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Determination of the Effects of Genistein on the Longevity of *Drosophila melanogaster* Meigen (Diptera; Drosophilidae)

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Abstract In this study, the effects of genistein on the longevity of *Drosophila melanogaster* were investigated. The effects of different concentrations of genistein (1, 3, 5 and 10 μM/100 mL medium) were separately administered one by one to female and male populations of *D. melanogaster* for application groups. In the control group, the maximum life span was determined to be 57 days for ♀♀, 46 for ♂♂. The maximum life span for the lowest (1.0 μL) and highest (10.0 μL) application groups among the adult populations of *D. melanogaster* subjected to genistein were observed to be 54, 50, 40 and 36 days for ♀♀ and 51, 48, 40 and 33 days for ♂♂. These values indicate a negative correlation ($R = 0.513$ for ♂♂ and $R = 0.509$ for ♀♀) between the maximum life span of the application groups and changing genistein concentrations.

Keywords Aging · *Drosophila melanogaster* · Genistein · Longevity

Plant estrogens (phythoestrogens) are substances that have different chemical forms which set out their effects by connecting to estrogen receptors (An et al. 2001). There are many naturally occurring plant derived estrogenic substances with similar biological effects in the natural and cultivated plants and seeds used for feeding humans and animals (Turner et al. 2007). Colborn et al. (1996) have reported 20 different substances including plant estrogens obtained from about 300 different plants. These estrogenic substances can be listed as isoflovanes (genistein, glisitein, bichanin A, prunetin, daidzein and formononetin), triterpenes, stilbens, fenantrens, steroids, cumestanes, galangin and bitter acids (Albertazzi and Purdie 2002). The first scientific articles about plant estrogens that are also named phytohormones were written during the 90s (Bedigian 2007). Although plant derived estrogens can be found in various plants in small quantities, it has been determined in large quantities inside soy and flax seed (Colborn et al. 1996). Epidemiological data show that the consumption of plant estrogens offer protection especially for hormone based diseases (Adlercreutz et al. 1991). In societies with irregular eating habits, this has resulted in the excessive consumption of foods that contain high amounts of these estrogenic effective substances (Davis et al. 1999). As a result of some undesired consequences of this excessive consumption, scientists have taken an interest in the question about whether these substances are really beneficial or not (Wanibuchi et al. 2003). Today, it has been shown that in some cases these substances cause more harm than good. Especially the plant estrogens genistein, daidzein and zearalenon have been characterized to be genotoxic (Stopper et al. 2005). Several environmental factors and chemicals affect the metabolisms of various organisms, and investigations in this regard have increased recently (Ekinci and Beydemir 2010). Also, genotoxic effects of various substances have been characterized over the past years (Ceyhun et al. 2010; Aksakal et al. 2010). Genistein is an aquatic pollutant plant estrogen which can

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be found in large quantities in especially soy and soy based food (Liu et al. 2010). In some soy manufacturing Asian countries, such as China and Japan, this plant estrogen is naturally taken due to high amounts of soy consumption (Adlercreutz et al. 1991). Besides the usage of this substance in alternative treatments of various cancer types, some ailments due to menopause and cardiovascular diseases (Adlercreutz and Mazur 1997) have also been determined.

In this study, the effects of different concentrations of the plant estrogen genistein on the longevity of *Drosophila melanogaster* were investigated.

Materials and Methods

The flies used in the experiments belong to Oregon R wild type (w.t.) strain of *Drosophila melanogaster* Meigen (Diptera; Drosophilidae). This stock had been maintained for many years in the laboratory of the Department of Biology, Atatürk University in Erzurum and highly inbred with little genetic variation. The flies were kept at a constant temperature of 25 ± 1°C on standard medium composed of maize-flour, agar, sucrose, dried yeast and propionic acid (Standard Drosophila Medium = SDM). The flies were kept in darkness, except during the transfers onto fresh medium (usually twice weekly). The humidity of the experimental chamber was 40–60%, and the females used in this experiment were virgins.

In this study, the effects of genistein (purity: 98%, Sigma) on longevity were studied separately in female and male populations. The experiments were repeated three times. To obtain the even aged flies, adult individuals were mated in the culture vials including only SDM, and prestocks were prepared. On an average, 100 individuals were collected from among the even aged female and male flies which were not mated and obtained from pupa. Then, the gathered individuals were put into the empty culture vials, and they were starved for 2 h before genistein application. For application groups, two layers of blotting papers were placed into each culture vial, and genistein in different concentrations was absorbed into these papers. Afterwards, the gathered flies put into the application vials were left there for 2 h. Following the application, 100 individuals put into one vial for application (separately applied for female and male flies) were placed into the culture vials containing only SDM as 25 by 25. The experiments for both control and application groups were initiated at the same time. All vials were kept in appropriate thermal cabins. During the experiments, food was refreshed twice a week. The number of individuals was controlled both at the beginning and at the end of each application day, and dead individuals were registered and then removed from the culture vials. The application was carried out until the last individual died. The experiments were repeated three times for each group. The obtained data were analyzed with SPSS (version 13.0). One way analysis of variance and Duncan's multiple range test was used for the mean longevity values of the control and applications groups (Duncan 1955). $p < 0.001$ was considered to be a significant difference.

Results and Discussion

We have determined in this study that genistein decreased the maximum mean life span of the female and male *D. melanogaster* populations in comparison to the control.

In the control group, the maximum life span was determined to be 57 days for ♀♀, 46 for ♂♂; whereas in the Control + DMSO group it was determined to be 57 days for ♀♀ and 55 days for ♂♂. The difference between the Control and Control + DMSO groups was not statistically significant ($p > 0.001$) (Table 1).

The maximum life span for the lowest (1.0 μL) and highest (10.0 μL) application groups among the adult

Table 1 The longevity of male and female populations of *D. melanogaster* and the probability levels between groups

Experiment groups	Female population				Male population			
	N	Max. life span	SD	Mean life span ± SE	*N*	Max. life span	SD	Mean life span ± SE
Control	100	57	11.97	40.18 ± 1.19^{a}	100	56	11.35	38.18 ± 1.13^{a}
Control + DMSO	100	57	12.05	39.62 ± 1.20^{a}	100	55	11.51	37.65 ± 1.15^{a}
1.0	100	54	11.05	34.03 ± 1.10^{b}	100	51	10.07	32.18 ± 1.00^{b}
3.0	100	50	11.81	$31.50 \pm 1.82^{b,c}$	100	48	9.88	30.50 ± 0.98^{b}
5.0	100	43	8.84	$26.96 \pm 0.88^{c,d}$	100	40	7.78	25.99 ± 7.78^{c}
10.0	100	36	6.53	22.64 ± 0.65^{d}	100	33	5.90	21.93 ± 5.90^{c}

N Total number of individuals; *C* Control, *Max*. Maximum; *SE* Standard error; ^{a,b,c,d,e} Means each column followed by the same letter are not different by Duncan's one way range test alpha = 0.001

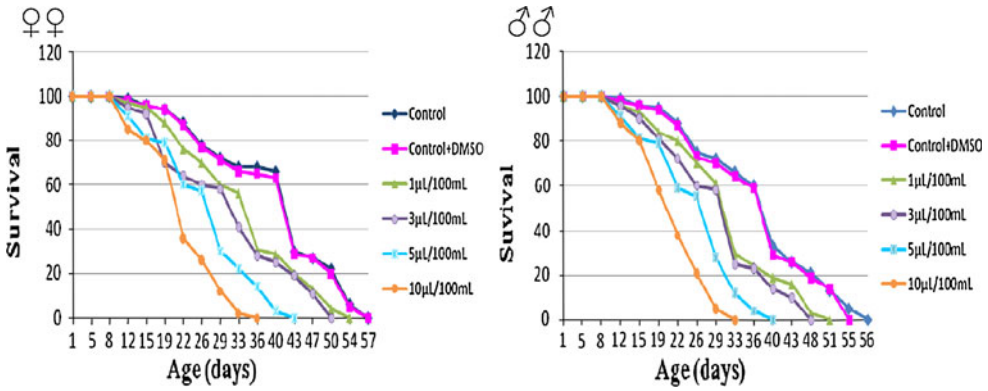

Fig. 1 The survival curves of female and male individuals of the *D. melanogaster* living medium applied with different concentrations of genistein during adult stages. Both female and male flies belong to application groups showed significantly shorter life spans than the control (both $p < 0.001$)

populations of *D. melanogaster* subjected to genistein were observed to be 54, 50, 40 and 36 days for ♀♀ and 51, 48, 40 and 33 days for ♂♂. These values indicate a negative correlation ($R = 0.513$ for ♂♂ and $R = 0.509$ for ♀♀) between the maximum life span of the application groups and changing genistein concentrations. As seen in Fig. 1, mean life span of the female and male *D. melanogaster* populations decreased with increasing concentrations of genistein. According to the results obtained from the application groups, the maximum mean life span in the ♀♀ population has dropped from 34.03 ± 1.10 to 22.64 ± 0.65 whereas the maximum mean life span of ♂♂ population has been observed to drop from 32.18 ± 1.00 to 21.93 ± 5.90. Table 1 and Fig. 1 shows the maximum difference in mean life span.

In the current study, the environmental and internal factors that might affect the longevity of the groups have been dropped down to a minimum at the application medium. The only variable parameter in our experiments is the genistein which was applied in different concentrations. Data of this study demonstrate that high concentrations of genistein plays a role in governing the lifespan of *D. melanogaster* during normal metabolism and in the case of environmental stress. In present study, we can say that the reason that application groups survive shorter than control group is due to the oxidative damage of high concentrations of genistein.

Genistein is found in large quantities in some milk and infant formulas with additives sold for use on babies and also in some foods with additives used as an alternative to hormone renewal treatments on women (Setchell et al. 1997). After various studies showing that these substances can cause cancer, serious proofs have been found especially for the dangers of milks and infant formulas with soy additives (Kulling et al. 2001). Genistein has displayed various in vitro effects on female mice and rats such as decrease of fertility, anomaly in the estrous cycle, decrease of ovary size and loss of function of ovary (Jefferson et al. 2007). Lazzari et al. (2008) observed the toxic effects of fifteen different chemicals including genistein on cattle, and reported that genistein has negative effects on fertilization and oocyte maturing at doses of 0.4–40 µM.

Dietary factors, including restriction of caloric intake, restriction of protein or methionine intake, or the ingestion of specific nutrients as genistein, have been shown to alter animal lifespans (Le Bourg 2001; Page et al. 2010). There were found no direct results concerning the effect of genistein on the lifespan of *Drosophila* in the literature review. Because of this, our findings were evaluated in the light of the results derived from different organisms and related compounds. Similar to our study, Wu et al. (2002) did not observe a positive effect of quercetin, a soy isoflavone as genistein on the lifespan of *Caenorhabditis elegans*, a model organism as *Drosophila*. Longevity and aging are two of the most important issues studied and are related to all living organisms. With this study, we evidence specific aspects of genistein on aging and longevity of *Drosophila melanogaster.*

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