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C.elegans'a Genel Bakış ve Oğul Otu (*Melissa Officinalis*) Bitki Ekstraktlarının *Caenorhabditis Elegance*'ın Termotoleransı Üzerindeki Etkilerinin Araştırılması

Overview of *C.elegans* and Investigation of the Effects of Plant Extracts of Lemon Balm (*Melissa Officinalis*) on the Thermotolerance of *Caenorhabditis Elegance*

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ÖZ

Amaç: Bu çalışmanın amacı, Melissa officinalis bitkisinin fonksiyonel bitki ekstraktlarının, model organizma C.elegans'ın termotoleransı (ısı stresine karşı direnç) ve yaşam süresi üzerindeki etkilerini araştırmaktır. Termotolerans çalışmaları, bu tür model organizmalarda yaşam süresi analizi çalışmalarının ilk adımı ve kısa sürelisi olarak yer almaktadır. Oğul otu (Melissa officinalis) bitkisinin metanol ekstraktlarının çeşitli konsantrasyonlarının (sırasıyla 5, 50, 200 µg/ml olarak belirlenmiş olup) C.elegans termotoleransı (ısı stresi) üzerine olumlu etkilerinin olabileceği düşünülmektedir.

Yöntem: Bu çalışma kapsamında, antioksidanlar açısından zengin ve aynı zamanda fonksiyonel özelliklere sahip oğul otu (Melissa officinalis) bitkisinin metanol ekstraktları çeşitli konsantrasyonlarda (sırasıyla 5, 50, 200 µg/ml olarak) hazırlanmış ve canlı organizmalar üzerinde ısı stresi de oluşturularak (35°C'de) kontrol grubu ile birlikte, bu 3 farklı konsantrasyondaki deney grupları ile test edilmiştir. Her bir petriye, (FudR) maddesi 200 µg/ml ilave edilmiştir. Bu bitkinin C.elegans'ın termotoleransı üzerindeki etkileri yapılan bu çalışmada incelenmiş ve sonuçları yorumlanarak yaşam süresi üzerindeki etkisi değerlendirilmiştir. .

Bulgular: Yapılan deneyler sonucunda, termotolerans (heat stress) etkisinin en güçlü olduğu ekstre konsantrasyonlarının 50 ve 200 µg/ml olarak bulunduğu; ancak oğulotu ekstrelerinin 5 µg/ml gibi düşük konsantrasyonda, C.elegans termotoleransı üzerindeki etkinliğini kaybettiği tespit edilmiştir.

Sonuç: Araştırma sonucunda, oğulotu ekstraktlarının 50 ve 200 µg/ml konsantrasyonlarının, C.elegans termotoleransını arttırdığı ancak, 5 µg/ml konsantrasyonu kontrol grubu ile benzer sonuçlar verdiği görülmüştür. Çünkü 5 µg/ml, 50 µg/ml'nin 1/10 oranında seyreltilmiş halidir ve bu düşük konsantrasyon nedeniyle etkisiz olacağı buradan anlaşılabilir. Bu açıdan bakıldığında, Melisa bitki ekstraktlarının C.elegans termotoleransı üzerinde önemli bir oranda artırıcı etkiye sahip olduğu ve bu etkinin de 50-200 µg/ml seviyelerinde olduğunu söyleyebiliriz. Ancak, 5 µg/ml gibi düşük konsantrasyonlarda, ekstraktların C.elegans termotoleransı üzerindeki etkisi kaybolmaktadır.

Anahtar Kelimeler: Antioksidan, Bitki ekstresi, Caenorhabditis elegans, Oğulotu, Termotolerans, Yaşam süresi.

ABSTRACT

Objective: The aim of this study was to investigate the effects of functional plant extracts of Melissa officinalis on thermotolerance (resistance to heat stress) and lifespan of the model organism C. elegans. Thermotolerance studies are the first step and a short one in the life span analysis of such model organisms. It is thought that various concentrations (5, 50, 200 µg/ml, respectively) of methanol extracts of the plant (Melissa officinalis) may have positive effects on C.elegans thermotolerance (heat stress).

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Methods: In this study, methanol extracts of Lemon Balm (Melissa officinalis), which is rich in antioxidants and also has functional properties, were prepared at various concentrations (5, 50, 200 µg/ml, respectively) and tested with experimental groups at these 3 different concentrations together with the control group by creating heat stress on living organisms (at 35°C). In each petri dish, 200 µg/ml of (FudR) was added. The effects of this plant on the thermotolerance of C. elegans were examined in this study and the results were interpreted and the effect on the li
fe span was evaluated.

Results: As a result of the experiments, it was found that the extract concentrations at which the thermotolerance (heat stress) effect was the strongest were 50 and 200 µg/ml; however, it was determined that at low concentrations such as 5 µg/ml, the extracts lost their effectiveness on C.elegans thermotolerance.

Conclusion: As a result of the study, it was observed that 50 and 200 µg/ml concentrations of the extracts increased the thermotolerance of C.elegans, but the concentration of 5 µg/ml gave similar results with the control group. Because 5 µg/ml is a 1/10 dilution of 50 µg/ml and it can be understood that it will be ineffective due to this low concentration. From this point of view, we can say that lemon balm plant extracts have a significant enhancing effect on C. elegans thermotolerance and this effect is at 50-200 µg/ml levels. However, at concentrations as low as 5 µg/ml, the effect of the extracts on C.elegans thermotolerance is lost.

Key words: Antioxidant, Caenorhabditis elegans, Lemon balm, Lifespan, Plant extract, Thermotolerance.

1. INTRODUCTION

Caenorhabditis elegans (C.elegans) is a microscopic nematode about 1 mm in length and is a transparent, non-pathogenic model organism that is used in aging genetics and medical studies, living freely in the soil and tree bases in nature. Under laboratory conditions, N2 wild-type ones can live for about 18 days on average (1,2). One of the most important reasons why they are used in various scientific researches is that their genes are very similar (around 83%) to human genes (3,4). In addition, the discovery of the molecular basis of apoptosis (programmed cell death) was also possible thanks to the use of the C. elegans model organism (5).

Functional nutrients have long been known for their health benefits, which are now supported by scientific studies, also show positive effects on the life span of living organisms (2,6). The main subject and aim of this study was to investigate the effects of functional herbal extracts of Lemon Balm (Melissa officinalis) on the thermotolerance (resistance to heat stress) and lifespan of C. elegans model organism.

C. elegans model organism

It is an ideal organism with metabolically active reproductive, digestive, endocrine, sensory and neuromuscular systems and is economical. The C. elegans nematode offers researchers several opportunities for aging and lifespan studies. C.elegans is a nematode that feeds on the bacterium Escherichia coli (E.coli) OP50 strain in a petri dish under laboratory conditions (7).

Thermotolerance is the resistance against heat stress in simple invertebrates. In such organisms, as the resistance to stress increases, aging is delayed and normal life span is prolonged. Antioxidant compounds can create a kind of resistance against heat stress with the effect of various mechanisms, delay aging and thus increase the life span of living organisms (5,8).

2. METHOD

Material

In our study, N2 wild-type C. elegans were used and obtained from the Caenorhabditis Genetics Center (CGC) of the University of Minnesota. The rest of the laboratory materials were obtained from Faculty of Pharmacy (Istanbul) and the plant extracts were obtained from Ankara University, Faculty of Pharmacy, Department of Pharmacognosy (Prof. Dr. Ayşegül Köroğlu Güvenç). 5-Fluoro-2-deoxyuridine (FudR) was purchased from Sigma Chemical Company (St. Louis, MO).

Laboratory materials and equipment used

Autoclave (Core- OT 40L), etuv (Core- EN 120), precision scale (Kern-PFB120-3), parafilm, centrifuge device (Hitachi-CF15RN), automatic pipettes (Isolab-Capp), automatic pipette tips, sterile petri dishes (60 mm*15 mm), microscope (Zeiss- Stemi 508), Ph meter (Hanna- HI2211) , spectrophotometer (Genesys-10S-VIS), vortex (Scilogex- MXS), mezur, erlen, distilled water device (Nüve- ND 12), refrigerator (Ugur- USS 374 DTKE), incubator (Nüve- EC 160), laminar flow cabinet (Nüve-MN 120), falcon tubes of 15 ml and 50 ml.

Chemicals used

Bacterial Grade Agar (Multicell), Bacterial Peptone (Multicell), NaCl (Carlo Erba), MgSO4 (Sigma-Aldrich), KH2PO4/K2HPO4 buffers (Sigma), CaCl2 (Merck), MgSO4 (Merck), Cholesterol (Sigma-Aldrich), LB Broth (Sigma), LB Agar (Sigma), Fluorodeoxyuridine (Sigma-Aldrich), Methanol (Merck).

Method of experiment

Inoculation of E.coli on NGMs with Lemon Balm plant extract

In the present study, the prepared OP50 strain of E. coli bacteria was added to NGMs with pre-prepared melissa plant extract and dried in a laminar flow cabinet.

Addition of FudR (fluorodeoxyuridine)

After E.coli was cultured and dried in the NGM (medium) preparation stage, 200 µl of 5-Fluoro-2'-deoxyuridine (FudR), a primidine analog and an inhibitor of DNA synthesis, was added to each 60 mm petri dish in order to prevent the egg development of adult C.elegans and to prevent mixing of generations.

Synchronization (decontamination) phase

C.elegans embryos are isolated according to various protocols. This ensures that the worms grow at the same stage, i.e. synchronization. In the synchronization stage;

•The petri to be isolated is washed with 3.5 ml sterile water. The petri is kept slightly tilted so that the worms slide downwards and pipetted.

•Collect all the washing liquid in the Falcon tube (conical tube).

•The Falcon tube volume is filled to 3.5 ml with sterile water.

•In a separate falcon tube, 0.5 ml of 5N NaOH and 1 ml of bleach are added and mixed. This mixture is then added to the 3.5 ml of washing liquid.

•The tube is shaken with a vortex for a few minutes. This process is repeated once every 2 minutes for 10 minutes and the falcon tube is also turned upside down once every minute.

•After vortexing, the homogeneously mixed liquid was centrifuged at 1300 xg for 30 seconds.

•Discard the upper part of the pellet (supernatant), leaving 0.1 ml at the bottom of the Falcon tube.

•The remaining pelleted liquid is added to 5 ml with sterile water and vortexed for a few seconds.

•Then centrifuge again at 1300 xg for 30 seconds and discard the upper part of the pellet (supernatant) leaving 0.1 ml at the bottom of the tube.

•With the help of a pasteur pipette, all 0.1 ml of eggs remaining at the bottom are transferred to the prepared NGMs (medium) and finally observed under a microscope.

•After they reach the adult stage, they are used in experimental studies.

Thermotolerance (heat stress) experiment and general follow-up of the experiment

Thermotolerance experiment without FudR

Forty synchronized animals (C. elegans) were transferred to each of 6 unextracted petri dishes in which FudR was not added and E. Coli was inoculated. All petri dishes were placed in an incubator set at 35°C. Then, every 2 hours, the animals that died in the petri dishes removed from the incubator were noted and this counting process was continued until all of them died. The experiment lasted for 16 hours in total, and the number of dead and live animals was recorded at 6, 8, 10, 12, 14 and 16 hours. As a result of this thermotolerance experiment, it was determined that 50% of the animals removed from the incubator at the 12th hour were alive and 50% were dead. According to the time determined as a result of the experiment (12th hour); FudR substance was added and thermotolerance experiment was performed without extract. Animals that escaped from the petri dish were excluded from the study (Figure 1).

Thermotolerance experiment without Lemon Balm extract

Forty synchronized animals (C. elegans) were transferred to each of 5 unextracted petri dishes supplemented with FudR and inoculated with E. Coli. All petri dishes were placed in an incubator set at 35°C. Then, every 2 hours, the animals that died in the petri dishes removed from the incubator were noted and this counting process was continued until all of them died. The experiment lasted for a total of 10 hours and the number of dead and live animals was recorded at 2, 4, 6, 8 and 10 hours. As a result of this thermotolerance experiment, it was determined that 50% of the animals removed from the incubator at the 6th hour were alive and 50% were dead. According to the time determined as a result of the experiment (6th hour), plant extract thermotolerance experiments were carried out. In the experiment, no animal escaped from the petri dish (Figure 2).

Lemon Balm plant extract Thermotolerance experiment

In the thermotolerance experiment with the extracts of the plant, the study was carried out with 12 petri dishes in 4 different groups (200 µg/ml, 50 µg/ml, 5 µg/ml concentrations and control group (without extract) (Table 1). At the 0th hour of the experiment, 40 C. elegans were transferred to each petri dish with and without the extract and C. elegans were exposed to the extract at 25°C for one day in the petri dishes with the extract. The next day, the C. elegans in the petri dishes with and without extract were kept in an incubator set at 35°C for 6 hours and then removed from the incubator. The number of live and dead animals was also noted and the thermotolerance experiment with plant extract was completed. Animals that escaped from the petri dish were excluded from the study (Figure 3).

Statistical analyses

In the data obtained from the experimental results, the results of the control and experimental groups were compared with difference tests (t-Test, One-way ANOVA and Tukey's HSD post hoc test), and $p<0.05$ was considered statistically significant. IBM SPSS Statistic program version 22.0 was used for statistical analysis of the data. Related graphs were drawn in Microsoft Office Excel 2010 program.

3. RESULTS

As a result of the research, it has been determined that the Lemon Balm plant can increase C. elegans thermotolerance (resistance to heat stress) and extend its life span to a certain extent depending on the dose. Within the framework of the data findings, it is revealed that Lemon Balm extracts increase C.elegans thermotolerance, but this increase is directly related to the concentrations of the extracts. This is the most important data revealed in the thesis research. It is not surprising that 50 and 200 µg/ml concentrations of the extracts increased C.elegans thermotolerance, while 5 µg/ml concentration of the extracts gave similar results with the control group. Because 5 µg/ml is a 1/10 dilution of 50 µg/ml and it is easily understandable that it will be ineffective due to this low concentration. From this point of view, we can say that the plant extracts of the Lemon Balm have an increasing effect on C.elegans thermotolerance and this effect is at the levels of 50-200 µg/ml. However, at concentrations as low as 5 µg/ml, the effect of the extracts on C.elegans thermotolerance is lost.

4. DISCUSSION

Thermotolerance is the resistance to heat stress in simple invertebrates. In such organisms, as the resistance to stress increases, aging is delayed and normal life span is prolonged. Antioxidant compounds can provide a kind of resistance against heat stress with the effect of various mechanisms, delay aging and thus increase the life span of living organisms (9). In the present study, it was aimed to test the effects of different concentrations of Melissa officinalis (M. officinalis) plant on thermotolerance, i.e. lifespan assay, due to its various strong antioxidant activities.

Melissa officinalis is a lemon-scented plant that is also used for medicinal purposes. It is a perennial herbaceous plant originating from Southern Europe, Caucasus, Central Asia, North Africa and North America. Another name for it is 'sonderwort'. In our country, it is also known as lemon balm, lemon mint and hive weed. The Latin name of the plant, Melissa, means 'bee leaf'. The word 'mel' in its origin means 'honey' and since the plant is rich in nectar, it was

Table 1. Quantities of *E.coli* and Lemon Balm Plant Extract Added to NGMs for Final Concentrations of Experimental Groups

Groups	Final Concentrations (NGM)	E.coli+ Extract Amount (For 50 petri dishes)
Group 4	200 µg/ml	15 ml *E.Coli* + 150 µl extract
Group 3	50 µg/ml	15 ml *E.coli* + 30 µl extract+120 µl methanol
Group 2	5 µg/ml	15 ml *E.coli* + 3 µl ekstre + 147 µl methanol
Group 1	Non-extracts (0 µg/ml)	15 ml *E.coli* + 150µl methanol

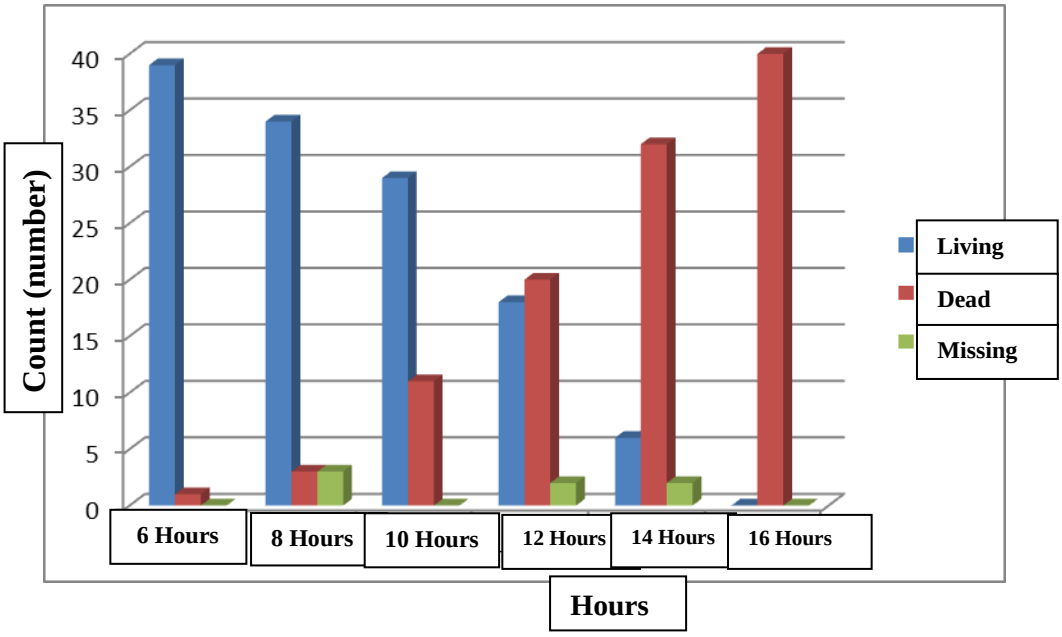

Figure 1: Thermotolerance Experiment Result Graph without FudR

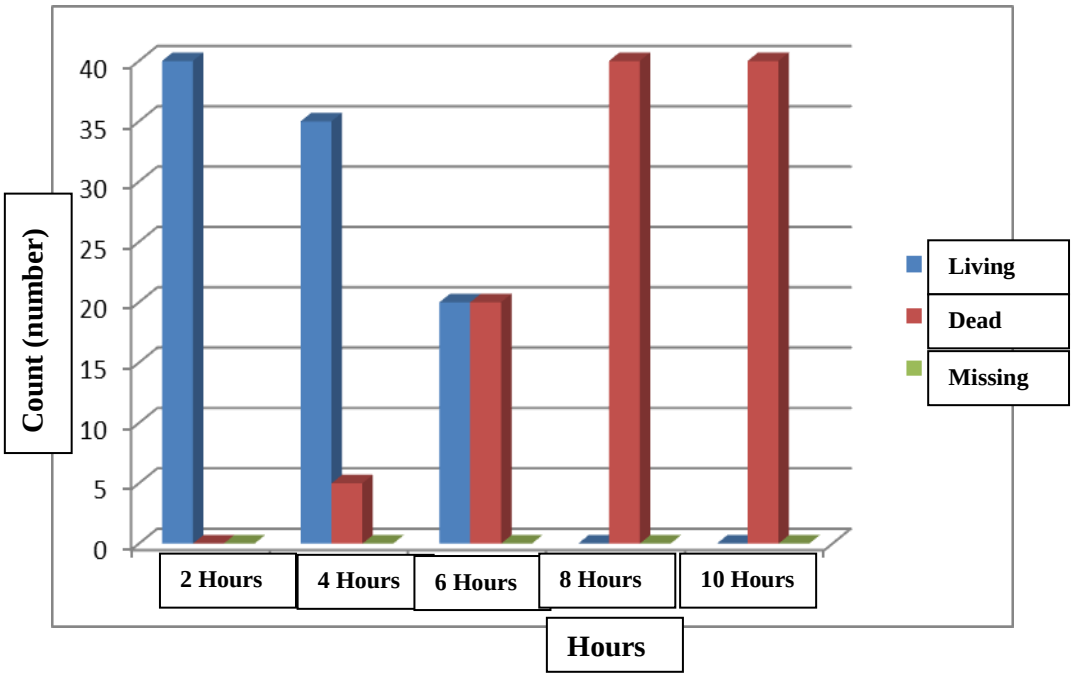

Figure 2: Unextracted Thermotolerance Experiment Result Graph

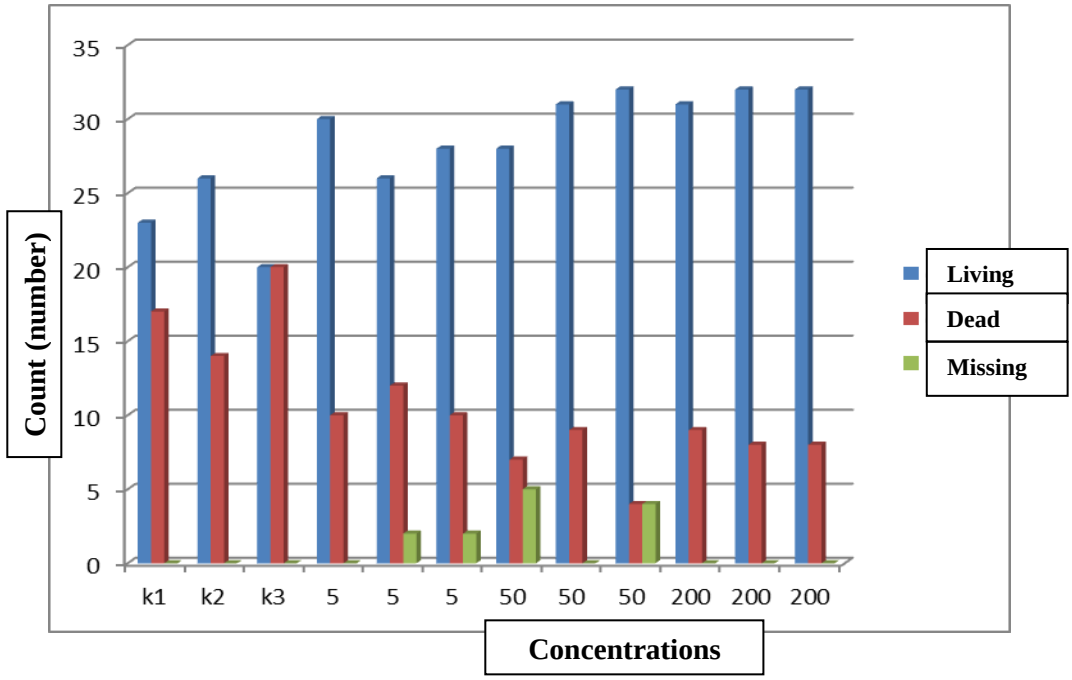

Figure 3: Thermotolerance Experiment Result Graph with Lemon Balm Extract and FudR

generally used to feed bees. In Greek, it means 'honey bee'. Its distribution area in Turkey is the Mediterranean Region and the coastal parts of Anatolia (10,11,12).

The leaves of the Melissa officinalis L (Lamiaceae) plant; digestive, carminative, antispasmolic, sedative, hypotensive, hypoglycemic, hypolipidemic, anticancer, anxiolytic, antinociceptive, cytotoxic, analgesic, vasorelaxant, memory booster, anti-inflammatory, antiseptic, antiviral, carminative, tonic, menstrual stimulant, spasmolytic, antiulcer, antifungal, antiparasitic, antipyretic, refreshing and it has diuretic properties and is also used for the treatment of functional gastrointestinal disorders, colds, asthma, bronchitis, heart failure, amenorrhea, arrhythmias, ulcers, wounds, headaches and rheumatism. Methanol extract of the plant, on the other hand, has a high concentration of flavonoid antioxidants (13,14). In addition, the above-ground parts of the plant are used in public health; around the Sakarya region, in the treatment of migraines and depression; around the Kırklareli region, in the treatment of diseases such as diabetes, heart diseases, asthma, bronchitis and cancer (15,16,17).Other phenolic components have also been reported in Melissa officinalis, it has a complex chemical composition. Some of them are; luteolin 3-O-glucuronide, apigenin, tannins, hydroxycinnamic acids (4-7%), polyphenolic acids (rosmarinic acid up to 6%, p-coumaric, protocatechic and caffeic acids) and monoterpene and monoterpene glycosides up to 0.37% (more than 40%), sesquiterpenes (more than 35%), triterpenes (ursolic and oleanolic they are volatile essential oils consisting of acids) and flavonoids (quercitrin, rhamnocitrin, luteolin). Among the most important terpenoids, Decapitated; there are citral, citronelal, geraniol, neral, linalool, farnesyl acetate, humulene, caryophyll and eremophylline. Phenolic acids are secondary metabolites that make up a large proportion of naturally occurring compounds that exhibit a wide range of biological activities. Phenolic acids are important bioactive components of Melissa officinalis L, among which rosmarinic, decaffeic, chlorogenic and ferulic acids are particularly interesting. In a literature review, rosmarinic acid has been shown to show anti-inflammatory, antibacterial and antiviral activity, reduce atopic dermatitis and prevent Alzheimer's disease to a certain extent (18,19). Ferulic acid has powerful antioxidant, antimicrobial, anti-inflammatory, antithrombotic and anticancer activities. Chlorogenic acid has anti-inflammatory, anti-bacterial and antiobesity properties, while caffeic acid has anti-inflammatory, antioxidant and immunomodulatory effects (20).

According to a study by (21), different concentrations (50, 100 and 200 mg/ml) of blueberry extract were tested on C.elegans using RNAi technology. A 22.2%, 36.5%, and 44.4% increase in life span was observed in a dose-dependent manner. At the same time, the animals exposed to blueberry extract showed a greater thermotolerance effect against heat stress than those not exposed. In the present study, a similar result was obtained in parallel with this effect in the extract of the lemon balm plant and was found to be statistically significant.

According to another study by (17-19), C.elegans exposed to spinach extract, whose antioxidant activity was increased by applying Ascophyllum nodosum (a type of seaweed) treatment, showed a higher thermotolerance effect against oxidative stress and extreme heat stress (thermotolerance) than those not exposed. In a dose-dependent manner at the concentrations; 50% of the organisms showed a tolerance effect against oxidative stress and 61% against extreme heat stress. In the control group, no tolerance effect against oxidative stress was observed, and a 38% thermotolerance effect against extreme heat stress was observed. In the present study, a similar effect was observed in the control groups; and in the

extracted groups, the swede plant extract showed a parallel effect to the spinach plant extract. This result may indicate that the lemon balm has a protective effect on C. elegans thanks to its unique constituents.

In another study on extracts of plant adaptogens such as Eleutherococcus senticosus (or Acanthopanax senticosus) and Rhodiola rosea, both plant extracts extended the average life span of C. elegans, again in a dose-dependent manner. In at least 4 independent experiments, 250 μg/ml of Eleutherococcus (SHE-3) and 10-25 μg/ml of Rhodiola (SHR-5) significantly increased the life span by 10-20% ($p<0.001$), the maximum life span was increased by 2-3 days, and the mortality of the first individual to die in the population was delayed. At the highest concentrations tested (2500 μg/ml Eleutherococcus and 250 μg/ml Rhodiola), the life span was shortened by 15-25% ($p<0.001$), while less effect was observed at higher concentrations. Both plant adaptogen extracts were tested in C. elegans subjected to a brief heat shock at 35°C for 3 h and chronic heat treatment at 26°C and increased thermotolerance (22). In the present study, the highest concentrations (50 μg/ml and 200 μg/ml) of the Lemon Balm increased thermotolerance in C. elegans exposed to heat shock at 35°C for 6 hours, showing an effect opposite to the highest concentrations of plant adaptogens in terms of thermotolerance effect, and a statistically significant increase was observed ($p<0.05$).

In another study, using Bacopa monnieri plant, plant extracts at concentrations of (0.1, 0.01 and 0.001 mg/ml) were tested on C.elegans exposed to thermal stress at 37°C. In the experimental results, the plant extracts statistically significantly ($p<0.001$) increased thermotolerance and prolonged the average life span of the organisms. In terms of thermotolerance effect, the plant extract with a concentration of (0.1 mg/ml) provided the highest effect. An increase of 22.5%, 20.27%, and 25.37% was also observed on the average life span of the animals compared to the control group in a dose-dependent manner (23). In this study, a concentration-dependent increase in thermotolerance effect was observed and the thermotolerance experiment was carried out at 35°C for 6 hours. Statistically significant results ($p<0.005$) were also obtained at the concentrations (50 and 200 μg/ml) of the lemon balm plant.

In a study using Juniper berry extracts, doses of the plant extract (0, 10, 50, 100 ppm) were investigated on C.elegans thermotolerance and life span. The study revealed that the low dose (10 ppm) was highly effective, increasing the life span by 18.54% when compared to the control group. Similarly, the same concentration, i.e. 10 ppm, also exhibited an effective potential against various oxidative and thermal stresses. When compared to the control group, it showed a thermotolerance effect of 30.40% (24). According to the data of the present study, the highest concentration (200 μg/ml) of the swordwort plant showed the highest thermotolerance effect ($p<0.005$), showing an opposite effect to the lowest concentration (10 ppm) in Juniper berry extracts. This difference may be due to the difference in antioxidant components in the plant extracts.

In another study, an RNAseq dataset comprised of expression patterns of 2900 E. coli genes in the strain OP50, which were seeded on either nematode growth media (NGM) plates or on FUdR (50 μM) supplemented NGM plates, was analyzed. Analysis showed differential gene expression in genes involved in general transport, amino acid biosynthesis, transcription, iron transport, and antibiotic resistance. We specifically highlight metabolic enzymes in the l-histidine biosynthesis pathway as differentially expressed between NGM and FUdR exposed

OP50. This study shows that OP50 exposed to FUdR results in differential expression of many genes, including those in amino acid biosynthetic pathways (25).

In the present study, the chemical FUDR was utilized so that the C. elegans life cycle would not affect the experimental results and the half-life was determined. In the group without FUDR as the control group, the LD50 value, i.e. the time during which half of the initial organism number of 40 individuals died and half survived (half-life) was determined as 12 hours. In contrast, in the treatment with FUDR, the LD50 value was determined as 6 hours. In the absence of FudR, although the temperature negatively affects the life cycle of C.elegans, the newly formed individuals delay the decrease in the number of C.elegans. However, since the addition of FudR to the medium prevents cell division and thus the formation of new individuals by blocking DNA synthesis, the LD50 value is smaller and this value is theoretically only due to the ambient temperature. Various internal and external (environmental) factors that may affect the life span of the groups were also kept to a minimum in the experimental environment studied.

The Lemon Balm extracts were applied on C.elegans for 6 hours, taking into account the FUDR-LD50 value. In these applications, 5 μg/ml, 50 μg/ml and 200 μg/ml extracts were prepared. In the control group, no extracts were added (0 μg/ml). When the data obtained were checked, it was observed that the number of C. elegans individuals surviving at the end of 6 hours gradually increased from the control group to the 5-50-200 μg/ml group. Therefore, at first glance, it can be concluded that the Lemon Balm extracts caused an increase in thermotolerance in C.elegans and accordingly, the number of surviving individuals increased compared to the control. However, in order to confirm this statement, detailed statistical analyses are needed, in which ANOVA and t-Test analyses were performed in SPSS 22.0.

ANOVA test is a type of analysis that allows us to reveal whether the variances of different groups are different. However, when a difference is detected between groups, it does not give information about which groups this difference is between. Post-hoc comparisons and t-Test were conducted to determine the groups between which there was a difference. Before ANOVA and t-Test, normality plots with test and homogeneity of variance test were performed to determine the normality (whether the data were normally distributed) and homogeneity of variance (whether the variances were homogeneous) of the data, respectively. In the normality test, Shapiro-Wilk Sig. values (1.000, 1.000 and 0.463) were found to be greater than 0.05 for the group Lemon Balm-Control, Lemon Balm-5 μg/ml, Lemon Balm-50 μg/ml and Lemon Balm-200 μg/ml, while the Sig. value of 0.000 could not be obtained for the group 200 μg/ml. According to these results, it is understood that the data belonging to the experiments carried out to determine the effect of the extracts of the swede on the thermotolerance of C.elegans have a normal distribution.

In the variance homogeneity test, Levene's Sig. value (0.436) was determined to be greater than 0.05 confidence value. Therefore, it was accepted that the variances between the groups were homogeneous. After the normality of the data and homogeneity of the variances were determined, ANOVA and t-test were started. In this thesis study, 4 groups were analyzed: group1 (Lemon Balm-Control), group2 (Lemon Balm-5 μg/ml), group3 (Lemon Balm-50 μg/ml) and group4 (Lemon Balm-200 μg/ml). In these groups, the results were investigated by one-way ANOVA analysis to determine whether the extracts of the swede extracts made a significant difference on the thermotolerance of C.elegans. ANOVA sig. value was determined

as 0.005. Since this value was less than 0.05 (p0.005<0.05), it was understood that there was a statistically significant difference between the means of the groups. In other words, C.elegans thermotolerance trait is significantly different in one or more groups compared to others. However, ANOVA Sig. value cannot be interpreted between which groups this difference is between. For this reason, post-hoc comparison was made by looking at the LEVENE Sig. value obtained in the variance homogeneity test. Because if the Levene sig. value is greater than 0.05, i.e. if the variances are homogeneous, the Post-hoc TUKEY test is performed, while when this value is less than 0.05, i.e. if the variances are not homogeneous, the Post-hoc TAMHANE test is performed. The Levene Sig. value found in this study is 0.436 and since it is greater than 0.05, the TUKEY test was conducted here.

In the Tukey test, each of the 4 groups was compared with each other in pairs. While Sig. values were greater than 0.05 in all pairwise comparisons, a statistically significant difference was found between the Lemon Balm-Control group and Lemon Balm-50 µg/ml group, between the Lemon Balm-Control group and Lemon Balm-200 µg/ml group and between the Lemon Balm-5 µg/ml group and Lemon Balm-200 µg/ml group. The sigma value between other binary groups is greater than 0.05.

In order to verify the results of the TUKEY test, the Bonferroni test, which can also be used instead of the TUKEY test and is preferred in special cases, was conducted. In the Bonferroni test, similar to the results of the TUKEY test, the Sig. values (0.016, 0.006, 0.039) between the Lemon Balm-Control group and Lemon Balm-50 µg/ml group, between the Lemon Balm-Control group and Lemon Balm-200 µg/ml group, and between the Lemon Balm -5 µg/ml group and Lemon Balm -200 µg/ml group were found to be less than 0.05, while this value was greater than 0.05 in other pairwise comparisons.

When the t-Test results are examined, the sigma value between the Lemon Balm-Control group and Lemon Balm-5 µg/ml groups is 0.074 (p>0.05), sigma value between Lemon Balm-Control group and Lemon Balm-50 µg/ml group is 0.025 (p<0.05), sigma value between Lemon Balm-Control group and Lemon Balm-200 µg/ml group was 0.008 (p<0.05), sigma value between Lemon Balm-5 µg/ml group and Lemon Balm-50 µg/ml group was 0.234 (p>0.05), sigma value between Lemon Balm-5 µg/ml group and Lemon Balm-200 µg/ml group was 0.038 (p<0.05), sigma value between Lemon Balm-50 µg/ml group and Lemon Balm-200 µg/ml group was 0.345 (p>0.05). As can be seen, in these pairwise comparisons made in the t-test, the values with sigma values less than 0.05 were determined between the Lemon Balm-Control group and Lemon Balm-50 µg/ml group, between the Lemon Balm-Control group and Lemon Balm-200 µg/ml group and between the Lemon Balm-5 µg/ml group and Lemon Balm-200 µg/ml group. Therefore, the Lemon Balm-5 µg/ml group did not differ from the Lemon Balm-Control group.

The antioxidant effects of compounds such as flavonoids, rosmarinic acid, and benzodioxol found in the capsicum were found to be 10 times stronger than the effects of vitamins B and C (26). In addition, the ability of M. officinalis to prolong life span and its important feature in restoring memory have also been noted in the sources (27).

In line with these data, in the future, in more comprehensive and more detailed studies on the thermotolerance of C. elegans thermotolerance of Lemon Balm extracts, analyses focusing on concentrations of 50-200 µg/ml and determining lower limits such as 45-40-35-30 µg/ml and upper limits such as 250-300-350-400 µg/ml can be performed. Also, the optimum

effect concentration can be investigated. In addition, the biochemical compounds in the extracts can be investigated in more detail and the molecular mechanisms that cause the thermotolerance-enhancing effect of these compounds in C. elegans can be examined at the level of gene expression, genome and proteome. Advanced purification techniques and various biological activation methods can also be used to identify the effective chemical compounds (28,29).

In line with these data obtained, in the future, in more comprehensive and more detailed studies to be carried out on the thermotolerance of C. elegans, analyses determining upper limits such as 160-170-180-180-200 µg/ml and lower limits such as 70-60-50-40-30 µg/ml can be performed. Also, the optimum effect concentration can be investigated. In addition, the biochemical compounds in the extracts can be investigated in more detail and the molecular mechanisms that cause the thermotolerance-enhancing effect of these compounds in C.elegans can be examined at the level of gene expression, genome and proteome. Advanced purification techniques and various biological activation methods can also be used to identify the effective chemical compounds.

5. CONCLUSION

The fact that the results of the application of lemon balm plant extracts on C. elegans increased thermotolerance suggests that this plant may be a source for other studies to be conducted with the same organism (obesity, diabetes, cardiovascular diseases, cancer, depression, Alzheimer's, etc.). The results of the present study show the benefit of antioxidant compounds in the plant and will shed light on the elucidation and investigation of various mechanisms in anti-aging, other aging and thermotolerance studies.

At the same time, plant foods rich in antioxidants, such as Lemon Balm may modulate stress responses in extreme conditions, potentially enhancing cellular defense mechanisms and increasing organismal stress tolerance in living organisms. These findings may help in the development of new herbal medicines for various stress-related complications in humans. In addition, the therapeutic consumption of Lemon Balm could be expanded across the country. Further studies should focus on the bioactive components in the extracts to elucidate the underlying physiological and molecular signaling mechanisms. The fact that plant extracts can cause longevity in living organisms and regulate various stress-related genes is also promising for researchers. Molecular studies to be carried out on C. elegans and other experimental animals with the plant are also important in terms of contributing to the subject.

Recent studies have examined the effects of temperature on altering various signaling pathways through specific gene expression programs that promote stress resistance and longevity.

One of the main characteristics of aging is the loss of protein homeostasis, or proteostasis. In fact, thermosensory neurons in C. elegans also regulate proteostasis pathways like the heat shock response and the aggregation of metastable proteins. It is believed that prolonged heat stress is a major factor in the development of human neurodegenerative protein misfolding illnesses. The most recent research on the relationship between temperature and proteostasis and aging is presented in this article. It also discusses how the herb lemon balm improves C. elegans thermotolerance, or its ability to withstand heat stress, and how it dose-

dependently lengthens the organism's life span by a specific percentage. It also covers the ways in which research on poikilothermic species can benefit vertebrates and lead to the development of fresh approaches to treating human illnesses. In this way, this study adds new knowledge to the literature and is unique in the information it provides.

In conclusion, due to the high genetic similarity between C. elegans and humans, the studies conducted and to be conducted on this model organism may give researchers ideas and the research results can be transferred to mammalian systems and re-examined. With the findings obtained from the research, various formulations of these plants can be developed and used for anti-aging products.

Extensive studies have helped identify the main players regulating longevity at cold and hot temperatures. Cultivation at low temperatures is beneficial, while warm temperatures affect longevity. However, this relationship is not always valid. When C. elegans is exposed to high temperatures during early developmental stages, adult longevity increases. This transient heat stress early in life activates long-term defense responses that promote longevity through the histone acetyltransferase CBP-1 and the chromatin remodeling complex SWI/SNF. These studies also show that temperature-induced effects on aging are a well-regulated event controlled by genetic and epigenetic factors. These pathways provide important avenues for understanding the effect of the Lemon Balm plant on thermotolerance.

In the light of the positive results obtained from the present study and other researches that have been conducted or will be conducted with the plant, it is thought that commercial preparations can be developed for this plant. Further studies at different doses will also give us more guidance. It is also recommended to investigate the effects of different Melissa officinalis species on thermotolerance. It is also recommended to examine the effects of different genetic variants of model organisms used in such studies.

This research may also help as a method for future studies targeting aging and age-related disorders in C. elegans using extracts from different plant sources. Again, such studies may also serve as an example for studies on the discovery of functional foods with C. elegans..

Limitations

There were some limitations in our study. Analyses determining upper limits such as 160-170-180-200 µg/ml and lower limits such as 70-60-50-40-30 µg/ml could not be performed. The optimum effect concentration can also be investigated. Using the same method and applying different plant extracts, observation of the effects of these extracts on C. elegans can be carried out by making dose and hour-based comparisons. In addition, the biochemical compounds in the extracts can be investigated in more detail and the molecular mechanisms that cause the thermotolerance-enhancing effect of these compounds in C. elegans can be examined at the level of gene expression, genome and proteome. Advanced purification techniques and various biological activation methods can also be used to analyze these effective chemical compounds and make the study more comprehensive.

Ethical Consideration of the Study

Ethics approval: Not applicable.
Consent to participate: Not applicable.

Consent for publication: All authors have given consent for publication

Conflict of Interest Statement

No conflict of interest was declared by the authors.

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