

## ***Drosophila melanogaster*'de *Cetraria islandica* (L.) Ach. Likenin Koruyucu Etkisinin Belirlenmesi**

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**Özet:** Bu çalışmada, *Drosophila melanogaster*'in gelişimi üzerine, *Cetraria islandica* (L.) Ach. likeninin su ekstraktının (Ciwe) etkileri araştırılmıştır. Büyüme ortamına farklı konsantrasyonlarda (0.25; 0.5 ve 1.0 mL/100 mL besiyeri) Ciwe ilave edildiğinde F<sub>1</sub> nesline ait gelişim evrelerinde bir gecikme meydana gelmemiştir. Uygulama gruplarında artan Ciwe konsantrasyonuna bağlı olarak ortalama yavru birey sayısında bir artış olduğu gözlenmiştir. Ayrıca kontrol grubunda görülen malformasyonlu bireyler, farklı konsantrasyonlarda Ciwe ilave edilen uygulama gruplarında giderilmiştir. Uygulama gruplarında, kontrol grubuna göre gözlenen bu farklılıklar istatistiksel olarak ( $p < 0.05$ ) önemli bulunmuştur

**Anahtar Kelimeler:** *Drosophila melanogaster*, *Cetraria islandica*, Gelişim Evreleri, Terapötik Ajan, Antioksidan Etki

## **Determination of Protective Activity of *Cetraria islandica* (L.) Ach. on *Drosophila melanogaster***

**Abstract:** In this study, the effects of water extract obtained from *Cetraria islandica* (L.) Ach. (Ciwe) on the development of *Drosophila melanogaster* were investigated. When the different concentrations of Ciwe (0.25; 0.5 and 1.0 mL/100 mL medium) were added to growth medium of adult individuals, no elongation of metamorphosis of F<sub>1</sub> progeny was determined. It was observed that the mean number of offspring increased depending on an increase of Ciwe on the application groups. Furthermore, malformed individuals seen in the control group were not observed by adding the different concentrations of Ciwe in the application groups. These differences were statistically significant ( $p < 0.05$ ) according to control group.

**Keywords:** *Drosophila melanogaster*, *Cetraria islandica*, Development Stages, Therapeutic Agent, Antioxidant Effect

## Introduction

In recent years, many investigations have been done for new substances from various sources, like medicinal plants which are good sources of therapeutic agent (Shukla et al., 2002; Schempp et al., 2005; Menegazzi et al., 2006). For this purpose, both in medical research and in biological research, more attention has been paid to the antioxidant properties of medicinal plants to minimize the harmful effects of free radicals. Lichen-forming fungi are unique organisms, producing biologically active metabolites with a great variety of effects, including antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative, antioxidant and cytotoxic activities (Boustie & Grube, 2005). However, rather few lichen substances have been screened for their biological activities and their therapeutic potential in medicine. *Cetraria islandica* is well known in Turkish alternative medicine and used for treatment of diseases such as hemorrhoids, bronchitis, dysentery and tuberculosis (Dülger et al., 1998).

Many scientists have investigated the chemical composition of the lichen *C. islandica* beginning from the XIX century till today. However, so far the nature of the lichen has not been elucidated exactly (Stepanenko et al., 1997). In addition to this, there are some pharmaceutical studies about composition of this lichen species. Protolichesterinic acid isolated from *C. islandica* has inhibitory effects on arachidonate 5-lipoxygenase in vitro. Protolichesterinic acid,  $\alpha$ -methylene- $\gamma$ -lactone, fumarprotocetric acid and  $\beta$ -orcinol depsidone are considered to be the major biologically active secondary metabolites in the lichen *C. islandica* (Ogmundsdottir et al., 1998). Several lichen metabolites of *C. islandica* exhibited highest antimycobacterial activity (Ingolfsdotti et al., 1998). Aliphatic  $\alpha$ -methylene- $\gamma$ -lactone isolated from the lichen *C. islandica* was found to be potent inhibitors of the DNA polymerase activity of human immunodeficiency virus-1 reverse transcriptase (HIV-1RT) (Pengsuparp et al., 1995).

The aim of the present study is to investigate the possible mechanisms of the effects of the water extract of *C. islandica* (Ciwe) in different concentrations on the development biology of *D. melanogaster*.

## Methods

### Flies

The wild type Oregon- R flies were used in all experiments. Adult flies were grown at  $25 \pm 1$  °C and aged in culture bottles containing maize-flour, agar, sucrose, dried yeast and propionic

acid (Standard Drosophila Medium= SDM) (Bozcuk, 1978). The flies were kept relative humidity of 40-60% and dark conditions, except during the transfers to fresh medium (usually twice weekly) and the females used in this experiment were virgins.

### **Plant material**

The lichen *C. islandica* was collected from Erzurum regions of Turkey in 2000. The plant was identified by Dr Ali Aslan and a voucher specimen has been deposited in the herbarium of the Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey.

### **Extraction of plant material**

To prepare extracts, 1.0 g of powdered lichen sample was added to 250 mL flasks containing 100 mL water. The mixtures were incubated at room temperature in a Soxhlet extractor for 2 days. The final suspension was sterilized by filtration with a membrane filter (0.2 µm). The filtrate was lyophilized and stored at -18°C (Srinivasan et al., 2001). The experiments were carried out using appropriate amount of lyophilized materials.

### **The application of Ciwe to adult individuals**

For the control group including only SDM and application groups including different concentrations of Ciwe (0.25; 0.5 and 1.0 mL/100 mL medium) ten pairs male and virgin female of adult *D. melanogaster* were placed into the culture bottles prepared in this way. Then, the F<sub>1</sub> progenies were allowed to develop and followed daily. Parent flies were cleared from the bottle when first pupa was appeared. At the end of this period, the surviving flies were counted from the vials on days 10 to 12 after egg laying and examined as phenotypic. Chronic experiments were repeated three times for each application group.

### **Statistical analyses**

Statistical calculations were done by using SPSS 11.5 software. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied.

### **Results**

Different concentrations (0.25; 0.5 and 1.0 mL/100) of Ciwe were applied to the adult individuals of *D. melanogaster*. In control and application groups laid eggs were observed on the second day from mating. Then, in these groups, first, second, third instar larvae, prepupa,

pupa and adult individuals were formed, respectively. The first adult belonging to F<sub>1</sub> progeny was observed at the 9<sup>th</sup> day after mating. The development stages of all application groups were completed at the same time together with control group (Table 1.).

Table 1. The effect of different concentrations of Ciwe on egg- adult developmental stages and the mean number of offspring of *D. melanogaster*

F <sub>1</sub> generation									
Concentration (mL/100mL medium)	Egg	1 <sup>st</sup> L	2 <sup>nd</sup> L	3 <sup>rd</sup> L	Prepupa	Pupa	Adult	Mean F <sub>1</sub> +SE	Σ Malf. (%)
Control	+	+	+	+	+	+	+	1682± 0.57 <sup>a</sup>	21 <sup>a</sup> (1.86)
0.25	+	+	+	+	+	+	+	1746± 2.30 <sup>b</sup>	2 <sup>b</sup> (0.11)
0.50	+	+	+	+	+	+	+	1758± 2.30 <sup>b</sup>	- (-)
1.0	+	+	+	+	+	+	+	2204± 1.73 <sup>c</sup>	- (-)

It has arranged as horizontal used letter in statistic evaluation. The difference of among groups is statistically significant (p<0.05).  
L:Larvae Σ Malf.: Total malformed individuals Mean F<sub>1</sub>:Mean number of offsprings (male+female) SE: Standard error

It was observed that the mean number of offspring obtained from application groups belonging to F<sub>1</sub> progeny increased when compared to control group (Table 1.). While the number of the offspring in the control group was 1682, as parallel to the increase in the concentrations of Ciwe, this number increased at the application groups of 0.25, 0.5 and 1.0 (1746, 1758 and 2204, respectively) (Fig.1.). These results obtained from Ciwe application showed statistically significant differences between groups (p<0.05).

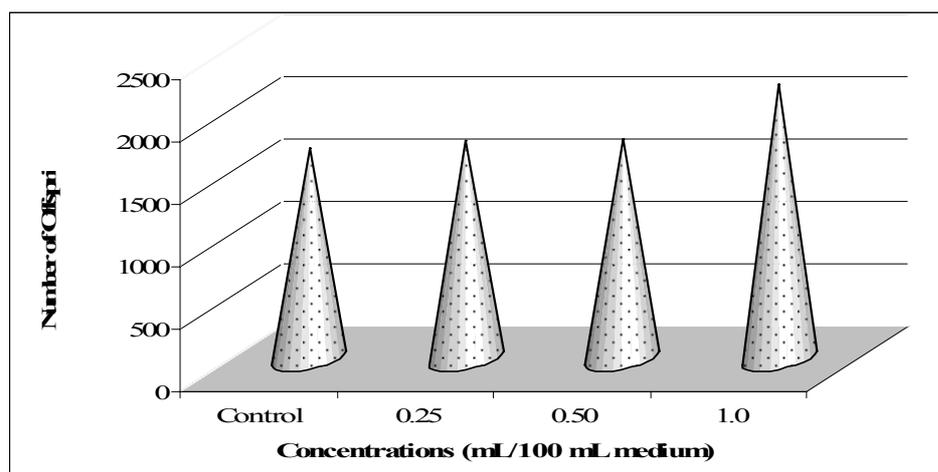


Fig. 1. Comparison of the mean number of offspring in the progeny F<sub>1</sub> exposed with different concentrations of Ciwe

On the other hand, some malformations (especially in unopened/unformed wing, lack

thorax and diffuse leg segments) were shown both in control (1.86%) and in application group of 0.25 (0.11%). The number of these malformations were presented in Table 1. As seen in Table 1., the ratio of abnormal individuals were decreased with increasing concentrations of Ciwe and there was no malformed individuals at the 0.5 and 1.0 application groups. The difference between the groups was statistically important ( $p < 0.05$ ).

## Discussion

According to above results, it could be said that none of Ciwe has toxic and mutagenetic effects on *D. melanogaster*. Because, *i*) life cycle from egg to adult of all groups was completed in nine days (Table 1.); *ii*) the number of offsprings increased with the increasing Ciwe. Besides, malformed of F<sub>1</sub> progeny decreased and even was not shown at high doses (Table). In this case, we can say that the Ciwe have not adverse effect on the fecundity and viability of *D. melanogaster*.

According to (Hatanoet et al., 1989), phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. For example, neither teratogenic effect nor malformations were observed at the fetuses of the wistar rat pregnancy feed with extracts of rosemary, *Rosmarinus officinalis* L. (Lemonica et al., 1996). It was suggested that polyphenolic compounds had inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables (Tanaka et al., 1998). It was reported that aqueous extract of *C. islandica* showed strong antioxidant activity, reducing power, DPPH radical and superoxide anion scavenging activities when compared with different standards such as  $\alpha$ -tocopherol, BHA, BHT, and quercetin (Gülçin et al., 2002). The results of the study showed that aqueous extract of *C. islandica* could be used as an easily-accessible source of natural antioxidants, and it could be considered as a possible food supplement or it could be used in pharmaceutical industry.

There are many data in the literature concerning the antioxidative properties of the phenolic compounds isolated from many plants (Chen & Ho, 1995). A lot of medical plants such as *Pimpinella anisum* L., *Rosmarinus officinalis* L., *Achillea millefolium* L., *Acorus calamus* L., *Hypericum perforatum* L. have antioxidant and recovery properties with active ingredients. Depending on an increase of the plant extracts on the application groups (1.0, 5.0 and 10 mL/100 mL medium), the number of offsprings belong to *D. melanogaster* increased (Uysal et al., 2007).

Recent studies have also indicated that exposure to various agents generates excess

reactive-oxygen species (ROS) in organisms. Due to the phenolic nature of most of the lichen compounds, many antioxidant activities (Hidalgo et al., 1993; Hidalgo et al., 1994; Jayaprakasha & Rao, 2000) and superoxide scavenging properties have been described (Behera et al., 2004). Antioxidant properties of medical plants, such as *Usnea longissima* (diffractaic acid and usnic acid, major active ingredients) and *C. islandica* (protolichesterinic acid,  $\alpha$ -methylene- $\gamma$ -lactone, fumarprotocetric acid and  $\beta$ -orcinol depsidone, major active ingredients), prevent negative effects of ROS. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis, and the aging processes (Stajner et al., 1998; Sanchez-Moreno et al., 1999; Malencic et al., 2000). The mean number of offsprings belonging to *D. melanogaster* obtain from application groups including different concentrations of the water extract of *Usnea longissima* (0.5; 1.0 and 1.5 mL/100 mL medium) increased according to control group. It was considered that the increase of the mean number of offsprings in the three application groups depends on the therapeutic effect of secondary metabolites contained the lichen (Uysal et al., 2009). In the aqueous extract of *C. islandica* (1.0 mg), 0.0387  $\mu$ g pyrocatechol equivalent of phenols was detected (Gülçin et al., 2002). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999). However, there is no information about the effects on development biology of Ciwe. It can be said that the recovering effects of this lichen on malformations and the increase in the number of individuals may be due to the antioxidant substances that they contain.

The results indicated that the lichen from Turkey may be a new medium for diet of *D. melanogaster* and a new source for compounds having a recovery potential against teratogenic effects. However, our results also showed that Ciwe can be used to obtain more individuals of *D. melanogaster* for short time test methods.

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