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The Effects of *Punica granatum* L. Ethanol Extract Including the Antioxidant Flavonoids on *Drosophila melanogaster* Lifespan

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Abstract
The present study investigated the effects of ethanol extract obtained from *Punica granatum* (Pge) on the longevity of *Drosophila melanogaster* Meigen. The effects of different concentrations of Pge (10.0; 30.0; 50.0 and 100.0mg/mL) were administered separately to female and male populations of *D. melanogaster* for the control and Pge groups. In all application groups, each population's longevity increased, depending on the concentration of Pge. These findings demonstrate that the constituents of *P. granatum* have great potential as a source for natural health products for *D. melanogaster* management.
Keywords: Antioxidant, *Drosophila melanogaster*, Longevity, *Punica granatum*

INTRODUCTION

The consumption of fruits and vegetables and their extracts is considered to be a safeguard against various diseases [1]. *Punica* is a small genus of fruit-bearing deciduous shrub or small trees [2]. Its better-known species is the pomegranate. The pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively in the folk medicine of many countries [3]. Polyphenols are the major constituents of fruit and vegetable diets and are believed to elicit a number of biological properties due to their antioxidant and anti-carcinogenic activities [4]. Pomegranate fruit consists of two major classes of polyphenols, flavonoids and ellagitannins. The flavonoids include quercetin, kaempferol, andmyricetin [5]. The biological activities, viz. Antibacterial [6-8], antifungal [9-11], anthelmintic [12, 13], anti-tumour [14], anti-diarrhoeal [15], antiulcer [16], antifertility [17, 18], antioxidant and anti-inflammatory [19, 20] of the various extracts/constituents of different parts of this plant have also been reported.

The pomegranate is a symbol of life, longevity, health, femininity, fecundity, knowledge, morality, immortality and spirituality, if not divinity [21]. The aim of this study was to investigate the effects of *Punica granatum* on longevity, in particular the antioxidant effects of male and female of fruit flies.

MATERIALS AND METHODS

Experimental animals

The flies used in the experiments were the Oregon R wild type (w.t.) strain of *Drosophila melanogaster* Meigen (Diptera; 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. The flies were kept at a constant temperature of 25 ± 1°C on a 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 a 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 *Punica granatum* (pomegranate) were collected in Erzincan region of Turkey in July 2009 and their identities were confirmed by Dr Meryem Şengül from the Department of Biology, Atatürk University, Erzurum, Turkey. The freshly picked flowers and leaves of the plant were shade dried at room temperature for 3 weeks.

Preparation of the extract

The aerial parts of the plant sample (50g) were separately extracted with 150mL 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, a 50g plant sample was kept in 250mL boiling water for 10 minutes and filtered. The water solution was then lyophilized using a Labconco 117 freeze-dryer [22]. The dried extract was later dissolved in dimethyl sulphoxide (DMSO) (99.9%, Sigma, St. Louis, MO) followed by a culture medium and prepared in different concentrations.

The application of Punica granatum extract to adult individuals

In this study, the effects of Pge on longevity were studied separately in female and male populations. The experiments were repeated 3 times. In order to obtain flies of the same age, adult individuals mated in the culture vials including only SDM and pre-stocks 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. The gathered individuals were then put into the empty culture vials and starved for 2 hours before the Pge application. For the application, two layers of blotting papers were placed into each culture vial; Pge in different concentrations was absorbed into these papers. Afterwards, the gathered flies that were put into the application vials were left for 2 hours. 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 X 25. The experiments for both the 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 controlled 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 was analysed 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<0.05).

RESULTS AND DISCUSSION

In this study, it was determined that the ethanol extract obtained from *Punica granatum* (Pge) increased the maximum lifespan of the male and female population according to control group, which belongs to *D. melanogaster*. It was observed that the maximum female lifespan of the control and DMSO control groups was 60 days, while the maximum lifespan of males belonging to the control and DMSO control groups was 57 days, respectively. The difference between the control and DMSO control groups is not statistically significant (p>0.05) (Table 1).

According to results obtained from application groups, in the female population of *D. melanogaster* applied with Pge, the maximum lifespan was 78 days for the lowest application group (10.0mg/mL) and the maximum lifespan was 92 days for the highest application group (100.0mg/mL). It was also found that the maximum male lifespan in 30.0 and 50.0mg/mL application groups was 81 and 88 days, respectively (Figure 1).

In the male populations of *D. melanogaster* applied with Pge, the maximum lifespan within the lowest (10.0mg/mL) and highest (100.0mg/mL) application groups was 74–88 days (Figure 2). These values indicate a positive correlation (R = 0.513 for ♀♀and R = 0.509 for ♂♂) between the maximum lifespan of the application groups and changing Pge concentrations. As seen in Figures 1 and 2, the mean lifespan of the female and male *D. melanogaster* populations increased with increasing concentrations of Pge.

The mean lifespan of the male populations was determined to be shorter than the female populations (Table 1). For example, the maximum mean lifespan of the male flies increased from 56.52± 1.62 days to 74.33± 1.61 days, while the maximum mean lifespan of the female flies increased from 64.05± 1.95 days to 81.78± 1.72 days in the application groups. The difference observed in terms of mean lifespan of fly sex was statistically significant (p<0.05).

These values indicate a negative correlation (R=0.119 for ♂♂ and R=0.185 for ♀♀) between the maximum life span of the application groups and changing Pge concentrations.

In our study, the external (environmental) or internal factors that may affect the longevity of ♀♀ and ♂♂ *D. melanogaster* were reduced to minimum levels in the

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

Experiment Groups (mg/mL) (No)	Female population					Male population				
	N	Max. life span	Std. dev.	Mean life span±SE	*p*	N	Max. life span	Std. dev.	Mean life span±SE	*p*
Control (1)	100	60	10.77	44.23±1.07	1-2*	100	57	10.27	42.98±1.02	1-2*
C+DMSO (2)	100	60	10.76	44.23±1.09		100	57	10.28	42.97±1.03	
10.0 (3)	100	78	19.51	64.05±1.95		100	74	16.28	56.52±1.62	
30.0 (4)	100	81	16.84	69.95±1.68		100	78	15.06	62.99±1.50	
50.0 (5)	100	88	18.76	75.92±1.87		100	85	17.52	68.83±1.75	
100.0 (6)	100	92	17.24	81.78±1.72		100	88	16.18	74.33±1.61	

N: Total number of individuals, C: Control, Max.: Maximum, S.E.: Standard error, Std. dev.: Standard deviation, *p*: Probability levels between groups*: The mean difference is not significant at the 0.05 level.

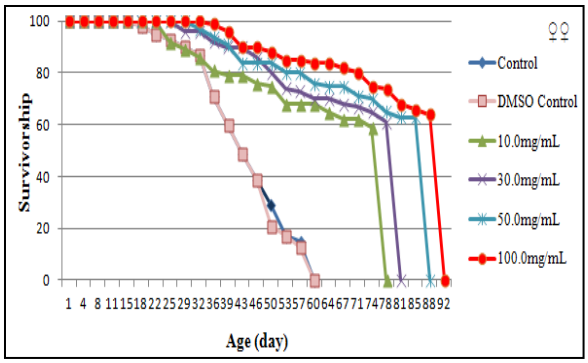

Figure 1. The survivorship lines of female individuals of *D. melanogaster* living medium applied with different concentrations of Pge during adult stages

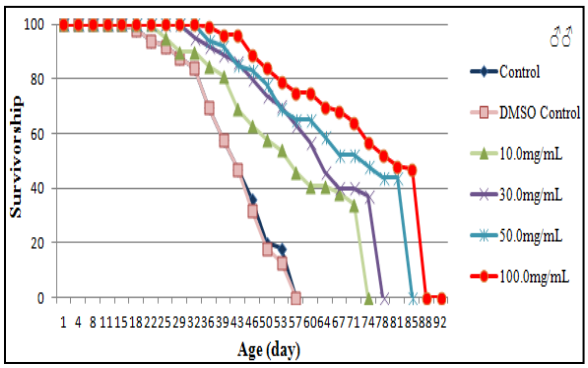

Figure 2. The survivorship lines of male individuals of *D. melanogaster* living medium applied with different concentrations of Pge during adult stages

application environment. The only variable parameter is *P. granatum* extract, applied with different concentrations, in our experiments. In recent years many plants have been examined, due to the aging effects of antioxidants and their antioxidant properties were observed [23]. For example, extract of *Rosa damascena* mortality of adult [24], green tea and broccoli extract reduces the high mortality rate caused by excess fatty foods by increasing the activity of antioxidant enzymes in *D. melanogaster*, lichen extracts of *Lobaria pulmonaria* and *Usnea longissima* increases the longevity of *D. melanogaster* [25], extracts of *P. granatum* were shown to reduce mutation and chromosomal aberrations and delay the aging process in genotoxic agents-treated mice and rats [26].

Some researchers have determined that various phenolic acids (gallic, caffeic, chlorogenic, and punikalajin punikalin, etc.) and some flavonoids (catechin, quercetin and phloridzin, etc.) in *P. granatum* by the phytochemical studies and these components because of the strong antioxidant properties have shown a protective effect against disease and aging was delayed [27]. In addition, it has also been reported that extract of *P. granatum* is a potential bioactive substances and there can be used as anticancer agents [28]. In a study by *in vitro* models, it has been shown that extracts *of P. granatum* by inhibiting the tumour cells, due to their biochemical components prevent breast, prostate, colon, lung, and skin cancers [29]. Again, there are many studies on *P. granatum* extract of bark, which show that it is capable of a high degree of free radical scavenging and is effective in the prevention of tissue damage in the organism [30].

It was determined that activities of antioxidant enzymes (catalase, glutathione reductase) and the amount of total glutathione decreased with aging in *D. melanogaster* [31]. To increase the amount of these enzymes, it a protective effect can be created for the organism by taking fruits and vegetables, a source of antioxidants, in addition to endogenous antioxidant defence system [32]. Pomegranates have that antioxidant activity due to their content of flavonoids and phenolic compounds [19]. They carry out these activities by eliminating the radicals that cause lipid peroxidation, by connecting metal ions or by inhibiting enzymes that free radical production [33] because free oxygen radicals that increase the amount in the cell are able to generate a variety of toxic effects. As a result, this accelerates the aging process by leading to genetic and metabolic disorders [34].

We can say that the occurrence of long life application groups other than the control groups, due to antioxidant activity of the extract that obtained from the flowers of *Punica granatum* in our study. *Punica granatum* most likely reduces the formation of free oxygen radicals and has a protective effect by preventing oxidative damage with antioxidant properties in the organism.

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