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RESEARCH ARTICLE

Extended longevity of *Drosophila melanogaster* by water and ethanol extracts of *Stachys lavandulifolia*

Deniz Altun[1], Arif Ayar[2], Handan Uysal[2], Ayşe Aydan Kara[2], and Elif Leman Ünal[2]

[1]*Department of Biology, Faculty of Art and Science, Erzincan University, Erzincan, Turkey, and* [2]*Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey*

Abstract

Context: *Stachyss* species have been used as a medicine for centuries throughout the world. *Stachys lavandulifolia* Vahl. var. *lavandulifolia* (Lamiaceae) is of interest to researchers because the constituents such as betulinic acid, oleanolic acid, rosmarinic acid, and ursolic acid are similar to other *Stachys* species commonly used as an alterative in medicinal preparations.

Objective: The present study investigated the effects of water extract (SLE_w) and ethanol extract (SLE_e) obtained from *S. lavandulifolia* (*SL*E) on the longevity of *Drosophila melanogaster* Meigen.

Materials and methods: The effects of different concentrations of *SL*E (Control+DMSO; 4.0; 12.0 and 20.0 µL/100 mL medium) were administered separately to female and male populations of *D. melanogaster* for control and *SL*E groups.

Results: In all application groups, each population's longevity increased, depending on the concentration of *SL*E. The mean life-span of the extract groups which are applied with $SLE_{(w)}$ was determined to be shorter than the extract groups which are applied with $SLE_{(e)}$. For example, the maximum mean life-span applied with $SLE_{(w)}$ increased from 31.86 ± 0.92 days to 43.21 ± 1.33 days and the maximum mean life-span applied with $SLE_{(e)}$ increased from 31.86 ± 0.92 days to 49.62 ± 1.62 days in females.

Conclusion: These findings demonstrate that the constituents of *S. lavandulifolia* have great potential as a source for natural health products for *D. melanogaster* management.

Keywords: *Lamiaceae; aging; antioxidant*

Introduction

Phytotherapy is now accepted as a part of medicine rather than an alternative medicine. It has been used in the treatment of many human and animal diseases including cancer treatment (Phillipson & Anderson, 1989). Even in Germany, where there is less plant diversity, more than 500 plants are utilized for medical purposes. In Turkey, which has a rich diversity of plants, there are approximately 10,000 species and 30% of these species are not found in other countries (Bhattacharjee, 1982).

These plants are utilized as regional remedies, but since little is known about the characteristics of these plants, the usage is limited. *Stachys lavandulifolia* Vahl. (Labiatae) is widely used by the people of south Anatolia (Mabberly, 1997) for the treatment of gastrointestinal diseases, stomach aches, and as an appetizer.

In this study, the effects of water and ethanol extracts of *S. lavandulifolia* aerial parts on the longevity in male and female population of *Drosophila melanogaster* are evaluated.

Materials and methods

Experimental animals

The flies used in the experiments were Oregon R wild type (w.t.) strain of *Drosophila melanogaster* Meigen (Diptera;

Address for Correspondence: Handan Uysal, Department of Biology, Faculty of Science, Atatürk University, 25240 Erzurum, Turkey. Tel.: +904422314336; Fax: +904422360948; E-mail: hauysal@atauni.edu.tr

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Drosophilidae). This stock had been maintained for many years in the Laboratory at the Department of Biology of the Atatürk University in Erzurum and was, therefore, highly inbred with little genetic variation.

Laboratory conditions

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%. The females used in this experiment were virgins.

Plant material

The flowering aerial parts of *Stachys lavandulifolia* was collected in Erzurum region of Turkey (Eastern Anatolia) near the Palandöken Mountain at 1980 m height in July 2005 during the time of flowering. The plant was identified and a voucher specimen was deposited in the Ata Herbarium (no. ATA-9808) by Meryem Şengül from the Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey. The freshly picked flowers and leaves of the plant were shade dried at room temperature for 3 weeks.

Extract preparation

The aerial parts of the plant sample (50 g) were separately extracted with 150 mL ethanol (96%, analytical grade, Merck, Darmstadt, Germany) at room temperature three times. The organic solvent was evaporated to dryness under vacuum at low temperature using a rotary evaporator. To obtain the water extract, 50 g plant sample was kept in 250 mL boiling water for 10 min and filtered. Then the water solution was lyophilized by using a Labconco 117 freeze-dryer (Cakır et al., 2003). The dried extract was later dissolved in dimethylsulfoxide (DMSO) (99.9%, Sigma, St. Louis, MO) followed by culture medium and prepared in different concentrations.

The application of Stachys lavandulifolia *extract to adult individuals*

In this study, the effects of *SL*E on longevity were studied separately in female and male populations. The experiments were repeated three times. To obtain the same age flies, adult individuals mated in the culture vials including only SDM and prestocks were prepared. On average, 100 individuals were collected from among the same 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 the *SL*E application. For the application, two layers of blotting papers were placed into each culture vial, $SLE_{(w)}$ and $SLE_{(e)}$ in different concentrations was absorbed into these papers. Afterwards, the gathered flies put into the application vials were left 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 × 25. The experiments for both control and application groups were started synchronically. All the vials were kept in appropriate thermal cabins. During the experiments, food was replaced with fresh food twice a week. The number of individuals was counted both at the beginning and at the end of each application day, and the dead individuals were registered and then removed from the culture vials. The application was carried out until the last individual died.

Statistical analyses

The obtained data were analyzed with SPSS version 13.0 (Statistical Package for the Social Sciences Software, SPSS, Chicago, IL). The mean longevity values of the control and application groups were subjected to Duncan's one-way range test ($p<0.001$).

Results

In this study, it was determined that the water extract (SLE_w) and the ethanol extract (SLE_e) obtained from *Stachys lavandulifolia* (*SL*E) increased the maximum lifespan of male and female population according to control group which belongs to *D. melanogaster*. It was observed that the maximum female lifespan of the control group was 50 days; the maximum lifespan of males belonging to the control group was 47 days and the maximum male and female lifespan of the Control+DMSO group were 47 and 43 days, respectively. The difference between control and Control+DMSO groups is not statistically significant ($p>0.001$) (Table 1).

According to results obtained from application groups, in the female population of *D. melanogaster* applied with $SLE_{(w)}$, the maximum lifespan was 50 days for the lowest application group (4.0 μL) and the maximum life-span 57 days for the highest application group (20.0 μL). It was also found that the maximum male life-span in 4.0 and 20.0 μL application groups were 50 and 54 days, respectively (Figure 1).

In adult populations of *D. melanogaster* applied with $SLE_{(e)}$, the female life-span within the lowest (4.0 μL) and highest (20.0 μL) application groups is 57–68 days, whereas the male life-span is 50–64 days (Figure 2).

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

Experiment groups (µL) (no.)	Female population N	Max. life-span	SD	Mean life-span ± SE	p	Male population N	Max. life-span	SD	Mean life-span ± SE	p
Control (1)	100	50	9.267	31.86 ± 0.92		100	47	9.273	30.93 ± 0.92	
C+DMSO (2)	100	47	9.571	30.57 ± 0.95		100	43	8.848	28.2 ± 0.88	
4 w (3)	100	50	9.246	32.27 ± 0.92		100	50	10.058	32.0 ± 1.0	1vs.2*
4 e (4)	100	57	12.807	36.96 ± 1.28	1-2*	100	50	12.037	34.75 ± 1.2	1vs.3*
12 w (5)	100	54	12.105	38.83 ± 1.21	1-3*	100	52	12.686	37.76 ± 1.26	2vs.3*
12 e (6)	100	61	15.221	41.84 ± 1.52	2-3*	100	57	14.944	39.23 ± 1.49	4vs.5*
20 w (7)	100	57	13.312	43.21 ± 1.33		100	54	13.514	40.84 ± 1.35	
20 e (8)	100	68	16.28	49.62 ± 1.62		100	64	15.729	46.0 ± 1.57	

N, total number of individuals: C, control; Max., maximum; SE, standard error; SD, standard deviation; w,water extract, e,ethanol extract; p, probability levels between groups; *the mean difference is not significant at the 0.001 level.

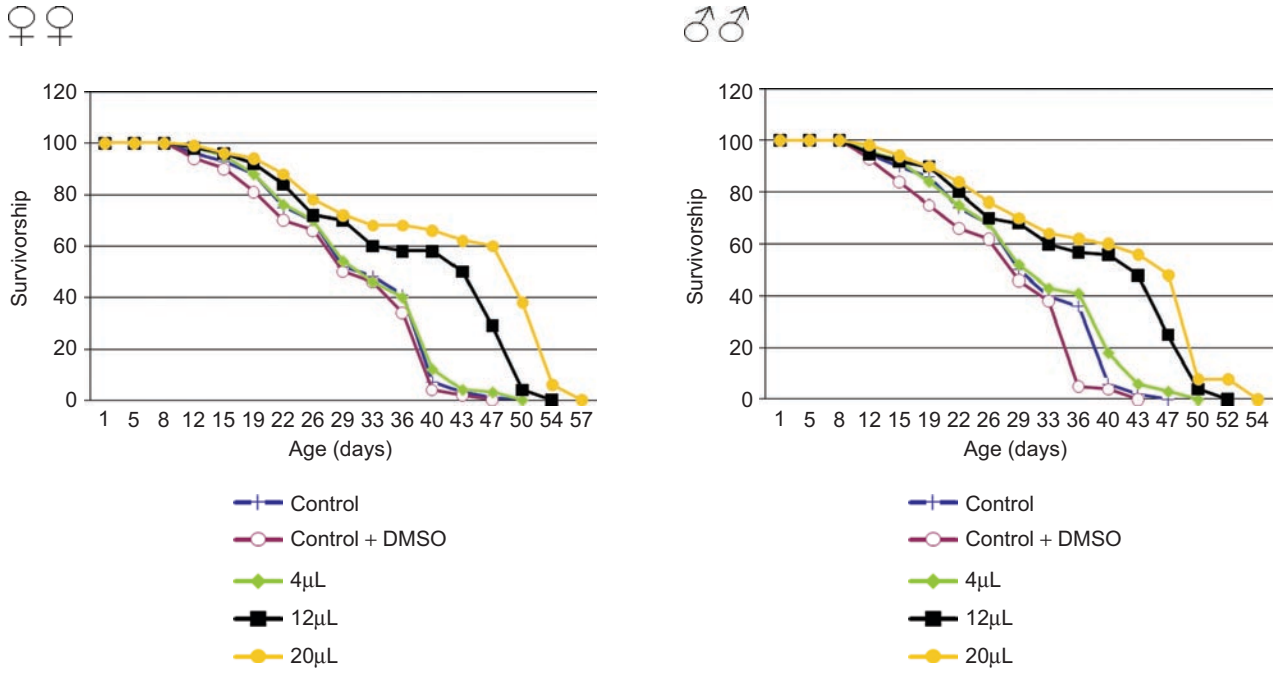

Figure 1. The survivorship lines of female and male individuals of *D. melanogaster* living medium applied with different concentrations of $SLE_{(w)}$ during adult stages.

On the other hand, the present results indicated that $SLE_{(e)}$ was more potent than $SLE_{(w)}$ (Table 1). When the application groups of the females were compared with control group separately, the maximum mean life-span applied with $SLE_{(w)}$ increased from 31.86 ± 0.92 days to 43.21 ± 1.33 days and the maximum mean life-span applied with $SLE_{(e)}$ increased from 31.86 ± 0.92 days to 49.62 ± 1.62 days. As in the females, the maximum mean life-span applied with $SLE_{(w)}$ increased from 30.93 ± 0.92 days to 40.84 ± 1.35 days, and the maximum mean life-span applied with $SLE_{(e)}$ increased from 30.93 ± 0.92 days to 46.0 ± 1.57 days in the males. The difference observed in terms of mean life-span was statistically significant ($p<0.001$) in both sexes.

Discussion

In our study, external (environmental) or internal factors that may affect the longevity of the groups were reduced to minimum levels in the application environment. One of these factors is photoperiod and it was determined to be effective during the period of laying egg and coming out of pupa (Qiu & Hardin, 1996), on metabolic velocity (Lanciani et al., 1991) and the longevity (Sheeba et al., 2000) of *D. melanogaster*. Individuals of control and experimental groups were removed from the incubator only during nutrient exchange and thus the impact of the light on the longevity was corrected. In our study standard drosophila

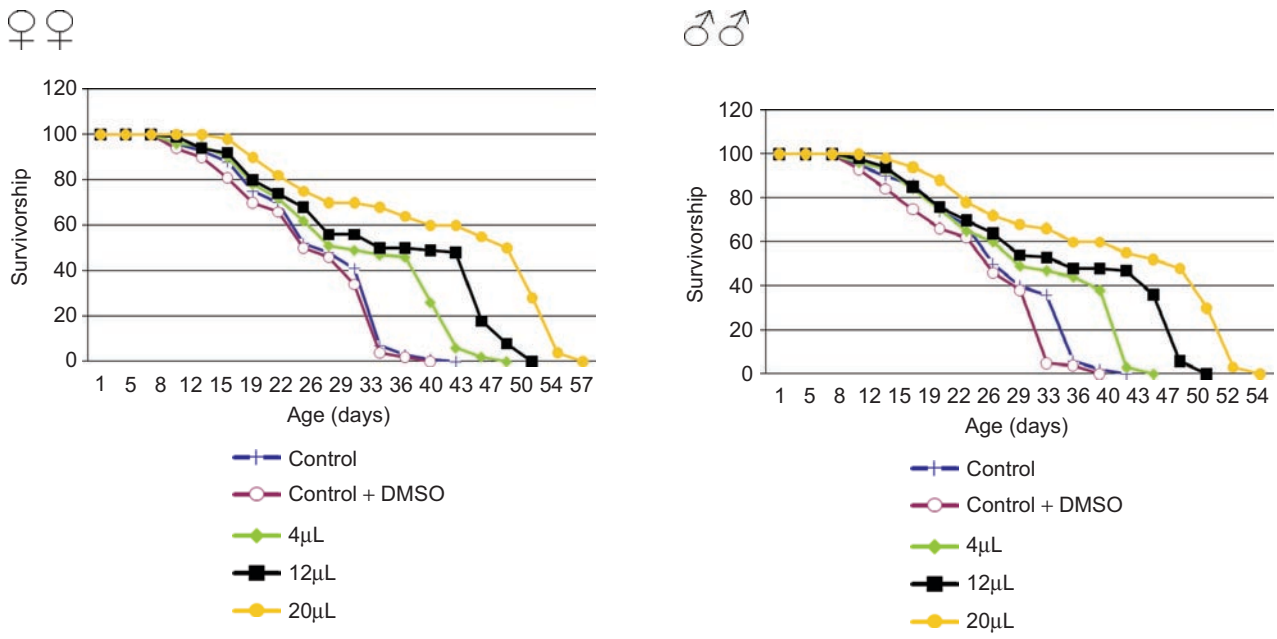

Figure 2. The survivorship lines of female and male individuals of *D. melanogaster* living medium applied with different concentrations of $SLE_{(e)}$ during adult stages.

medium (SDM) was used instead of different types of food, which might affect longevity (David et al., 1975). Maternal age is known to be an important factor on longevity of offspring (Sorensen & Loeschcke, 2002). Therefore, in our experiments, the individuals which were handled as a result of the match between virgin males and females of the same age (3 days) were used. Bradley TJ, Simmons FM (1997), determined by ten different populations of *Drosophila,* ascertained that longevity was increased when metabolic wastes did not exist in the medium. So, the negative effect of waste on the longevity was corrected via frequent renewal of the medium.

The above-mentioned internal and external factors that may affect longevity were fixed during our experiment. The only variable parameter in our experiment was *S. lavandulifolia* extract, which was practiced at different concentrations.

Plants belonging to the genus *Stachys* have long been applied to treat various diseases such as sclerosis of the spleen, genital tumors, inflammatory tumors, and cancerous ulcers (Skaltsa et al., 1999). Saeedi et al. (2008) observed that methanol extracts of various *Stachys* species such as *S. byzantina* K. Koch, *S. inflata* Benth., *S. lavandulifolia* and *S. laxa* Boiss. & Buhse had antimicrobial effects especially on Gram (+) microorganisms. In addition, Hajhasemi et al. (2006) determined analgesic and anti-inflammatory effects of *Stachys lavandulifolia* extracts on mice. Many studies have confirmed that extracts or components of *Stachys* species display significant antitoxic (Zinchenko et al., 1981), antinephritic (Hayashi et al., 1994; Savchenko & Khvorostinka, 1978), anti-anoxia (Yamahara et al., 1990), hypotensive (Takeda et al., 1997), and anti-anxious (Rabbani et al., 2005) effects.

Phytochemical investigations show that *Stachys* species consist of flavonoids, terpenes, phenyl ethanoid glycosides, and saponins (Khanavi et al., 2005). Janicsak et al. (2006) found higher amounts of oleonolic and ursolic acids in many *Stachys* and *Salvia* species. Both oleonolic acid and ursolic acid, which are rather similar because of the closeness of their chemical structures, have many important pharmacological effects. The literature includes numerous data on anti-inflammatory (Safayhi & Sailer, 1997), antitumor (Ovesna et al., 2004), anti-HIV (Kashiwada et al., 1998), antimicrobial (Mallavadhani et al., 2004.), antifungal (Rocha et al., 2004), hypoglycemic (Perez et al., 1998) and antihyperlipidemic (Ma, 1982) properties.

α-Pinene, myrcene, β-phellandrene, and β-caryophylene were isolated at different stages of growth as pre-flowering, flowering, and post-flowering of *S. lavandulifolia* by hydro distillation (Meshkatalsadat et al., 2007). Seventy-nine compounds were identified, representing 98.2% of the essential oil of *S. lavandulifolia*, in which the major components were germacrene-D, β-phellandrene, β-pinene, myrcene, α-pinene, and *Z*-*β*-ocimene (Javidnia et al., 2004). Forty-four components were identified representing 89.6% of the oil of *S. lavandulifolia*, which was particularly rich in monoterpenes and sesquiterpenes. Among the monoterpene fractions, oxygenated compounds were only in small percentages as compared with the hydrocarbons. α-Pinene, β-pinene, spathulenol, and germacrene-D were found

to be the major constituents (Feizbaksh et al., 2003). Also, Mortaza-Semnani et al. (2006) identified major components such as 4-hydroxi-4-methyl-2-pentanone, α-pinene, and hexadecanoic acid in the essential oil of *Stachys lavandulifolia*.

In a study on rats with the α-pinene obtained from the essential oils of *Foeniculum vulgare* Mill. var. *dulce* (Apiaceae), it was reported that α-pinene had a protective effect on hepatotoxicity caused by carbontetrachlorore (Ozbek et al., 2006).

Jafari et al. (2008) found that the extract obtained from *Rosa damascena* Mill. (Rosaceae) reduced mortality in the adult *Drosophila melanogaster*. In previous studies, it was determined that the water extract obtained from *Usnea longissima* Ach. (Ascomycota, Parmeliaceae) had remedial effects on longevity and some development stages of *D. melanogaster* (Uysal et al., 2009a, 2009b). Uysal et al. (2010) also showed that the extract obtained from *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae) increased the longevity of male and female population of *D. melanogaster*. The extracts of green tea and broccoli increased the activity of antioxidant enzymes thus reducing the high percentage of mortality in *D. melanogaster* caused by the consummation of fatty nutrients, (Man Li et al., 2008).

The balance between oxidants and antioxidants changes depending on age. Metabolism functions as oxidant by-products of normal energy metabolism extensively damage DNA, proteins, and other molecules in the cell, and this damage accumulates with age. Therefore, antioxidant activity is a basic requirement for life (Cook & Samman, 1996). Antioxidant defense systems including antioxidant enzymes, food, and drugs are important for the prevention of many diseases (Yen & Hsieh, 1998). By aging, *in vivo* activities of the antioxidant enzymes (catalase, glutathione peroxidase, etc.) and the levels of reduced glutathione were observed in *D. melanogaster*. For the organism, the consumption of fruit and vegetables, which are the main source of antioxidants in the diet, may create a protective effect with endogenous enzymatic antioxidant defense systems (Ames et al., 1993). Mantle et al. (2000) determined that with the help of flavonoids and saponins, the extracts of the some *Stachys* species have shown antioxidant activity. The antioxidant activities of these compounds can exert their antioxidant activity through various mechanisms, for example, by scavenging radicals, which initiate lipid peroxidation and lipid peroxide radicals, binding metal ions, and inhibiting enzymatic systems responsible for free radical generation (Lebeau et al., 2000).

In the present study we can say that the reason the *Stachys lavandulifolia* extract groups survived longer than the control group is due to the antioxidant activity of *Stachys lavandulifolia* mentioned above. Most likely, *S. lavandulifolia* antioxidant properties cause healing effects on organisms and delay aging. This effect may act by reducing the formation of free oxygen radicals which increase with ageing and preventing oxidative damage.

Conclusions

Longevity and aging are two of the most important issues studied, and are related to all living organisms. Although there is much published regarding the chemical content of *Stachys lavandulifolia*, there are not many studies on the effects of the chemical content on longevity and aging of living organisms. With this study, the findings obtained may create a basis to analyze the subject further.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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