. As a result of our study, no decrease was observed in the larval mortality rates at any concentration we applied. This result has been interpreted as that white tea does not have toxic effects in the experimental groups. In the results obtained from the second phase of the study, statistically, significant increases were observed in the lifespan parallel to the increase in concentration. This result was interpreted to have been related to the antioxidant content in white tea.

Keywords: White tea, *Drosophila melanogaster*, Lifespan, Larval mortality

Beyaz Çay ile Beslenen *Drosophila melanogaster*'de Hayatta Kalma Oranı ve Yaşam Süresi

Öz
Beyaz çay, *Camellia sinensis* L. Kuntze bitkisinin bazı varyetelerinin tomurcuk ve genç yapraklarından yapılan özel bir çaydır. Bu çalışmada beyaz çayın *Drosophila melanogaste*r'de larval mortalite ve ömür uzunluğu üzerine etkilerinin belirlenmesi amaçlanmıştır. Beyaz çayın ömür uzunluğu üzerine etkisi, *D. melanogaster'* in dişi ve erkek populasyonlarında ayrı ayrı çalışılmıştır. Pupadan çıkan aynı yaştaki (1-3 günlük) çiftleşmemiş dişi ve erkek sineklerden, her bir grup için ortalama 100 birey toplanmıştır. Daha sonra, bu bireyler farklı konsantrasyonlarda (0.5; 1.0; 1.5 ve 2.0 mL/100mL besiyeri) beyaz çay ile kontrol grubu olan su ekstrelerini içeren şişelerde 2 saat beslenmişlerdir. Kontrol ve uygulama gruplarının tümünde, sayımlara ve uygulamaya en son birey ölene kadar devam edilmiştir. Çalışmamızın sonucunda, uyguladığımız hiçbir konsantrasyonda kontrol grubuna göre larval mortalite oranlarında herhangi bir azalış gözlenmemiştir. Çalışmanın ikinci aşamasından elde ettiğimiz sonuçlarda ise konsantrasyon artışına paralel olarak ömür uzunluğu oranlarında istatistiksel olarak anlamlı artışlar gözlenmiştir. Bu sonucun beyaz çaydaki antioksidan içeriği ile ilgili olabileceği düşünülmüştür.

Anahtar kelimeler: Beyaz çay, *Drosophila melanogaster*, Ömür uzunluğu, Larval mortalite

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1. Introduction

Camellia sinensis (Tea), which has been used in Far East countries for years and spread over Europe, America, and other regions over time, is the most consumed drink in the world after water (Anesini et al. 2008; de Godoy et al. 2013). The beverage of tea is usually consumed in three different forms known as green tea, black tea, and oolong tea. Besides, the use of white tea some of the varieties of which are made from buds and young leaves has been increasing nowadays. It is reported that an average of 2 million tons of tea is produced annually around the world. White tea is the form of tea that is produced in the lowest amount after black, oolong, and green tea having different oxidation levels produced from buds and leaves of the appropriate varieties of the tea plants (Üstün and Demirci, 2013). White tea is a form of tea with slight oxidation grown and collected especially in the Fujian and Zhejiang regions of China (Sartippour et al. 2001; Dashwood et al. 2002). White tea which was first produced only in this region traditionally is produced in India, Sri Lanka, Kenya, Vietnam, Eastern Nepal, and too little in Turkey today (Çimen, 2014). Annual white tea production in the world is around 2000 tons and this amount corresponds to only 0.1% of black tea production. 90% of this production takes place in China (Chen et al. 2003).

The tea forms that resemble each other structurally show different antioxidant effects due to the different compounds they contain. Tea contains a generally large amount of polyphenols in the flavonoid group. Also, the availability rate of catechins from the polyphenol group and epigallocatechin gallate (EGCG) from catechins are the highest. Besides, epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), catechin (C), gallocatechin (GC) and gallocatein gallate (GCG), theaflavins (TF), and thearubigin (TB) are the other compounds found in different amounts (Cooper et al. 2005).

As well as green and black tea is very useful for human health, white tea is known as the least produced tea with the highest level of antioxidant (Chang and Szabo, 2000; Chow et al. 2005). Antioxidants are compounds that protect the body by blocking and neutralizing the harmful effects of free radicals, known as structures that damage the body by disrupting the DNA structure and accelerating aging (Halliwell, 2012). Many clinical experiments in the world have shown that white tea with high amounts of catechin, especially epigallocatechin gallate (EGCG), has many benefits to human health thanks to this component and other important tea components (Kumar et al. 2012; Pérez-Jiménez et al. 2012). It has been believed that white tea had the highest percentage of protein compared to the other forms of tea and this was thought to have resulted from the fact that the harvested part of the white tea was only the buds (Ilgaz et al. 2006; Shukla, 2007). It has been found that flavonoids which are a group of antioxidants in white tea have effective protection against many different types of cancer such as colon, prostate, and stomach cancer (Yang and Wang, 1993; Fassina et al. 2002; McKay and Blumberg, 2002; Almajano et al. 2008), block the growth of cancer cells and prevent the formation of new ones (Çaykur, 2013). Again, it has been determined that it has an antibacterial and antiviral effect due to the antioxidants it contains (Gramza et al. 2005; Nazer *et al.*2005), and it has been demonstrated to lower cholesterol due to its catechins which is another antioxidant group (Roghani and Baluchnejadmojarad, 2010). It is also reported that white tea contains a small amount of fluoride and other nutrients that make the teeth stronger and healthier, reduces blood pressure, protect the heart, strengthens the bones, and accelerates metabolism (Orner et al. 2003). Komes et al. (2009) compared five different types of tea like white tea, green, yellow, oolong, and black tea in terms of caffeine content, listed tea plant in terms of caffeine content as white (3.62%)> yellow (3.18%)> black (2.79%)> oolong (2.77%)> green tea (2.35%). They reported that the amount of caffeine varies according to the origin, genetic, environmental factors, harvesting time, method of processing, and the age of the tea leaf. They stated that young shoots contain higher amounts of caffeine and therefore the amount of caffeine made from young shoots is higher than the others (Üstün and Demirci, 2013). With all these properties, white tea stands out as a unique and special product among other tea forms (Kersten et al. 2000).

In this study, the effects of white tea extract on larval mortality and lifespan were investigated in *D. melanogaster*. Some characteristics that the *D. melanogaster* can study in the eukaryotic organism *in vivo*, have a small number of chromosomes to eliminate complexity; the enzyme systems responsible for the bioactivation have great similarity with the enzyme systems of the mammals and there is no need to take research ethics committee approvals that are required to be taken for the vertebrates used in the other biomedical studies are the important reasons for being preferred as a model organism in genetic studies (Jennings, 2011). About half of the fly proteins have similar sequences as mammalian proteins. Through *Drosophila* genome sequence analysis, it has been shown that more than 60% of the genes identified in humans are *Drosophila* orthologs. This means that around 287 genes that are changed by mutation, amplification, or deletion in human diseases are found similarly in *Drosophila* (Schneider, 2000; Bernards and Hariharan, 2001; Marsh and Thompson, 2006). Of these diseases, the leading ones include developmental disorders, cardiovascular and neurological diseases, metabolism and storage diseases, and genetic-based visual, auditory, and immune system dysfunctions (Bier, 2005). The similarity of all characteristics in *Drosophila* and humans and between cell cycle and cell cycle regulating proteins are among the reasons why *Drosophila* is frequently preferred in cancer research. Besides, since the basic biological mechanisms and molecular pathways of many physiological and neurological events are similar in *Drosophila* and mammalian organisms, this attracted the attention of the scientists studying especially on the fields of biology and medicine and *Drosophila* has become an important non-mammalian model organism (Pandey and Nichols, 2011).

2. Material and Methods

Animals

In this study, larvae and adults belonging to the Oregon-R wild-type (w.t.) strain of *Drosophila melanogaster* were used (Diptera, Drosophilidae).

Plant material

The tea brand Beyaz İksir used in the study was obtained from the ÇAYKUR company. The samples were kept in their original packaging in a dark and cool laboratory environment until analysis was carried out. After taking 20g of white tea, 500 mL of freshly boiled water was added and allowed to brew for 15-20 minutes. The brewed stock tea sample was kept at room temperature and the experimental groups were formed from this stock. Fresh stocks were prepared for each study.

Larval mortality tests

The male and female individuals taken from the stock *Drosophila* individuals in a sufficient number for larval mortality or survival rate tests were transferred to fresh medium for making cross-ways and kept in the incubator at the temperature of 25°C and having 40-60% of relative humidity for 25 days. The third stage larvae (72±4 h) obtained after 3 days were buried in a medium containing different concentrations of white tea solution (0.5; 1.0; 1.5 and 2.0 mL/100mL medium). Distilled water was used for the control group. 100 larvae were used for each experimental group. The mouths of the tubes were closed with cotton plugs and put into the loops and the larvae were ensured to become adults. During this process, all experimental groups were checked daily and counted for 7 days from the first adult fly that was seen in the tube. The count was recorded twice a day, by distinguishing between male and female. All experiments were repeated 3 times.

The experiments on the lifespan

The effect of the white tea solution on *D. melanogaster's* lifespan was studied separately in male and female populations. For this purpose, to obtain individuals of the same age, preliminary stocks were formed by crossing the culture tubes containing fresh food medium. After making cross-ways, all culture tubes were placed in the incubators at the temperature of 25°C and having 40- 60% of relative humidity. After 7 days, the parents were removed from the culture tubes to avoid the interlacing of the parents and the offspring to be coming out. About 10 days after making cross-ways, 100 individuals were recruited for each group from the same elderly (1 to 3 days) unpaired female and the male coming out of the pupa. Collected individuals were taken into empty tubes and left hungry for 2 hours before the application. These individuals were then fed for 2 hours in culture tubes containing White tea solution prepared at different concentrations (0.5, 1.0, 1.5, and 2.0 mL/100 mL medium). At the end of this period, 100 individuals were transferred for each tubes containing Standard Drosophila medium and they were taken to the incubator at the temperature of 25°C and having 40-60% relative humidity. The culture tubes were kept in the incubator during the experiment and the medium was exchanged twice a week. The number of individuals was checked at the beginning and end of each day and the dead individuals were recorded and removed from the medium. Counting and medium exchange were continued in control and experimental groups until the last individual died.

Statistical analysis

Statistical analyzes of the data obtained from the lifespan and larval mortality experiments of the investigated substances were performed with the SPSS 15.0 program. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied. The differences between groups were considered significant at $p<0.05$ level.

3. Results

In our study, it was determined that both the survival rate and lifespan in male and female individuals of *D. melanogaster* increased in parallel with the increase in the concentration of white tea. It was observed that the maximum life expectancy in the female population was 72 days and the mean lifespan was 56.23±1.16 days in the control group. According to the results obtained in the experimental groups, the maximum life span in females with white tea added to the medium was 73 days in the lowest experimental group (0.5mL/100mL) and 82 days in the highest experimental group (2mL/100mL); mean life span values were determined as 58.49±1.02 and 69.83±1.12 days, respectively (Table 1, Figure 1-2).

Table 1. The survival rate and lifespan of male and female populations of *D. melanogaster* and the probability levels between white tea groups

Experiment Groups (mL/100mL) (No)	Female population					Male population				
	N	SR (%)	ML_1	ML_2±SE	*P*	N	SR (%)	ML_1	ML_2±SE	*P*
Control (1)	100	95	72	56.23±1.16		100	94	71	55.79±1.16	
0.5 (2)	100	95	73	58.49±1.02		100	95	72	57.87±1.02	
1.0 (3)	100	96	76	64.58±1.09	1-2* 3-4*	100	95	74	63.71±1.07	1-2* 3-4* 4-5*
1.5 (4)	100	97	78	66.74±1.09		100	97	77	66.18±1.06	
2.0 (5)	100	99	82	69.83±1.12		100	98	80	68.56±1.03	

N: Number of individuals, SR: Survival rate, ML_1: Maximum lifespan, ML_2: Mean lifespan, SE: Standard error, P: Probability level, *: the mean difference is not significant at the 0.05 level

In the control group of the male population, the maximum lifespan was determined to be 71 days and the average lifespan was 55.79±1.16 days in the control group. When Table 1 is examined, it is seen that the maximum lifespan in individuals is 72 days in the lowest experimental group and 80 days in the highest experimental group, while average values of the length of time were found to be 57.87±1.02 and 68.56±1.03 days, respectively.

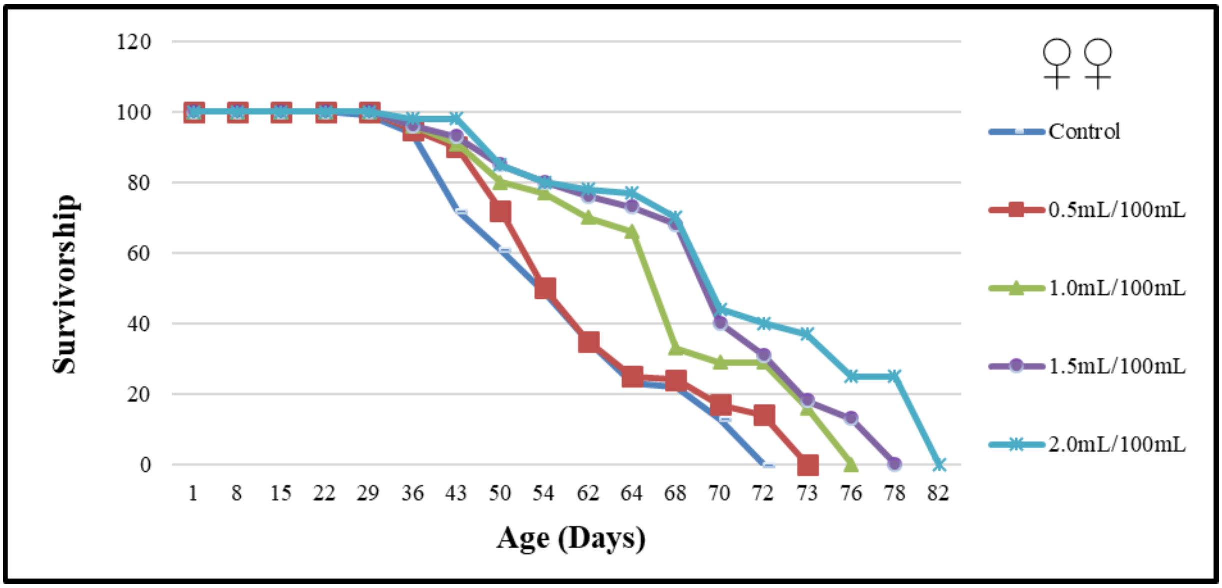

Figure 1. The survivorship lines of female individuals of *D. melanogaster* living medium applied with different concentrations of white tea during adult stages

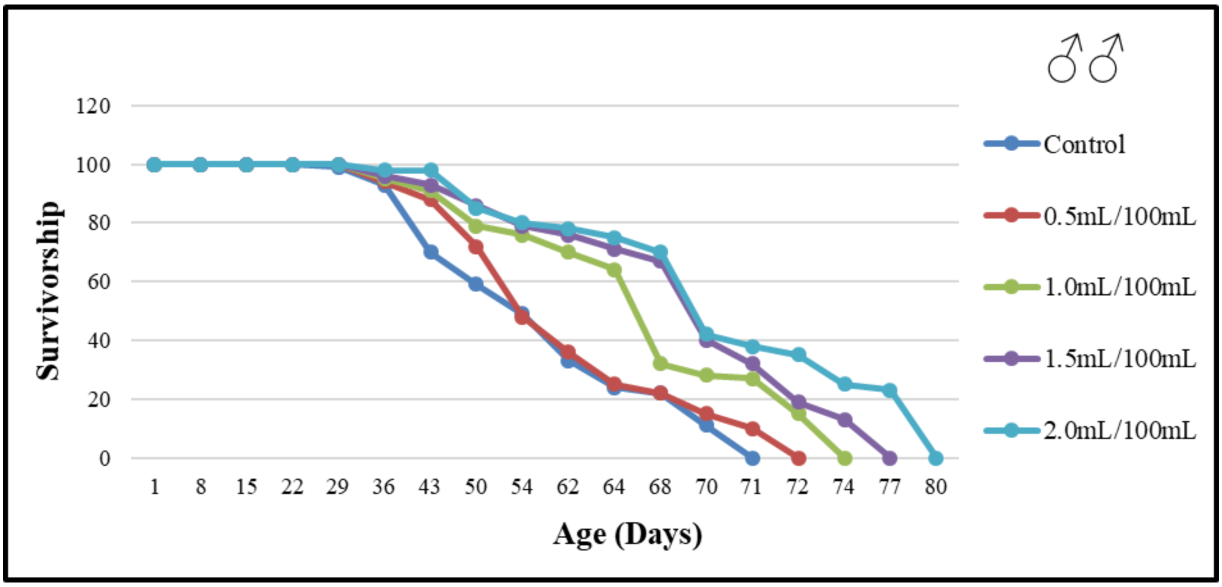

Figure 2. The survivorship lines of male individuals of *D. melanogaster* living medium applied with different concentrations of white tea during adult stages

With the increase in white tea concentration, this increase in the values of maximum and average lifespan belonging to both male and female populations was found to be statistically significant at the $p < 0.05$ level compared to the control groups. Besides, it was observed that in terms of lifespan, female individuals in the experimental groups containing white tea lived longer compared to the male individuals (Table 1). However, these differences observed in terms of average lifespan in sex groups are statistically insignificant ($p > 0.05$). It is also seen that there is a positive correlation between the white tea concentration and the lifespan values of the control and experimental groups. These values are $R = 0.415$ for ♀♀ individuals and $R = 0.408$ for ♂♂ individuals ($p < 0.01$).

4. Discussion and Conclusions

As in our study, in many studies in the literature, the effects of the antigenotoxic and antioxidative effects of various plant extracts having medical value and also used in the alternative medicine have been investigated in *Drosophila* by various test techniques, especially SMART (de Rezende et al. 2009; Patenkovic et al. 2009; Demir et al. 2013). As a result of our study, statistically, significant increases in lifespan were determined depending on the increasing white tea concentration. We believe that all these effects are due to the active compounds in white tea and the antioxidative activity of these compounds. The results obtained from several studies on tea and white tea support these data. For example, it has been detected that the tea contains more than 4000 bioactive compounds which are thought to have broad physiological properties (Çelik, 2006) and these compounds have several activities which are antidepressant (Zhu *et al.*2012), anti-inflammatory (de Magalhães et al. 2012), antioxidant (Costa et al. 2009; Cavet et al. 2011; Carloni et al. 2013), anti-arteriosclerosis (Curin and Andriantsitohaina, 2005), antihypertensive (Hodgson et al. 2005), antibacterial (Weber et al. 2003), antimutagenic (Bhattacharya et al. 2005), anticarcinogenic (Carvalho et al. 2010), antimicrobial (Von Staszewski et al. 2011), antidiabetic (Abolfathi et al. 2012), hypolipidemic (Huang and Lin, 2012), hypocholesterolemic (Maron et al. 2003), nervous system protecting (Almajano et al. 2011), and immune-enhancing (Sheikhzadeh et al. 2011).

With the studies carried out related to the anticarcinogenic effect of tea, it is revealed that the consumption of tea protects by reducing the DNA damage in the cell against the chemical carcinogens which cause the formation of the skin, lung, esophagus, stomach, liver, pancreas, breast, prostate and colon cancers (Sharangi et al. 2009). It is also thought that this preservation duty is made with strong antioxidant properties of tea, especially catechin-derivative polyphenols (Almajano et al. 2008). Vinson and Dabbagh (1998) stated that the antioxidant power of tea catechins is higher than vitamins and listed the antioxidant activity of tea catechins from large to small as EGCG> EGC> ECG> EC. In recent years, it is seen that EGCG has been found to show anti-tumor activity in approximately 20 different tumor lines including prostate cancer, melanoma, multiple myeloma, acute myelogenous leukemia, and chronic myelogenous leukemia (Kumazoe and Tachibana, 2016). Also, in a study in which the antimicrobial effectiveness against 111 different bacteria of the extract prepared from tea leaves by using methanol extraction method, it has been determined that tea extract shows antibacterial effectiveness against bacteria even at low doses (Bandyopadhyay et al. 2005). In other studies on catechins, it has been revealed that catechins protect cell membranes against oxidation by keeping reactive oxygen derivatives in closed regions; they block cell membrane receptors for the growth of cancer cells and suppress certain specific enzymes required for the initiation of carcinogenesis. Besides, it has been shown that the polyphenols that tea contains inhibit angiogenesis, metastasis, and cell proliferation and in different cell lines and animal models and induce apoptosis by regulating multiple signaling pathways (Almajano et al. 2011).

Despite the studies conducted on various forms of tea, especially black and green tea, there are very few studies on the anticarcinogenic potential of white tea in the literature. However, some studies have been carried out to reveal that white tea has antineoplastic effects in lung cancer cells (Mao et al. 2010) and can protect human skin from sunlight ultraviolet rays (Camouse et al. 2009). In a study comparing the antimutagenic activity of white and green tea with the Salmonella test, it was emphasized that white tea had stronger antimutagenic activity and that this power could be caused by higher concentrations of white tea components such as caffeine, gallic acid, theobromine, EGC and ECG (Santana-Rios et al. 2001). In a study carried out to determine the antioxidative activity of white tea, significant increases were observed in antioxidative enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) after the application of white tea to mice (Espinosa et al. 2014).

In similar studies previously carried out, it has been emphasized that white tea has higher protective properties against bacteria, fungi, and viruses in the body than other forms of tea (Enzveiler et al. 2011; Zhao et al. 2013; Mitra et al. 2016), and it is good for tooth and gingival diseases (Vanka and Vanka, 2012). As a result of a histopathological study with mice; white tea extracts have hepatoprotective effects in mercury-induced acute liver injuries and these results are related to antioxidant, antitoxic and antiapoptotic properties in white tea (Abdella, 2017). In the study performed with Streptozotocin and mice that were made in prediabetic patients, the epididymal sperm viability in mice increased and the negative effects of streptozotocin on sperm number and quality were reduced due to the use of white tea (Dias et al. 2016). As a result of the study carried out by Mao et al. (2010), it has been shown that white tea extracts can induce apoptosis in cell lines of non-small cell lung cancer (NSCLC). It has been determined that white tea performs this induction partly by upregulation of PPAR-γ and 15-lipoxygenase (15-LOX) signaling pathways and by increasing the activity of Caspase-3; therefore, it can be used as an antineoplastic and chemopreventive agent for lung cancer.

Today, alternative medicine is used as a new approach for the treatment of diseases. Plants and their antioxidant capacities among these types of treatments have an important role in the activities' expanding lifespan. For this reason, it is thought that white tea can be used as a medicinal plant in both the food and medicine sectors, and with becoming widespread of traditional usage, its gainings will be high in terms of both health and national economy.

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