LONGEVITY AND CIRCADIAN RHYTHM IN *Caenorhabditis elegans*: THE IMPACT OF LITHIUM CHLORIDE

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By
Elif Sena Temirci
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LONGEVITY AND CIRCADIAN RHYTHM IN *Caenorhabditis elegans*: THE IMPACT OF LITHIUM CHLORIDE

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June 2024

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

____

Seymur Jahangirov(Advisor)

____

Ayça Arslan Ergül (Co-Advisor)

____

Aykut Erbaş

____

Ahmet Acar

Approved for the Graduate School of Engineering and Science:

____

Orhan Arıkan

Director of the Graduate School

ABSTRACT

LONGEVITY AND CIRCADIAN RHYTHM IN *Caenorhabditis elegans*: THE IMPACT OF LITHIUM CHLORIDE

Elif Sena Temirci

M.S. in Material Science and Nanotechnology

Supervisor : Seymur Jahangirov

Co-Advisor : Ayça Arslan Ergül

June 2024

Lithium chloride (LiCl) is a popular treatment for various neurological disorders, especially bipolar disorders. While its complete mechanism of action remains partially elucidated, LiCl has been found to support new memory formation by triggering the construction of new neurons, reducing senescence, and regulating the circadian rhythm, particularly in bipolar patients, where it counteracts their abnormally fast biological clock. The circadian rhythm is vital in determining efficiency, understanding energy consumption, and biochemical balance for all organisms. This rhythm includes regulating body functions by the day/night cycle. *Caenorhabditis elegans* (*C. elegans*) is one of the most robust organisms for modeling circadian rhythm, although it lives in the soil. Therefore, by employing *C. elegans* as a model system, valuable insights could be gained for these complex processes.

This study aims to elucidate the complex relationship between LiCl, circadian rhythms, and longevity, as disruptions in these pathways are implicated in neurodegenerative diseases and age-related cognitive and motor decline. In this project, white light was employed to manipulate the circadian rhythm in *C. elegans*, with one group additionally receiving LiCl treatment in addition to light exposure. The study focused on longevity, response to environmental factors, and circadian rhythm. To elucidate the effect on longevity, lifespan measurements showed that LiCl treatment extended the lifespan of *C. elegans* under both light and dark conditions, with a shorter lifespan observed in the light. Additionally, when comparing the effect of specific developmental time points, the signs of aging appeared later in the dark compared to the light. The differential gene expression of longevity genes suggested that LiCl treatment could impact gene expression, particularly the age-1 gene, but not the daf-16 gene. Furthermore, the response to environmental changes was examined similarly and it was observed that *C. elegans* responded to the circadian rhythm disruption caused by light and LiCl administration. In conclusion, this study suggests that LiCl treatment has the potential to mitigate the adverse effects of circadian rhythm disruptions and reverse the aging process of *C. elegans.*

Keywords: Lithium chloride Administration, Circadian Rhythm, Longevity, *Caenorhabditis elegans*

ÖZET

Caenorhabditis elegans'TA YAŞAM SÜRESİ VE SİRKADİYEN RİTİM: LİTYUM KLORÜRÜN ETKİSİ

Elif Sena Temirci

Malzeme Bilimi ve Nanoteknoloji, Yüksek Lisans

Tez Danışmanı : Seymur Jahangirov

İkinci Tez Danışmanı : Ayça Arslan Ergül

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Lityum klorür (LiCl), bipolar bozukluklar başta olmak üzere çeşitli nörolojik bozuklukların tedavisinde kullanılan popüler bir tedavi yöntemidir. Etki mekanizması tam olarak anlaşılmamış olsa da LiCl'nin yeni nöronların oluşumunu tetikleyerek yeni hafıza oluşumunu desteklediğini, yaşlanmanın etkilerini azalttığı ve özellikle de bipolar hastaların sirkadiyen ritmi düzenleyerek hızlı biyolojik saatlere etki ettiği bilinmektedir. Sirkadiyen ritim, tüm organizmalar için verimliliğin belirlenmesinde, enerji tüketiminin anlaşılmasında ve biyokimyasal dengenin belirlenmesinde hayati öneme sahiptir. Bu ritim, gece/gündüz döngüsüyle vücut fonksiyonlarının düzenlendiğini ifade etmektedir. *Caenorhabditis elegans* (*C. elegans*), toprakta yaşamasına rağmen sirkadiyen modellemesi için en önemli organizmalardan biridir. Bu nedenle, *C. elegans* model

organizmasından faydanılarak bu karmaşık süreçlere ilişkin değerli bilgiler edinilmesi mümkündür.

Bu çalışma, LiCl, sirkadiyen ritim ve uzun ömür arasındaki karmaşık ilişkiyi açıklamayı amaçlamaktadır. Bu yolaklardaki aksaklıklar, nörodejeneratif hastalıklarda ve yaşa bağlı bilişsel ve motor gerilemede rol oynamaktadır. Bu projede, *C. elegans*'ta sirkadiyen ritmi değiştirmek için beyaz ışık kullanıldı ve ışığa maruz kalmanın yanı sıra ek olarak bir gruba LiCl uygulaması yapıldı. Mevcut çalışma, uzun ömür, çevresel faktörlere tepki ve sirkadiyen ritim üzerine odaklandı. Uzun ömür üzerindeki etkiyi açıklamak için yapılan yaşam süresi ölçümleri, LiCl tedavisinin *C. elegans*'ın ömrünü hem aydınlık hem de karanlık koşullar altında uzattığını, ışıkta ise daha kısa bir yaşam süresi ile ilişkilendiğini gösterdi. Ek olarak, belirli gelişimsel zaman noktalarının etkisi karşılaştırıldığında, yaşlanma belirtilerinin ışığa kıyasla karanlıkta daha geç ortaya çıktığı görüldü. Uzun ömürlü genlerin diferansiyel gen ekspresyonu, LiCl tedavisinin gen ekspresyonunu, özellikle de age-1 geni ile ilişkili olabileceği, ancak daf-16 genini etkilemediğini yönünde bulgular elde edildi. Ayrıca çevresel değişikliklere verilen yanıt da benzer şekilde incelendi ve *C. elegans*'ın ışık ve LiCl uygulamasının neden olduğu sirkadiyen ritim bozulmasına yanıt verdiği görüldü. Sonuç olarak bu çalışma, LiCl tedavisinin sirkadiyen ritim bozukluklarının olumsuz etkilerini azaltma ve *C. elegans*'ın yaşlanma sürecini tersine çevirme potansiyeline sahip olabileceği yönünde potansiyel etkiler göstermiştir.

Anahtar sözcükler: Lityum klorür Uygulaması, Sirkadiyen Ritim, Yaşam Ömrü, *Caenorhabditis elegans*

To my dear family…

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Contents

CHAPTER 1 1

1. INTRODUCTION 1

1.1. Caenorhabditis elegans (C. elegans) 1

1.2. C. elegans as a Model for Understanding Aging and Extending Lifespan 3

1.3. Lithium chloride (LiCl) treatment and Effect in C. elegans 8

1.4. Circadian Rhythm in C. elegans 10

1.5. Combinational Impact of Circadian Rhythm and LiCl Administration 12

1.6. Significance of the Study 13

CHAPTER 2 16

2. MATERIALS AND METHOD 16

2.1. Preparation of Buffers and Solutions 16

2.2. Maintenance of C. elegans and Application of LiCl 17

2.3. Circadian Rhythm Alterations 18

2.4. Age Synchronization of Nematodes 19

2.5. Lifespan Assay 20

2.6. Protein Isolation and Measurement of Autofluorescence Accumulation 20

2.7. Confocal Imaging 21

2.8. Sample Preparation and RNA Isolation 21

2.9. The synthesis of complementary DNA 23

2.10. Real-Time Quantitative PCR 23

2.11. Fertility Assay 26

CHAPTER 3 .... **27**

3. CIRCADIAN RHYTHM MANIPULATION 27

3.1. Experimental Groups and Treatment Conditions *27*

3.2. Ensuring Light Wavelength for Circadian Rhythm Entrainment *27*

CHAPTER 4 .... **30**

4. LONGEVITY 30

4.1. Impact of LiCl Administration and Light-Induced Circadian Rhythm Manipulation on Longevity *30*

4.2. The Autofluorescence Measurement of LiCl Treated and Changed Circadian Rhythm C. elegans as Aging Marker *33*

4.3. Understanding The Effect on Different Exposure Periods *38*

4.4. Determination of The Differential Gene Expression Patterns Change with LiCl Treatment and Circadian Rhythm Alteration *43*

4.4.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: age-1 Expression *45*

4.4.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: daf-16 Expression *47*

4.5. LiCl Treatment and Light-Induced Circadian Rhythm Affects the Fertility Efficiency of C. elegans *50*

CHAPTER 5 .... **55**

5. RESPONSE TO ENVIRONMENTAL CHANGES 55

5.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: lite-1 Expression *55*

5.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tax-2 Expression *57*

5.3. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tax-4 Expression *61*

CHAPTER 6 .... **64**

6. CIRCADIAN RHYTHM 64

6.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: lin-42 Expression *64*

6.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tim-1 Expression *67*

CHAPTER 7 .... **70**

7. CONCLUSION AND FUTURE PROSPECTS 70

7.1. Conclusions *70*

7.2. Future Perspectives *76*

BIBLIOGRAPHY **77**

List of Figures

Figure 1.1: The demonstration of highly crucial advantages of C. elegans model organism that establish its prominence in diverse research studies.. .... *2*

Figure 1.2: The representation of C. elegans' stages under stereomicroscope. A- The representation of all stages and size differences between stages. B- After egg laying, the stages are L1, L2, L3, L4, Young Adult and Adult in a developing manner. *5*

Figure 1.3: The schematic representation of the IIS pathway for longevity. When the signal is detected from the DAF-2/IGF1R receptor, this signal increases the activity of AGE-1/PI3K as represented in the right part of the graph (purple arrows). This increased activity triggers the intermediate elements and the inhibition of DAF-16/FOXO. On the other hand, when AGE-1/PIK3 activity is reduced, DAF-16/FOXO is phosphorylated (the left part, green arrows). This phosphorylation stimulates the translation of DAF-16/FOXO into the nucleus to induce gene activity. The figure is formed in Bio Render. .... *..7*

Figure 1.4: The illustration of LiCl treatment and Circadian rhythm association on longevity [78], [79]. Bipolar individuals experience periods of mania and depression, also has abnormal circadian rhythms. *14*

Figure 3.2: The spectra measurement of the utilized light source. The graph compares the wavelength of the light source in nanometers versus normalized light intensity in arbitrary units obtained from the light sources. *29*

*Figure 4.1.1: The lifespan determination of C. elegans under dark conditions. This graph displays the percent survival according to the days of adulthood of C. elegans. The green line indicates the control groups that did not undergo any treatment. On the other hand, the orange line represents the 10 mM LiCl-treated group at dark conditions. The experiment was performed at 20oC. **** denote a P-value lower than 0.0001.. .... 32*

*Figure 4.1.2: The analysis of C. elegans' lifespan under light conditions. This graph compares the percent survival versus the days of adulthood of C. elegans. The green line are the control groups that did not undergo any treatment at constant light. On the contrary, the orange line denotes the 10 mM LiCl-treated group at light conditions. The experiment was carried out at 20oC. **** symbolize a P-value lower than 0.0001..... 33*

Figure 4.2.1: The autofluorescence measurement of C. elegans at dark condition and LiCl application. The green points represent control groups and the orange color is for LiCl-treated groups. The autofluorescence level was investigated (ex 330/em 350–600) by worm protein samples at different time points. A- Blank solutions; distilled H2O and M9 buffer. B- 47 h. C- 53 h. D- 65 h. E- 73 h. F- 89 h. 36

Figure 4.2.2: The measurement of autofluorescence level of C. elegans at constant light conditions and LiCl treatment. The green points denote control groups and the orange color is for LiCl-treated groups. The autofluorescence level was investigated (ex 330/em 350–600) by worm protein samples at different time points. A- Blank solutions; distilled H2O and M9 buffer. B- 47 h. C- 53 h. D- 65 h. E- 73 h. F- 89 h 37

Figure 4.3.1: The Confocal images of 53 h groups at dark conditions. The scale bar is 100 µm. A- non-treated 53 h B- LiCl-treated 53h. 39

Figure 4.3.2: The Confocal images of 65 h groups at dark conditions. The scale bar is 100 µm .A- non-treated 65 h B- LiCl-treated 65h. 40

Figure 4.3.3: The Confocal images of 53 h groups at light conditions. The scale bar is 100 µm. A- non-treated 53 h B- LiCl-treated 53h 41

Figure 4.3.4: The Confocal images of 65 h groups at light conditions. The scale bar is 100 µm. A- non-treated 65 h B- LiCl-treated 65h. 42

*Figure 4.4.1: The relative gene expression of age-1 in a C. elegans model. Quantifications were performed according to LiCl treatment and dark as well as light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05, ** P<0.01, *** P<0.001. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h....* 46

*Figure 4.4.2: The relative gene expression of daf-16 in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05, *** P<0.001. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h.* 49

Figure 4.5.1: The total counts of brood size comparison between control and LiCl-treated groups at dark conditions. The green bars represent the control group that received no treatment and the orange bars are the LiCl-treated groups at constant dark. 51

Figure 4.5.2: The effect of LiCl administration on brood size of C. elegans in a daily manner. The green bars signify the control group that received no treatment and the orange bars are the LiCl-treated groups at constant dark 52

Figure 4.5.3: The total counts of the brood size comparison between control and LiCl-treated groups at light conditions. The green bars represent the control group that received no treatment and the orange bars are the LiCl-treated groups at constant light. 53

Figure 4.5.4: The effect of LiCl administration on brood size of C. elegans in a daily manner at light conditions. The green bars signify the control group that received no treatment and the orange bars are the LiCl-treated groups at constant light 53

Figure 4.5.5: The comparison of C. elegans' brood size at different light conditions and LiCl treatment. Green bars are the control groups and orange bars are the LiCl-treated groups 54

*Figure 5.1.1: The relative gene expression of the lite-1 expression in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h.* 56

*Figure 5.2.1: The relative gene expression of the tax-2 in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05, ** P<0.01, **** P<0.0001. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h.* 59

*Figure 5.3.1: The relative gene expression of the tax-4 in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h..... 62*

*Figure 6.1.1: The relative gene expression of the lin-42 in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05 ** P<0.01, *** P<0.001. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h 66*

*Figure 6.2.1: The relative gene expression of the tim-1 in a C. elegans model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05 ** P<0.01. A- at 47 h, B- at 53 h, C- at 65 h, D- at 73 h and E- at 89 h. 69*

List of Tables

Table 1. The details of RT-qPCR reaction mixture for different genes 26

Table 2. The statistical representation of alteration in the lifespan of wild-type C. elegans as a result of LiCl administration and light-induced circadian rhythm disruption. 31

Table 3. The detailed list of targeted genes of this study. **43**

CHAPTER 1

1. INTRODUCTION

1.1. *Caenorhabditis elegans* (*C. elegans*)

The nematode *Caenorhabditis elegans* (*C. elegans*) is a powerful model organism, especially, in the fields of molecular biology and genetics and even, in multidisciplinary areas. In the mid-1960s, Sydney Brenner sought a relatively small model organism that could be easily grown *in vitro*, had fewer neurons than other model organisms, and could be easily viewed with a microscope to understand how genes work [1]. With this aim, Brenner and his team integrated the *C. elegans* as a model organism [1], [2]. From this point, this new model incorporation makes *C. elegans* the main character of three different Nobel prizes. In 2002, Sydney Brenner, H. Robert Horvitz and John E. Sulston were awarded the prize. Genetic analysis was used to integrate *C. elegans* as a new model organism and illuminate the processes of cell division, differentiation, and organ development [3]. In 2006, Andrew Z. Fire and Craig C. Mello explored the power of double-stranded RNA to silence genes. This was the discovery of RNA interference which enabled the construction of novel insights into controlling gene expressions [4]. In 2008, Martin Chalfie revealed that the green fluorescent protein (GFP) marker could be

used as a luminous genetic tag for biological phenomena, and in the first experiments of this study, six different cells of *C. elegans* were marked with GFP [5].

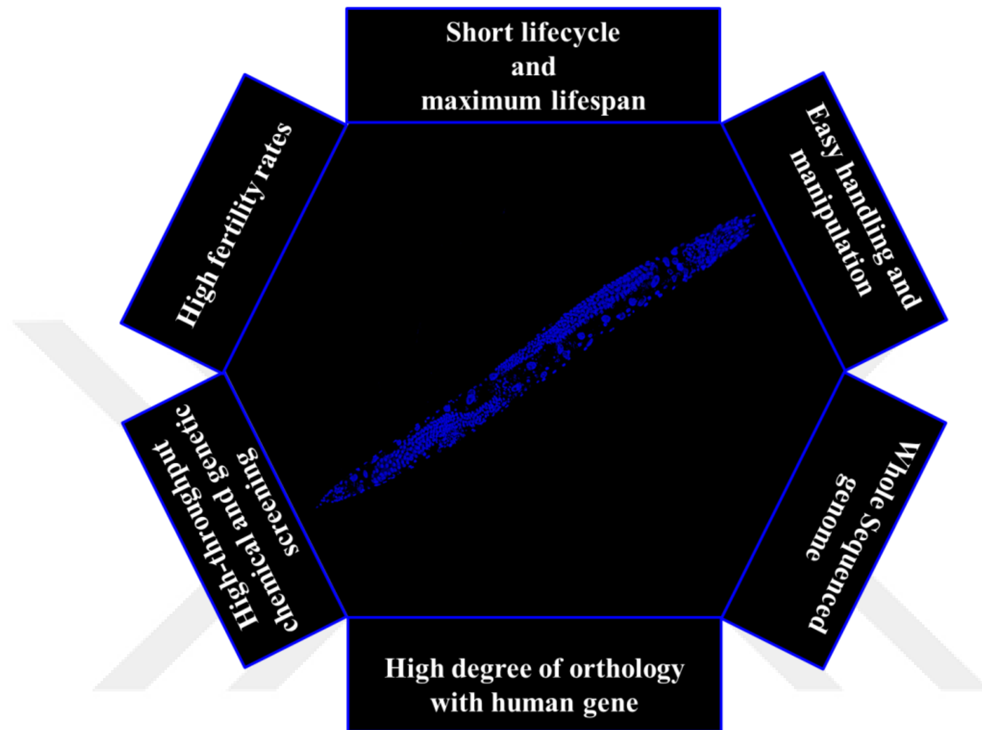


Figure 1.1: The demonstration of highly crucial advantages of *C. elegans* model organism that establish its prominence in diverse research studies

C. elegans has transparent and defined anatomy that helps to the simplicity of its manipulation for these studies. It is also known as the first multicellular organism to have its whole genome sequenced [6], [7]. Hence, the *C. elegans* genome has provided a basic template for other genome sequencing projects, including the Human Genome Project [7], [8]. The main reason behind this contribution was that many of the genes in the worm's genome have functional counterparts in the human genome [9]. The *C. elegans*

genome has revealed many functional counterparts in the human genome. It was estimated that genes are orthologous in 40-80% of all human genes and 40-50% of human disease-associated genes [9], [10]. The noteworthy degree of conservation present between *C. elegans* and humans increases its significance in the investigation of human diseases, lipid metabolism genes and signaling pathways such as the insulin signaling pathway [11]. Thanks to these benefits, there has been a great deal of studies with *C. elegans* regarding the characterization of behavioral as well as morphological mutants [2], the discovery of RNA interference (RNAi) methodology [12], aging [13], neurogenesis/neurodevelopment [14], [15], longevity [16]. Overall, the simplicity of its maintenance, high genetic conservation with humans and other advantages increase its value in many biological phenomena (Figure 1.1).

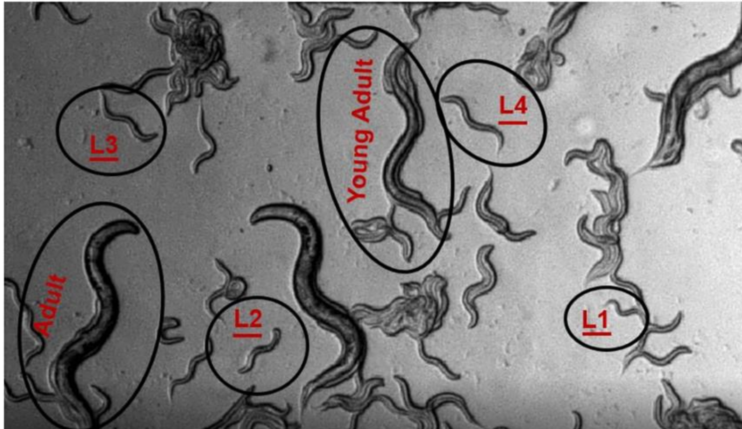
1.2. *C. elegans* as a Model for Understanding Aging and Extending Lifespan

Aging is an inevitable, universal part of our lives. Aging and the changes related to this process form the basis of many important studies and problems to be elucidated from the past to the present. It gradually weakens how well our biological systems function over time. This weakening is caused by a buildup of damage from different stresses that the body experiences throughout life [17], [18]. In the field of aging research, longevity is the key term. It implies the capacity of an organism to achieve an extended lifespan, approaching its species' biological maximum under normal circumstances [19], [20]. Model organisms are crucial players in revealing the underlying mechanism of this

complex process and effect. The short lifespan, ease of manipulation, and short generation cycle are some of the fundamental reasons why model organisms are useful for comprehending aging and longevity. As demonstrated in Figure 1.1, *C. elegans* is one of the most powerful species due to its completed genome sequence, a determined number of cells (959 somatic cells for hermaphrodites and 1031 somatic cells for males) and the presence of mutant strains [21], [22]. These nematodes have a short life cycle of around three days to reach adulthood and a life span of approximately three weeks at 20°C. Thanks to their sensitivity, these periods vary with the temperature change. In their short generation cycle, their developmental stages can be distinguished easily which mainly consist of six stages (L1, L2, L3, L4, Young Adult, and Adult) as indicated in Figure 1.2.B. This property helps to investigate and manipulate each development stage separately [13], [23], [24].

C. elegans primarily exists in two sexes, with the majority being hermaphrodites (around 99.8% ratio) and a small percentage being male (nearly at 0.2%). Ought to its hermaphrodite nature, the numbers increase rapidly, with a high reproduction rate, approximately each adult produces 300 progenies when self-fertilization occurs and more than 1000 offspring by mating with a male [11], [24]. The high number of progenies enables the investigation of the associated mechanism of fertilization efficiency and reproductive health [11]. Furthermore, the size of the organism, which is almost 1 mm in its adulthood is one of the biggest reasons for its widespread use. With the aid of its compact size, the whole life cycle and each developmental stage can be easily followed under the stereo-microscope as illustrated in Figure 1.2.A.

A-



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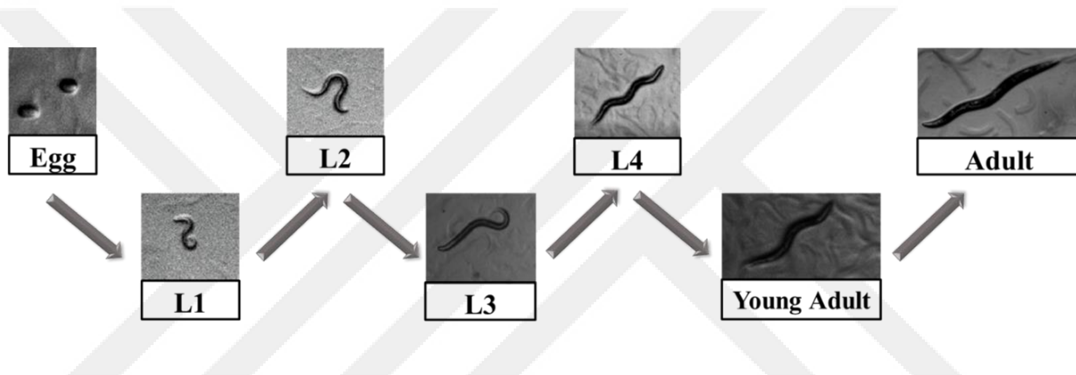

Figure 1.2: The representation of *C. elegans*' stages under stereomicroscope. **A-** The representation of all stages and size differences between stages. **B-** After egg laying, the stages are L1, L2, L3, L4, Young Adult and Adult in a developing manner.

During the aging period of worms, they demonstrate deterioration in movement/locomotion [25], rate of reproduction [26], behavioral response [27], increased auto-fluorescence accumulation in cells [28] and loss in vital functions such as learning, memory, and immunity constitutes another dimension of *C. elegans* aging and longevity [13]. Approximately two-thirds of all human disease genes have homologs in *C. elegans*, and age-related physiological changes at the molecular, cellular and tissue levels show human-like changes. As a result, *C. elegans* is a powerful model for studying aging,

lifespan and experimental research to understand several aging-related human diseases [13].

Investigating how the shift in gene activity patterns is valuable for assigning molecular signatures and potential effectors in *C. elegans*. In other words, these patterns can give insight into how gene expression alteration is related to an organism's lifespan and healthy aging [29]. Previous studies have highlighted that age-1 and daf-16 genes are the key players for better longevity. These genes are the effector of the insulin/IGF-1 signaling (IIS) pathway and necessary for the formation of dauer that developmentally arrested stage of *C. elegans* under severe conditions [30], [31]. The longevity regulator age-1 gene is known as the homolog of the catalytic subunit of human phosphatidylinositol 3-kinase (PI3K). PI3K signaling pathway has appeared as a critical player in several regulations of biological mechanisms. Fundamentally, PI3K signaling influences brain functioning by triggering the regulation of synaptic plasticity, increasing the capacity of adult neuronal progenitor cells. These relations indicate the influential role of PI3K in healthy brain functioning, aging and the longevity of organisms [31], [32], [33]. Studies exhibited that worms with the loss-of-function mutation at age-1 have survived longer than the wild-type *C. elegans* [34], [35]. This extension in longevity is mediated by the antagonistic relationship between age-1 and daf-16. daf-16 has been identified as producing a homolog of the human forkhead box O (FOXO) transcription factor and has a remarkable relationship with age-1. In humans, the role of FOXO expression in longevity studies was reported. In brief, the FOXO proteins behave as transcription factors and the expression of FOXO is governed by the IIS pathway. The increase in PI3K activity caused the phosphorylation of FOXO which acts as a negative regulator of FOXO

gene expression [36], [37], [38]. Meaningfully, in *C. elegans*, when the IIS pathway downstream element age-1 is inhibited, the IIS pathway activity decreases. This decrease diminishes the IIS pathway's inhibitory effect on daf-16 indirectly and daf-16 acts as a transcription factor that promotes turning on the genes associated with longevity (Figure 1.3). Therefore, as a conclusion, reduction function mutation in age-1 could disrupt the normal functioning of the IIS pathway, leading to the elevation of the longevity-promoting DAF-16 activity indirectly [13], [39], [40]. However, how the age-1 and daf-16 gene expression fluctuations and relation affect longevity are not fully elucidated.

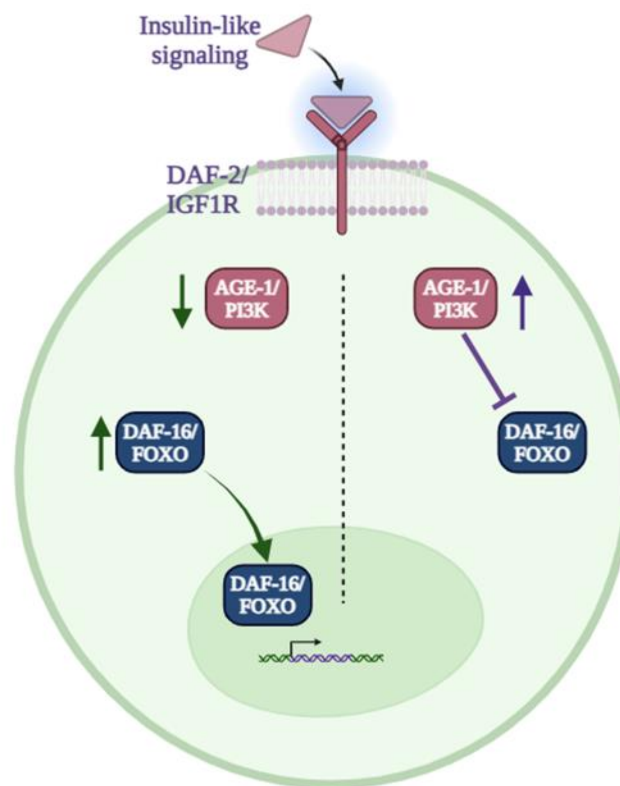

Figure 1.3: The schematic representation of the IIS pathway for longevity. When the signal is detected from the DAF-2/IGF1R receptor, this signal increases the activity of AGE-1/PI3K as represented in the right part of the graph (purple arrows). This increased

activity triggers the intermediate elements and the inhibition of DAF-16/FOXO. On the other hand, when AGE-1/PIK3 activity is reduced, DAF-16/FOXO is phosphorylated (the left part, green arrows). This phosphorylation stimulates the translation of DAF-16/FOXO into the nucleus to induce gene activity. The figure is formed in Bio Render.

On the other perspective, the elevation activity of DAF-16 is connected to germline inhibition. Studies assumed that when germline ablation happens, activation of DAF-16 is triggered by the translocating of DAF-16 protein into the nucleus. These outcomes cause an extension in the lifespan of *C. elegans*. This reproduction system's impact on the IIS pathway promises a vital route for uncovering the profound relationship between fertility and longevity [41], [42].

1.3. Lithium chloride (LiCl) treatment and Effect in *C. elegans*

Pharmaceutical applications became a prevalent trend in longevity research. Lithium chloride (LiCl) is emerging as a candidate drug within this rising trend of pharmaceutical applications in longevity research. LiCl become more and more popular in treating mood disorders like bipolar disorder and lithium's therapeutic effects extend beyond mood stabilization. For its action mechanism, various pathways are assumed as potential contributors of LiCl. One proposed mechanism is glycogen synthase kinase-3β (GSK-3β), a serine/threonine kinase that affects many different cellular functions such as growth, autophagy and apoptosis [43], [44], [45]. Fundamentally, LiCl inhibits GSK-3β.

In the inhibition of GSK-3β pathway, PI3K is one of the key elements. When the stimulator like insulin attracted to the receptor, PI3K becomes active and phosphorylates the Protein kinase B (AKT). AKT phosphorylation triggers further phosphorylation of GSK-3β at Ser 9 residue. This last phosphorylation causes the inactivation of GSK-3β and this dynamic relationship between PI3K and GSK-3β regulates the balance in cellular signaling pathways. Potentially, these interactions lead to enhanced cellular survival and stress resistance as hallmarks of longevity [46], [47].

LiCl is being investigated for its potential use in studies on aging, age-related diseases, and life span extension [48], [49]. The study revealed that LiCl treatment has an inverse relationship with mortality rates in individuals with Alzheimer's disease, leading to a reduction in overall mortality rates [48]. The neuroprotective properties and ability to modulate several cellular pathways implicated in longevity create this potential effect. These researches extend beyond human cells to include model organisms such as *C. elegans* and Drosophila melanogaster [49], [50]. Due to its benefits, *C. elegans* is the most powerful and popular model organism to reveal the LiCl effect. Studies suggested that LiCl can increase stress resistance, enhance protein quality control and influence gene expression through GSK-3β inhibition, known to regulate and extend lifespan in C. elegans [49], [51]. Furthermore, lithium treatment has been observed to decrease the expression of certain genes while increasing the expression of others [49]. As discussed in Chapter 1.2, the targeted genes of this study, age-1 and daf-16 are the milestone elements to regulate the longevity of C. elegans. However, the LiCl effect on these gene expressions and the precise mechanisms underlying its therapeutic effects remain elusive.

1.4. Circadian Rhythm in *C. elegans*

Circadian rhythm refers to the 24-hour cycle of physical, biological, mental, and behavioral changes in an organism, encompassing day/night cycles. Circadian rhythm describes behavioral and physiological changes that organisms make in response to environmental factors such as temperature, light, and food availability. These changes are also viewed as results of an internal, autonomous system that counteracts the passage of time [52], [53]. In 2017, Michael W. Young [54], Jeffrey C. Hall, and Michael Rosbash [55] were awarded the Nobel Prize in Medicine for their research on circadian rhythm. They isolated a gene that controls daily life rhythm, naming it 'period', and its corresponding protein 'PER'. It was shown that this protein accumulates at night and affects the activity of the period gene. Additionally, another gene affecting the function of the period gene, named 'timeless', and its protein 'TIM', was identified. The TIM protein binds to the PER protein, inhibiting the period gene activity [55], [56]. This groundbreaking research has the potential to explain how plants, animals, and humans adapt their biological rhythms to synchronize with the Earth's revolutions, providing insights into various aspects of physiology such as digestion, meal timing, endocrine hormone regulation, and energy expenditure [57].

A study conducted in 2002 demonstrated that, in addition to model organisms like fruit flies and mice, *C. elegans* also possess circadian rhythms by proving their disrupted circadian rhythm period with the alteration in light condition [58]. Subsequent research by various groups has supported this finding over time [59], [60], [61]. Due to its natural

habitat in soil, *C. elegans* is exposed to temperature fluctuations corresponding to sunlight during day-night cycles with a homolog of per gene; lin-42 and homolog of tim gene; tim-1 [62]. Additionally, when associated with surface-dwelling species, it is likely that *C. elegans* experience daily light-dark cycles [63] and have a photosensation ability [64]. Their transparent bodies enable them to penetrate light and influence the internal photoreceptor cells to respond to the alterations.

Significantly, LITE-1 is the responsible protein for this photosensation. It belongs to the invertebrate taste receptor family and shows exceptional efficiency about 10-fold higher than usual photoreceptors [65], [66]. At the molecular level, upon light exposure, lite-1 undergoes a conformational change that initiates a signaling cascade within the sensory neurons of *C. elegans*. Recruitment of the signaling cascade stimulates the downstream signaling molecules, ultimately leading to changes in gene expression and behavioral responses [66], [67], [68]. Therefore, lite-1 gene activity could be required for elucidating the association between sensory inputs and physiological and developmental processes in *C. elegans*. Detection of light through lite-1 provides adaptation and adjustment to worms' behavior and physiology as a result of light fluctuations in their environment [64], [66], [69].

1.5. Combinational Impact of Circadian Rhythm and LiCl Administration

Interruptions in circadian rhythm and duration of sleep have been widely observed across a range of neuropsychiatric disorders, including bipolar disorder [70], [71], [72]. Bipolar patients' difficulties induce less requirement for sleep by shortening their circadian phase. This shorting refers to the fast running of the internal clock which is the indicator of improper sleep cycle and early occurrence of daily rhythm. It has been observed that bipolar patients undergoing LiCl treatment experience better-adapted circadian phases by delaying this rhythm to a normal diurnal rhythm [73], [74], [75]. This and supporting studies highlight the link between these two concepts and suggest that LiCl's known effects on GSK-3β and the IIS pathway are both involved in circadian rhythm regulation, possible influencers of this period-lengthening [76], [77].

In *C. elegans*, TAX-2 and TAX-4 are subunits of a cyclic nucleotide-gated (CNG) channel. These channels are crucial for sensory transduction, particularly in chemo-sensation and photo-sensation. TAX-2 and TAX-4 work together to mediate responses to light condition changes as photoreceptors, which can influence the organism's internal biological clock. Fundamentally, they encode for subunits of a heterodimeric transcription factor complex that regulates the expression of clock genes. These clock genes, in turn, control the rhythmic expression of various downstream genes crucial for maintaining a healthy circadian cycle [61], [63]. Additionally, how LiCl treatment affects the activity of tax-2/-4 gene expression requires further investigation. Consequently, the

interplay between LiCl treatment and the TAX-2/TAX-4 signaling pathway in regulating circadian rhythms is a promising area of research. LiCl may enhance or stabilize the sensory signaling mediated by TAX-2 and TAX-4, leading to more robust entrainment of the circadian clock in response to the alteration of light conditions.

1.6. Significance of the Study

This study hypothesizes that LiCl treatment can alter the adverse effects of circadian rhythm disruptions and reverse the aging process of *C. elegans* (Figure 1.6). In order to test this hypothesis, there are three primary objectives. The first is to comprehensively identify the changes that occur in the circadian rhythm of *C. elegans* due to variations in light conditions. Despite research demonstrating the presence of circadian rhythms and some aging-related changes triggered by light alterations [66], the specific gene expression involved in this mechanism has yet to be fully elucidated. Remarkably, the reference research was conducted at a temperature of 22°C, while our current study was carried out at 20°C. This temperature change is a very critical factor to consider. This is because, as shown in studies, 20°C constitutes the standard optimum growth condition of *C. elegans*. At lower temperatures, there is a temperature-dependent extension in the typically determined lifespan (~ 4 weeks), and generation time (~ 5 days) compared to normal conditions. Conversely, higher temperatures result in a shortening of lifespan (~ 2 weeks), a reduction in egg production and life cycle (~ 2.5 days) and unexpected transcriptomic changes due to temperature differences [21], [78]. This variance in temperature is of significance due to its impact on the alteration of circadian rhythm.

Therefore, as an initial significance of this study, it will provide a vital understanding of the changes in circadian rhythm.

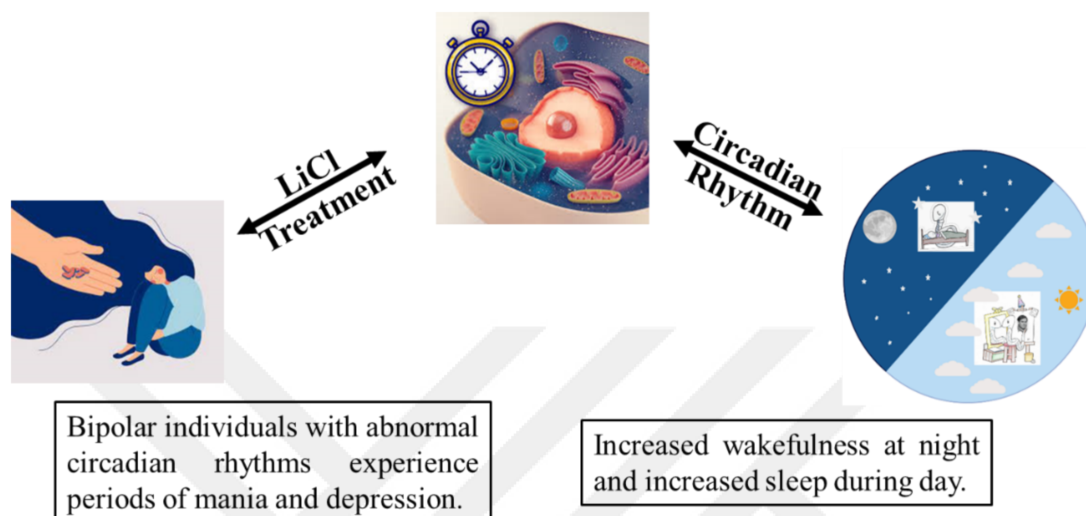

Figure 1.4: The illustration of LiCl treatment and Circadian rhythm association on longevity [73], [79]. Bipolar individuals experience periods of mania and depression, also has abnormal circadian rhythms.

The second objective is to determine the alterations caused by LiCl treatment and its role in circadian rhythm and aging. In this study, we aim to investigate the impact of LiCl administration on specific gene expression and its associated patterns in life span extension at various developmental time points. This research endeavors to provide valuable insights into the effects of LiCl on different stages of development.

Lastly, by combining the insights gained from the first two objectives, we aim to model the role of these changes in longevity by investigating their combinational effect. Hence,

this study aims to provide a comprehensive analysis of how circadian rhythm manipulations and LiCl treatment affect longevity and aging in *C. elegans*. This understanding could open new opportunities for future research on the genetic and environmental factors influencing longevity and health span.

CHAPTER 2

2. MATERIALS AND METHOD

2.1. Preparation of Buffers and Solutions

1 M LiCl solution: To prepare 1 M LiCl Solution, dissolve ~2,12 g LiCl with 50 ml distilled H_2O. The solution was sterilized by autoclaving at 121°C. The solution was stored at room temperature.

Nematode growth media (NGM): 8.5 g agar, 1.5 g NaCl and 1,25 g peptone were measured and dissolved in distilled H_2O. The final volume is 500 ml and the prepared NGM was sterilized by autoclaving at 121°C for 10 mins in liquid mode. After sterilization, the NGM was cooled to 55°C and 500 µl of 5 mg/mL cholesterol in ethanol solution, 500 µl of 1 M $MgSO_4$, 500 µl of 1 M $CaCl_2$, and 12.5 mL of 1 M KPO_4 buffer were added to cooled NGM, near the flame.

M9 medium: 1.5 g KH_2PO_4, 3 g Na_2HPO_4 and 2.5 g NaCl were dissolved in distilled H_2O. The final volume was 500 mL and autoclaved at 121°C. The prepared M9 medium

was stored at room temperature. Before utilizing, the 500 µl of 1 M MgSO4 solution was added near the flame.

KPO_4 buffer: 19 g KH_2PO_4, 10,5 g K_2HPO_4 and 160 ml distilled H_2O were mixed and sterilized by filter sterilization. The prepared solution was stored at room temperature.

5mg/ml Cholesterol solution: 0.25 g of cholesterol was softened in 100% ethanol. The final volume was 50 ml with 100% ethanol and filter sterilization was done. The solution was stored at 4°C.

1 M $CaCl_2$ solution: 1,47 g of $CaCl_2.2H_2O$ was measured and dissolved with distilled H_2O that fill up 10 ml distilled H_2O. The mixture was filter-sterilized and stored at 4°C.

1 M MgSO4 solution: 2,46 g $MgSO_4.7H_2O$ was weighted and dissolved with distilled H_2O that filled up 10 ml. The mixture was filter-sterilized and stored at 4°C.

2.2. Maintenance of *C. elegans* and Application of LiCl

In this study, Bristol isolate N2 wild-type strain of *C. elegans* was utilized and obtained from the *Caenorhabditis* Genetic Center (CGC) at the University of Minnesota. The nematodes were cultured on NGM agar plates. The NGM agar plates were prepared according to the explanation in Section 2.1. In general, NGM plates are maintained at 20°C and prior to utilization, the plates were brought to room temperature in order to

avoid the unplanned heat shock effect. Controlling temperature also aids in minimizing any additional effect of temperature variation on biological processes, especially circadian rhythms and aging. A lawn of OP50 bacterial strain of live *Escherichia Coli* was used as a food source for *C. elegans*. *C. elegans* was cultured on 6-cm NGM plates by using flame-sterilized platinum picks. Whole experiments were conducted at a constant temperature of 20°C.

To comprehend the pharmacological effect, 10 mM LiCl which was obtained from Thermo Fisher Scientific (Cat. No. AC449040000) was used in this experiment. This concentration was chosen based on previous studies indicating its efficacy in altering biological pathways relevant to aging and circadian rhythms [49]. For the administration of LiCl, the calculated amount of 10 mM LiCl was added to NGM plates prior to the solidification.

2.3. Circadian Rhythm Alterations

To understand the effect of circadian rhythm, the light condition changes were performed as a zeitgeber. The experiment was performed in two groups; one was exposed to constant darkness and the other to permanent light conditions. For whole experiments, white light LED was used as a mirroring of artificial office LED light (peak at 450 nm). In the present experimental setup, the spectra data of the used light source were collected in two distinct methods. Initially, direct measurements were taken using an Ocean Optics-Flame-S-XR1 spectrometer. Subsequently, measurements were conducted using a fiber connector

spectrometer with a 1-meter long, 400 µm core diameter silica fiber attached. The analysis of the spectrometer was performed by using Origin.

2.4. Age Synchronization of Nematodes

When the culture grown on 6 cm plates became gravid adult, plates were washed with 1 ml M9 buffer and collected into a 1.5 ml microcentrifuge tube. The collected samples remained for precipitation. When the gravid adults were precipitated, the supernatant was removed and washed again with 1 ml M9 buffer. These steps were repeated two more times to ensure the removal of the bacteria from the samples. During the final precipitation incubation, 1 ml bleaching solution was prepared freshly that contains 50 µl of 10M KOH, 200 µl of 15% sodium hypo-chloride and 750 µl of autoclaved distilled water. At the last removal of the supernatant, 1 ml of freshly prepared bleaching solution was added. Then, immediately, the tubes were vigorously shaken for 3 minutes. During this step, the condition of gravid adults was monitored with a stereomicroscope to ensure proper bleaching. After 3 minutes, the samples were instantly centrifugated for 30 seconds at 2000 rpm at room temperature. The supernatant was removed and the egg pellets were washed with 1 ml of M9 buffer. This washing step was repeated four more times to eliminate the sodium hypo-chloride solution from samples. At the last washing, the pellets were resuspended with 100 µl of M9 Buffer. The age-synchronized worms were then counted using a stereo-microscope. The same number of worms (~2000) was placed into each experiment group as explained in Chapter 3.1.1.

2.5. Lifespan Assay

Lifespan measurement was performed to understand the consequence of longevity of *C. elegans* as a direct method. After the synchronization of the age of worms, the lifespan assessment was started and both circadian rhythm manipulation and LiCl treatment were begun at this point. When worms reached the late L4 stage, 100 worms were transferred to new experimental plates. This day was called day 0 as a starting period of the experiment. Until day 7, the groups were checked and transferred to new plates every day to eliminate the interference of egg laying. Every day, the animals were scored as alive, dead and censored. If worms did not move or respond to gentle touch with a platinum pick, these animals were grouped as dead and if worms dug into the NGM, they were grouped as censored. After day 7, the experiment was checked every two to three days. Kaplan-Meier analysis of lifespan data was done with GraphPad Prism 8.0.

2.6. Protein Isolation and Measurement of Autofluorescence Accumulation

Approximately 2000 worms were subjected to protein extraction for each different time point (47 hours (h), 53 h, 65 h, 73 h and 89 h). The samples were boiled at 95°C for 10 minutes. Subsequently, the samples were vortexed for 10 minutes at room temperature. Then, the samples were centrifugated at 16,000 x g for 30 minutes at 4°C to separate crude protein from the debris. The supernatants contained crude protein extract. The isolated

proteins were stored on ice during the whole procedure to prevent degradation. To determine the effect of the aging process by protein release with its autofluorescence feature, a fluorescence spectrophotometer (Cary, Eclipse) was used. The obtained outcomes were illustrated by using GraphPad Prism 8.0.

2.7. Confocal Imaging

To understand the signs of aging at 53h and 65h in more detail, DAPI staining was performed. The slide preparation was performed under the stereomicroscope. 10 µl of C. elegans in M9 buffer was placed on top of pre-sterilized microscopy slides. The excess M9 buffer was removed with the aid of a tissue wiper (Kimtech™ Science Precision Wipes). After ensuring the dry slides, 10 µl of 90% EtOH was added to on top of the worms. 2 minutes of incubation was done for dry 90% EtOH and these two steps were repeated four more times. At the last incubation, the DAPI stain was prepared at a final concentration of 2 ng/µl. After the slides were dried, 8 µl of DAPI and mounting medium solution were added to the slides. Slides were covered with cover glass and samples were fixed with nail polish.

2.8. Sample Preparation and RNA Isolation

For this experiment, 47 h, 53 h, 65 h, 73 h and 89 h of *C. elegans* were collected. The relevant samples were washed with M9 Buffer at their appropriate stage. For each group, almost 2000 worms were utilized. The supernatant was removed when the precipitation

of worms occurred. The pellet was washed again with 1 ml M9 buffer. These steps were repeated until the bacterial content was removed from the samples. After allowing the worms to precipitate, the methodology of total RNA extraction was performed. The protocol was performed with the aid of Green et. al., 2020 [80]. 1 mL of RiboEx™ monophasic lysis reagent (Cat. No. 301-001) was added to each sample. The samples were vortexed vigorously for 20 minutes at room temperature to lysed forms of worms and make them soluble. Subsequently, 200 µl of chloroform was added and vortexed occasionally during 15 minutes of incubation at room temperature to facilitate phase separation. When the incubation period finished, the samples were centrifugated at 14,000 x g for 15 minutes at 4°C. The aqueous phase was carefully transferred into new microcentrifuge tubes. Isopropanol was added to these samples in an equal volume of aqueous phase. Samples were inverted several times to mix the solution and incubated for 10 minutes at room temperature. Following the incubation, the samples were centrifugated at 14,000 x g for 20 minutes at 4°C. The supernatant was discarded and the RNA pellets were washed with 1 ml ice-cold 75% ethanol. Then, centrifugation was performed at 14,000 x g for 5 minutes at 4°C. After centrifugation, supernatants were discarded and the pellets were dried. The dried RNA pellets dissolved with 20 µl of RNase-free water. This period was supported with the incubation at 65°C for 5 min. This step was followed by a vortex for 10 seconds at room temperature. Finally, samples were centrifugated for 1 minute at 4°C and 14,000 x g. The supernatant was utilized as the isolated RNA. The concentration of isolated total RNA was measured by Nanodrop 2000 (Thermo Fisher Scientific). The RNA concentration of each sample was adjusted to around 200 ng per µl.

2.9. The synthesis of complementary DNA

To produce complementary DNA (cDNA) from isolated total RNA samples, the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Cat. No. 1708841) protocol was applied for each sample. This reaction was performed with 0.5µg RNA template, 4µl of iScript RT Supermix and the mixture's final reaction volume was brought to 20 µl with the addition of nuclease-free water. The prepared reaction mixture was incubated at Bio-Rad C1000 Thermal Cyclers. The adjusted thermal cycling protocol was 5 minutes at 25°C for priming, 20 minutes at 46°C for reverse transcription and 1 minute at 95°C for reverse transcriptase inactivation. For Real-Time quantitative PCR assay, the synthesized cDNAs were diluted with nuclease-free water at a 1:2 ratio and samples were stored at -20°C for short-term usage.

2.10. Real-Time Quantitative PCR

GoTaq® qPCR Master Mix (Promega, Cat. No. A6001) was utilized for the RT-qPCR reaction. The qPCR condition was 5 minutes at 95°C, 15 seconds at 95°C, 1 minute at 60°C and 60°C to 95°C with increments of 0.5°C for 5 seconds so as to form melt curves. The gene interests were normalized according to the act-1 housekeeping gene. In order to analyze the meaning of the data, the $2^{\wedge-\Delta\Delta Ct}$ method was used. This method allows for the comparison of gene expression levels between different samples by normalizing the Ct values of the target genes to the Ct values of the housekeeping gene and then normalizing

the experimental group to the control group. Quantitative RT-PCR results were subjected to statistical testing using 2-way ANOVA.

For different genes, the reaction mix preparation was different and these used reaction mixtures indicated in Table 1. The efficiency of this experiment was checked with agarose gel electrophoresis. Mainly, seven targeted genes were used in this study that are age-1, daf-16, lite-1, tax-2, tax-4, lin-42, tim-1 and also, for the housekeeping gene, act-1 was used. Except act-1 gene, the primers of targeted genes were designed with the help of NCBI Primer Blast and OligoAnalyzer™ Tool. The primer sequence of these genes is represented as:

Longevity;

age-1

Forward Primer: 5’- GCCGAGTCAAGGACAATACAC -3’

Reverse Primer: 5’- CTCCACATCCAGACATGACG -3’

daf-16

Forward Primer: 5’- CATCGTTTCCTTCGGATTTC -3’

Reverse Primer: 5’- CATCCATTCGTAAACTTGTGC -3’

Response to environmental factors;

lite-1

Forward Primer: 5’- GCTTCCTGAATCGTCGTTAC -3’

Reverse Primer: 5’- CAACTTCGGACCAGCAAATC -3’

tax-2

Forward Primer: 5'- GGAAATAACCCAGCACCAAC -3'

Reverse Primer: 5'- GAATTTGACCGAGCAACAAG -3'

tax-4

Forward Primer: 5'- GGTCGTTGTCTGGTATATTG -3'

Reverse Primer: 5'- CGTAGTCAGAATTAGGGTTG -3'

Circadian Rhythm;

lin-42

Forward Primer: 5'- GTATCTGCTCCCTTCCAATC -3'

Reverse Primer: 5'- GCTCATCCTCGAAAGTCTTC -3'

tim-1

Forward Primer: 5'- CGTTGAGGAGTACAGAGAGC -3'

Reverse Primer: 5'- CTTCAATGCTCCTGTCTTCAC -3'

Housekeeping gene;

act-1 [81]

Forward Primer: 5'- CCAATCCAAGAGAGGTATCCTTAC -3'

Reverse Primer: 5'- CATTGTAGAAGGTGTGATGCCAG -3'

Table 1. The details of RT-qPCR reaction mixture for different genes.

Genes	qPCR Master Mix	10 µM Forward Primer	10 µM Reverse Primer	Nuclease-free Water
age-1	5 µl	0.20 µl	0.20 µl	3.6 µl
daf-16	5 µl	0.25 µl	0.25 µl	3.5 µl
lite-1	5 µl	0.20 µl	0.20 µl	3.6 µl
tax-2	5 µl	0.20 µl	0.20 µl	3.6 µl
tax-4	5 µl	0.25 µl	0.25 µl	3.5 µl
lin-42	5 µl	0.20 µl	0.20 µl	3.6 µl
tim-1	5 µl	0.15 µl	0.15 µl	3.7 µl
act-1	5 µl	0.15 µl	0.15 µl	3.7 µl

2.11. Fertility Assay

Fertility assay was carried out spontaneously with lifespan measurement experiments. The egg-laying occurred between day 2 and day 7. This reproduction activity was monitored. During this period, fertile gravid adults were transferred to new plates daily. After transferring the adults, the egg-containing plates were incubated for two days at 20°C. This incubation was conducted to ensure the counting of healthy brood size.

CHAPTER 3

3. CIRCADIAN RHYTHM MANIPULATION

3.1. Experimental Groups and Treatment Conditions

In the scope of this experiment, there are four main experimental groups. These groups are divided into light and dark conditions to induce circadian rhythm manipulation. In addition, each dark and light condition contains a control group and a LiCl-treated group.

3.2. Ensuring Light Wavelength for Circadian Rhythm Entrainment

Circadian rhythms are crucial for maintaining various biological processes by triggering the regulation of sleep-wake cycles, fertility, hormone release and longevity in living organisms [82], [83]. In *C. elegans*, circadian rhythms play a pivotal role in controlling gene expression, metabolism, and stress responses. In this study, the light condition of *C. elegans* was altered to understand the circadian rhythm effect.

In this experimental setup, measurements were performed using two different light sources; the white and the LED light. As humans, a considerable amount of our time is spent in artificial light environments, such as offices, schools, and homes. Therefore, office lights could be a powerful effector for our circadian rhythm. Consideration of how these artificial lights affect circadian rhythms can provide insights into modern lifestyles' impact on our biological clocks [84]. The spectra of these light sources were measured in two different ways. Measurements were first conducted directly using an Ocean Optics-Flame-S-XR1 spectrometer. Then, a 1-meter long, 400 µm core diameter silica fiber was connected to the output of the spectrometer with a fiber connector for further measurements. As indicated in Figure 3.1, the black, red, green, and blue solid lines represent the spectra of the direct white light source, the white light measured with the optical fiber, the direct office LED, and the office LED measured with the optical fiber, respectively. The spectra ranged from 400 to 750 nm in wavelength. The white light source demonstrates a peak at 450 nm and two broad regions between 480 and 750 nm. Similarly, the office LED exhibits a peak at 450 nm and two regions between 490 and 720 nm. The experiment's findings demonstrate that the artificial light source utilized closely resembles office lighting. This type of artificial light has a greater impact on people's circadian rhythm, as it is more frequently encountered in their daily lives and work environments [85].

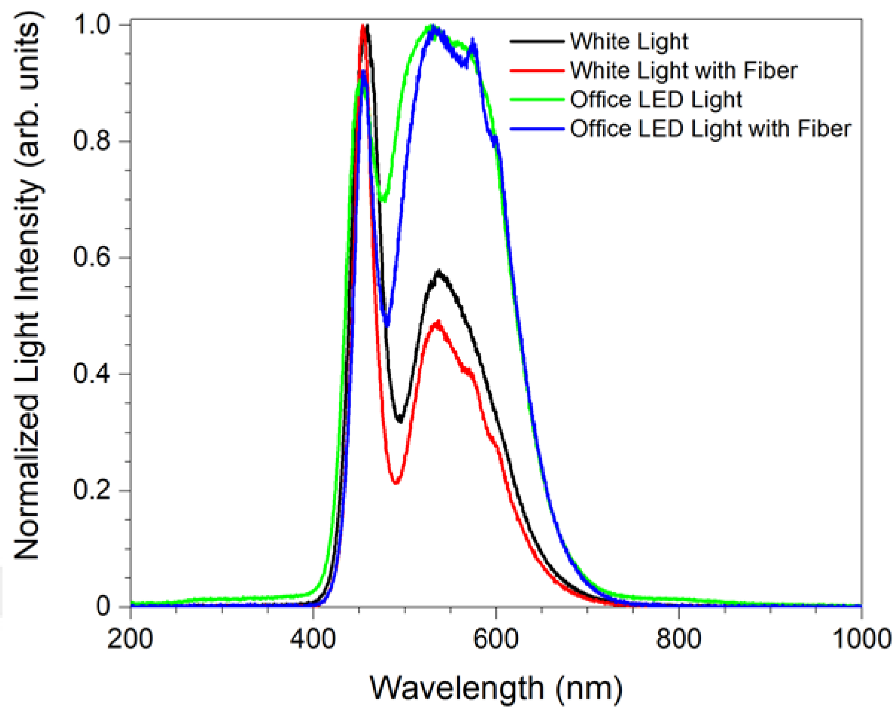

Figure 3.2: The spectra measurement of the utilized light source. The graph compares the wavelength of the light source in nanometers versus normalized light intensity in arbitrary units obtained from the light sources.

CHAPTER 4

4. LONGEVITY

4.1. Impact of LiCl Administration and Light-Induced Circadian Rhythm Manipulation on Longevity

In this section, the underlying effect of both LiCl treatment and light-induced circadian rhythm change on the lifespan of *C. elegans* was examined. In recent decades, attempts to improve the period of life passed in good health which is named as the health span of organisms have been elevated. Therefore, the search for novel interventions also arises to find how the organisms' lifespan increases and the possibilities to lower the devastating effects of aging. The pharmacological application is one of the possible pieces of addressing these biological questions [66], [86].

As stated in section 2.1, the 10 mM LiCl administration was applied thanks to its profound impact on longevity. Importantly, understanding the environmental factors' effect is another vital aspect. These environmental influences are fundamentally composed of temperature change, irregularity in eating habits, stress factors, and notably, time of exposure to light in daily life. Among these, light exposure has a critical role in revealing

this effect because this is directly linked with the biological clock of living organisms. The proper function of circadian rhythm is strongly correlated with better health span and longevity. When this rhythm is disrupted with light, the relevance of biological processes is also interrupted to trigger improper regulation in longevity. Therefore, the combinational impact of both LiCl treatment and alteration in circadian rhythm is the promising model for this inquiry.

As illuminated in Figures 4.1.1 and 4.1.2, the life span of *C. elegans* was determined in both dark and light conditions with the LiCl-treated and control groups. In Figure 4.1.1 and Table 2, at constant-dark conditions, the life span of LiCl-treated worms was increased by 33% when compared with the control group. This increase was exhibited with a P-value lower than 0.0001. This showed the significant impact of 10 mM LiCl treatment on the lifespan of *C. elegans*.

Table 2. The statistical representation of alteration in the lifespan of wild-type *C. elegans* as a result of LiCl administration and light-induced circadian rhythm disruption

Presence of Light	LiCl Administration	Median Lifespan (Days)	Median Lifespan Change	Number of worms	Biological Replicates
-	-	12	-	300	3
-	+	16	+33%	300	3
+	-	11	-8%	300	3
+	+	14	+27%	300	3

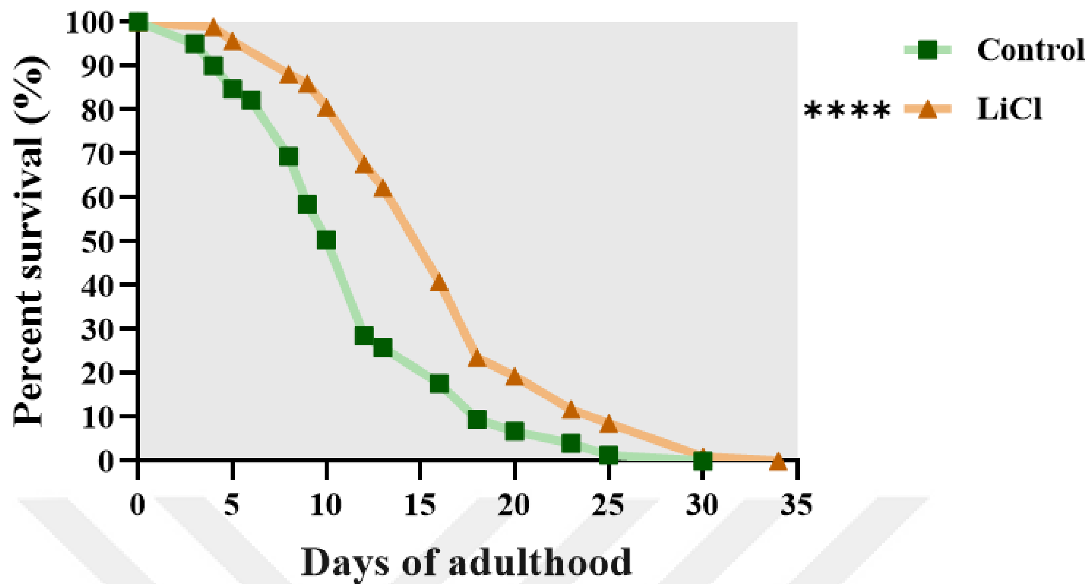

Figure 4.1.1: The lifespan determination of *C. elegans* under dark conditions. This graph displays the percent survival according to the days of adulthood of *C. elegans*. The green line indicates the control groups that did not undergo any treatment. On the other hand, the orange line represents the 10 mM LiCl-treated group at dark conditions. The experiment was performed at 20°C. **** denote a P-value lower than 0.0001.

At constant light conditions, the survival ratio was decreased by 8% according to their normal condition (Table 2). With the presence of LiCl treatment as an addition to light induction, the lifespan of *C. elegans* was 27% longer than the control group that lived at constant light conditions. This longer lifespan difference was statistically significant with a lower P-value than 0.0001 (Figure 4.1.2).

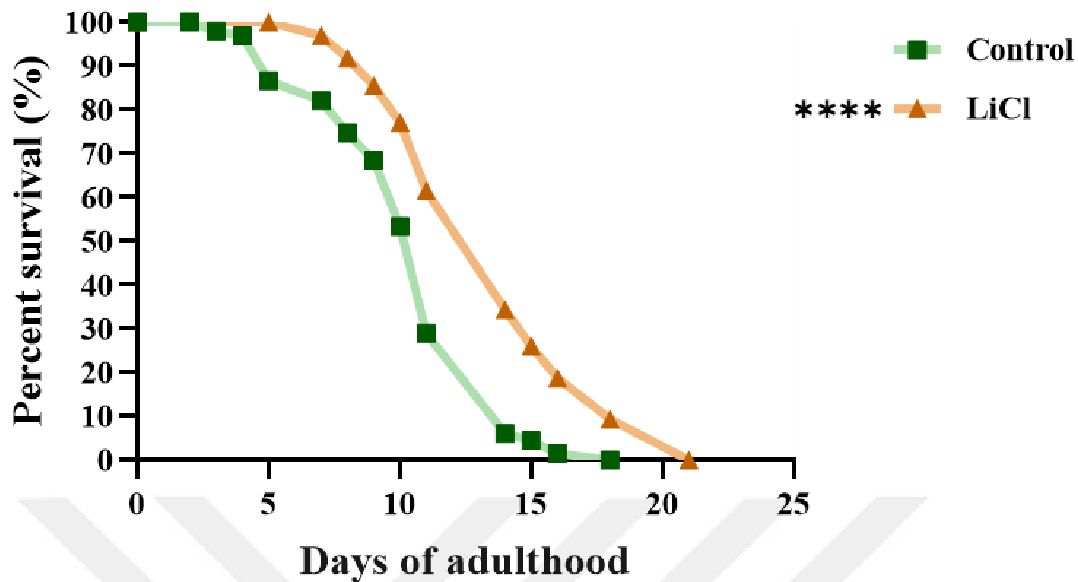

Figure 4.1.2: The analysis of *C. elegans*' lifespan under light conditions. This graph compares the percent survival versus the days of adulthood of *C. elegans*. The green line are the control groups that did not undergo any treatment at constant light. On the contrary, the orange line denotes the 10 mM LiCl-treated group at light conditions. The experiment was carried out at 20°C. **** symbolize a P-value lower than 0.0001.

4.2. The Autofluorescence Measurement of LiCl Treated and Changed Circadian Rhythm *C. elegans* as Aging Marker

Determination of the progressive pattern of aging is frequently challenging for *C. elegans*. A recent study demonstrated that the autofluorescence property of *C. elegans* is a useful

non-invasive biomarker for assessing this aging pattern [28], [87]. *C. elegans* displays another benefit for the determination of their change in the aging process. Significantly, *C. elegans* accumulates autofluorescence with age. This natural increase in fluorescence can be observed under specific wavelengths of light by using a fluorescence spectrophotometer. This method has significance due to its non-invasiveness. In other words, thanks to the functional property of non-invasive measurement, biological and physiological changes that happen naturally within the organism can be measured instead of the requirement of tissue biopsies or sample collection in an invasive way. These measurements are valuable to enhance effectiveness by maintaining the well-being of the *C. elegans* [28], [87].

In order to determine the impact of autofluorescence accumulation, fluorescence spectrometry of isolated proteins was measured at 225-390 nm excitation made worms fluoresce with the peak emission in the interval of 395-425 nm, at the highest peak 410 nm. In this experiment, distilled water and M9 buffer were used as blank solutions. The significant effect of LiCl administration on the aging pattern was even represented in Figure 4.2.1 (dark condition) and Figure 4.2.2 (light condition). In dark (Figure 4.2.1.B and Figure 4.2.1.C), when comparing the used blank solutions, there were no significant peaks at early response time points; 47 h and 53 h. For late response, control groups give a peak at 65 hours and this pattern was similar to 73 h and 89 h at dark conditions. For LiCl-treated groups, there was no difference between 47 h and 53 h at the early response. When the LiCl exposure time increases, the aging indicator peaks become apparent for the late response, particularly at 89 h. Prominently, the comparison between control and

LiCl-applied groups indicated that the LiCl-treated groups were lower than the normal effect of the prolonged exposure time according to control groups.

At light condition, there was no significance at 47 h. However, the autofluorescence signal became apparent at 53 h, earlier than dark groups. From this point, the appearance of peaks continues. Moreover, the difference was preserved between LiCl-treated and control groups as similar to dark condition. This suggested that the non-invasive aging biomarkers appeared in LiCl-treated groups, but the intensity was lower than in the control groups at both dark and light conditions.

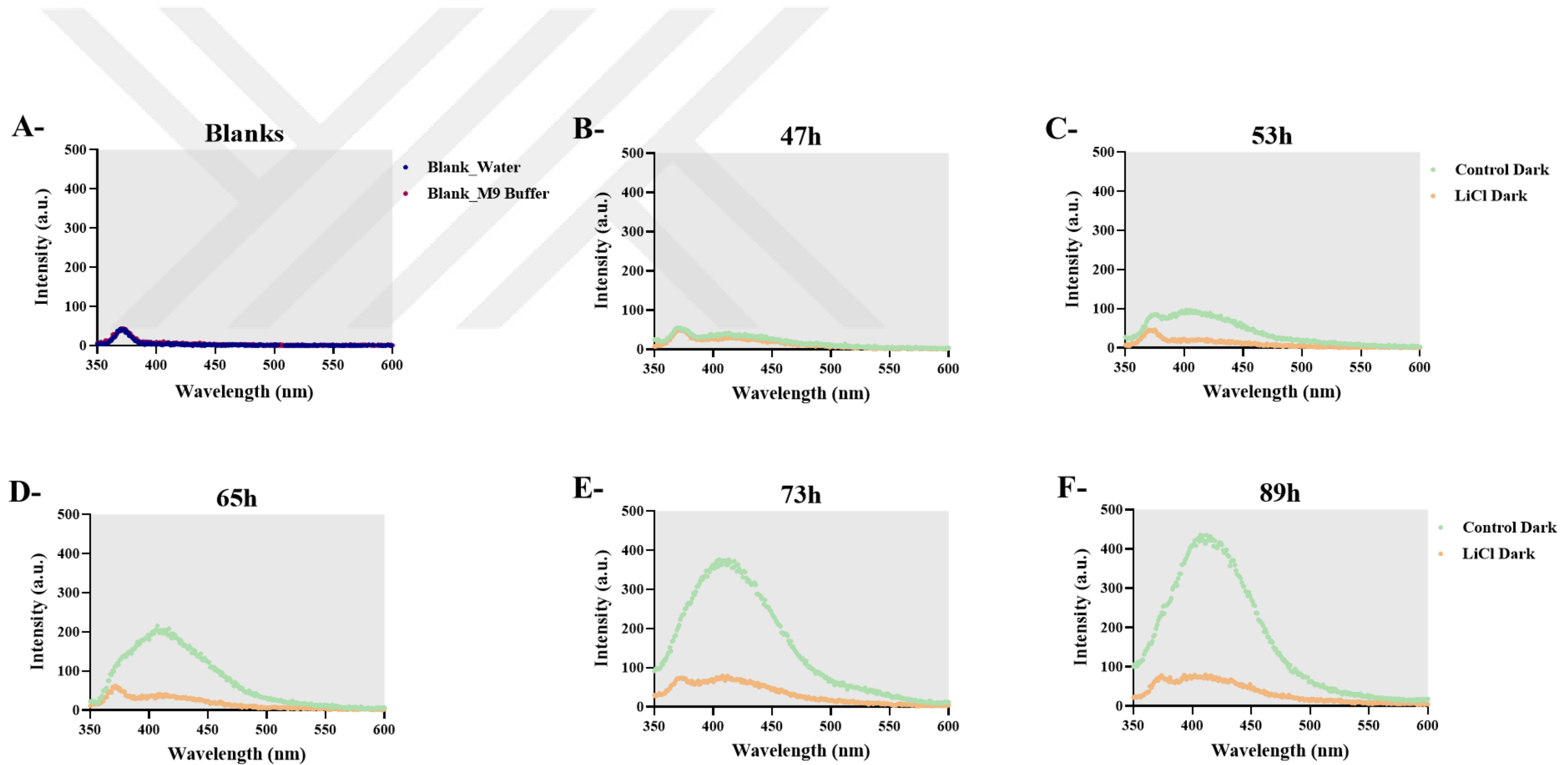

Figure 4.2.1: The autofluorescence measurement of *C. elegans* at dark condition and LiCl application. The green points represent control groups and the orange color is for LiCl-treated groups. The autofluorescence level was investigated (ex 330/em 350–600) by worm protein samples at different time points. **A-** Blank solutions; distilled H_2O and M9 buffer. **B-** 47 h. **C-** 53 h. **D-** 65 h. **E-** 73 h. **F-** 89 h.

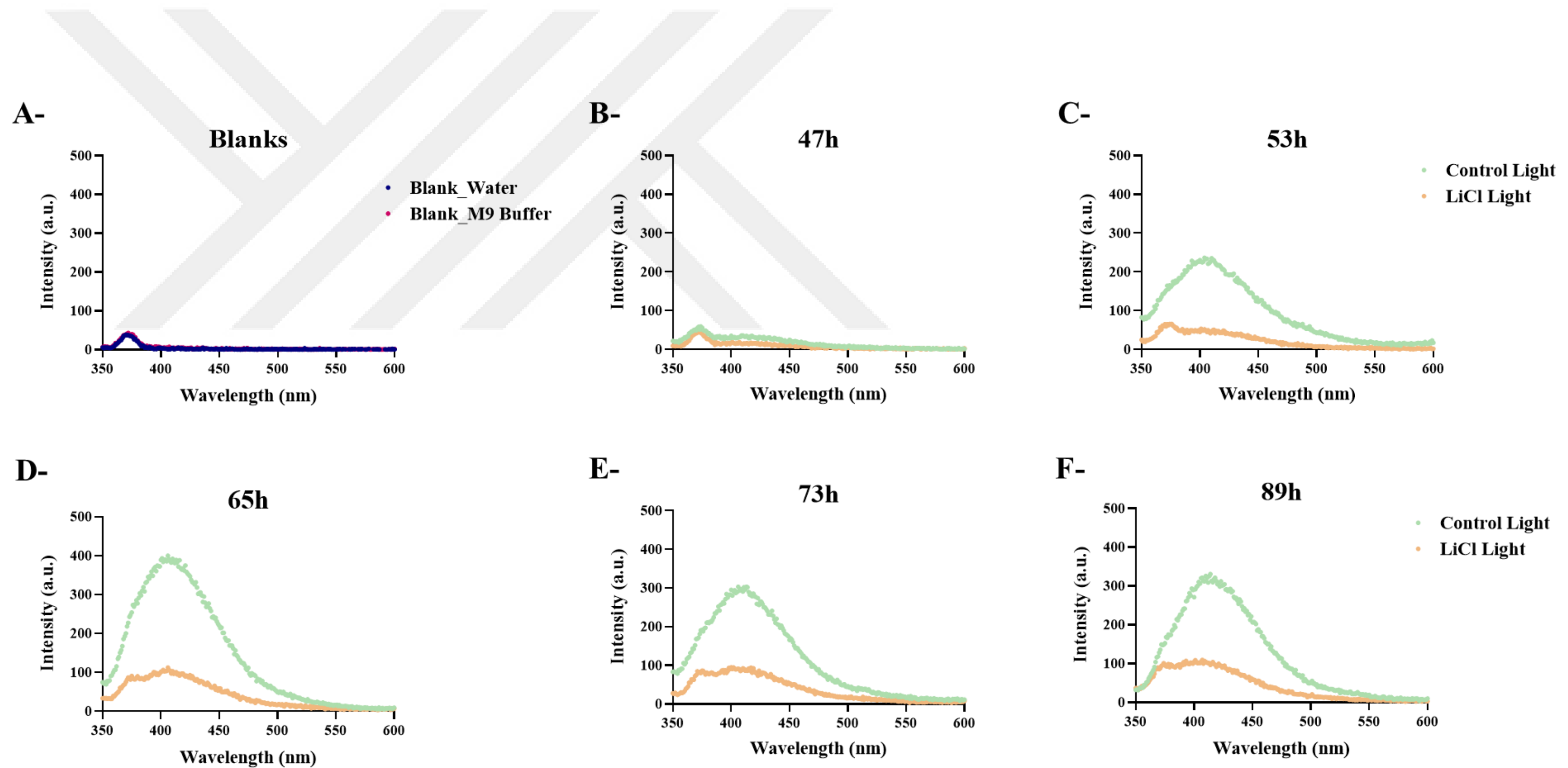

Figure 4.2.2: The measurement of autofluorescence level of *C. elegans* at constant light conditions and LiCl treatment. The green points denote control groups and the orange color is for LiCl-treated groups. The autofluorescence level was investigated (ex 330/em 350–600) by worm protein samples at different time points. **A-** Blank solutions; distilled H_2O and M9 buffer. **B-** 47 h. **C-** 53 h. **D-** 65 h. **E-** 73 h. **F-** 89 h.

4.3. Understanding The Effect on Different Exposure Periods

In Section 4.2, it was observed that the increase in autofluorescence signal, which is a non-invasive marker, occurred with different time points in the dark and light groups. Specifically, this significant accumulation started at 65 h in the dark group and at 53 h in the light group. To further understand this difference, DAPI staining was conducted. The main reason for this is that DAPI staining provides evidence about the cellular changes associated with aging. Studies display that DAPI staining aids in visualizing the damage caused by aging. the study conducted by McGee et al. showed that nuclear integrity and nuclei in *C. elegans* decreased with aging, especially in intestine. This effect acts in a manner related to cellular aging [88], [89].

In the dark groups, there was no difference in intensity between the LiCl-treated group and the control group at 53 h, as seen in Figure 4.3.1. At 65 h, it is seen that the integrity in the control group is higher than in the LiCl group (Figure 4.3.2). In the brightness group, it is seen that the higher integrity and nuclei in LiCl-treated groups difference started at 53 h (Figure 4.3.3) and continued at 65 h (Figure 4.3.4), proportional to the result obtained in section 4.2.

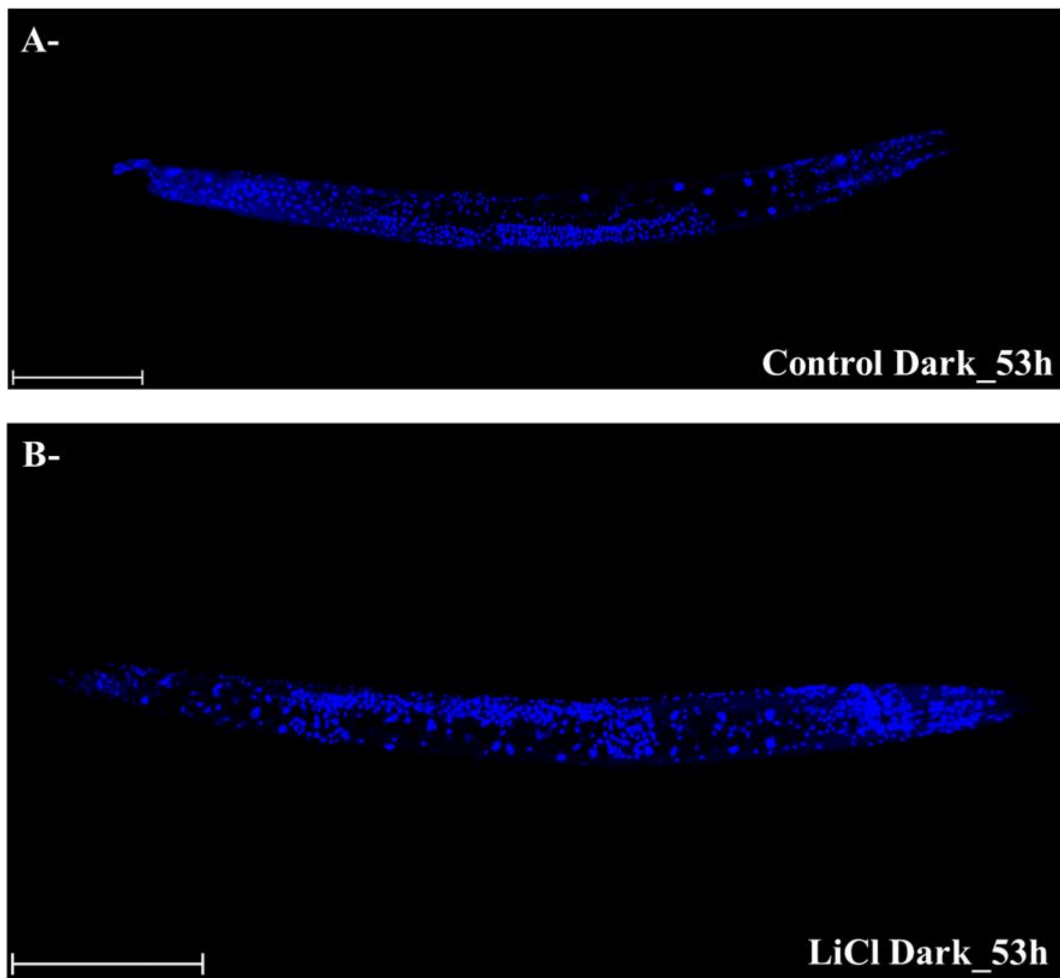

Figure 4.3.1: The Confocal images of 53 h groups at dark conditions.

A- non-treated 53 h **B-** LiCl-treated 53h. The scale bar is 100 µm.

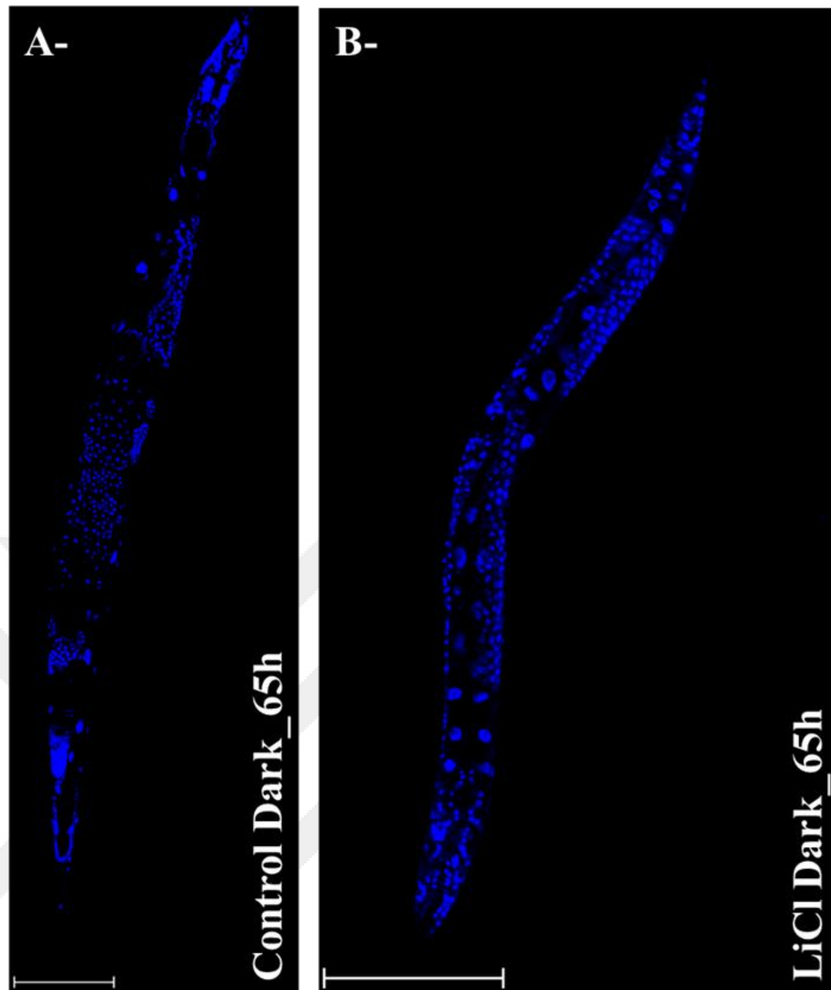

Figure 4.3.2: The Confocal images of 65 h groups at dark conditions.

A- non-treated 65 h **B-** LiCl-treated 65h. The scale bar is 100 µm.

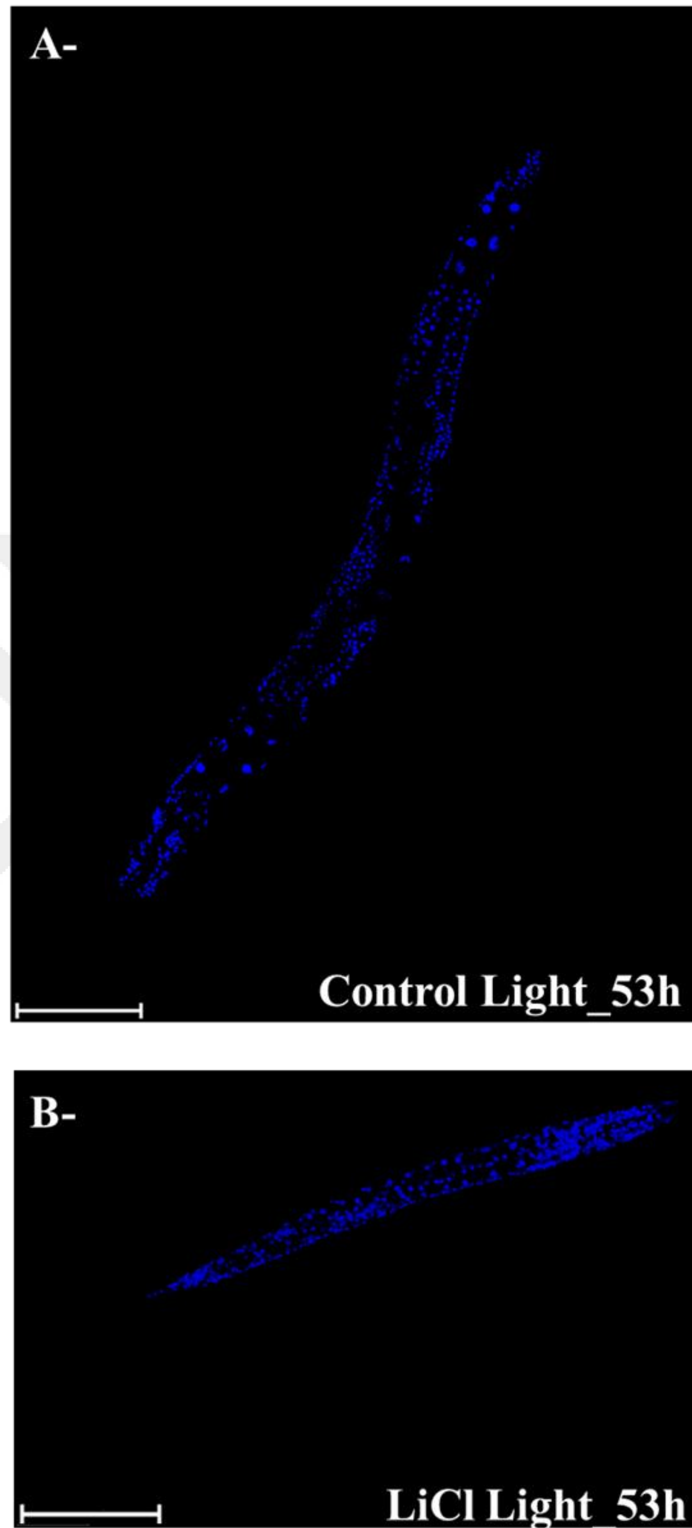

Figure 4.3.3: The Confocal images of 53 h groups at light conditions.

A- non-treated 53 h **B-** LiCl-treated 53h. (Scale bar: 100 µm).

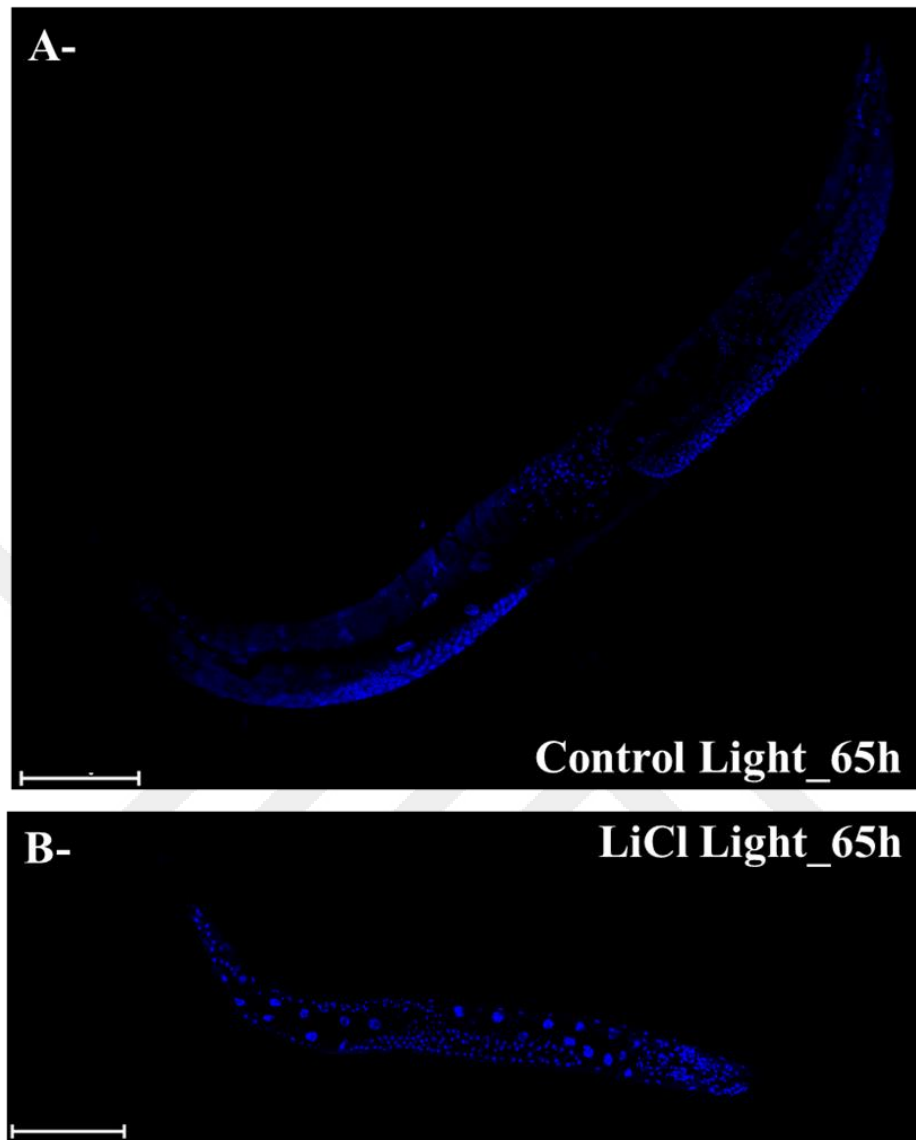

Figure 4.3.4: The Confocal images of 65 h groups at light conditions.

A- non-treated 65 h **B-** LiCl-treated 65h. (Scale bar: 100 μm).

4.4. Determination of The Differential Gene Expression Patterns Change with LiCl Treatment and Circadian Rhythm Alteration

Differential gene expression analysis is a critical experimental procedure for elucidating the underlying mechanism of biological processes and responses according to changes in normal conditions. In this section, the differential gene expression pattern was investigated for longevity. However, in this study, this differential gene expression pattern was investigated for other two categories: response to environmental changes and circadian rhythm. Table 3 presents these gene categories: age-1, daf-16, lite-1, tax-2, tax-4, lin-42, and tim-1, along with their functions and human homolog information.

According to the aim of this section, utilizing the advantages of *C. elegans*, differential gene expression pattern was investigated at different development stages to gain a comprehensive understanding of the effects. Fundamentally, 47 h, 53 h, 65 h, 73 h and 89 h' time points were investigated. Specifically, 47 h and 53 h were selected for early responses, focusing on the L4 - late L4 stage. On the other hand, 65 h, 73 h, and 89 h were chosen to study late responses, with a focus on young adulthood and adulthood.

Table 3: The detailed list of targeted genes of this study

Category	*C. elegans* Gene	Function	Human Homolog
Longevity	age-1	Lifespan regulator	PIK3CA
Longevity	daf-16	Lifespan regulator	FOXO3
Response	lite-1	Light sensitivity	-
Response	tax-2	Responsiveness to chemical- and photo-sensation	CNGB1/3
Response	tax-4	Responsiveness to chemical- and photo-sensation	CNGA1/2/3/4
Circadian Rhythm	lin-42	Circadian rhythm gene	PER2
Circadian Rhythm	tim-1	Circadian rhythm gene	TIMELESS

4.4.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: age-1 Expression

The age-1 gene is one part of the regulation of longevity and stress responses in *C. elegans*. The expression significantly influences lifespan and the development of *C. elegans* by connecting to the insulin/IGF-1 signaling pathway. Understanding the correlation between age-1 expression and various treatments or conditions provides valuable insights into the molecular mechanisms of aging and identifies potential targets for therapeutic interventions aimed at promoting healthy aging [90], [91].

Lithium causes an abrupt decrease in age-1 expression levels in all conditions (except 47h light). In most cases, the expression levels of age-1 were barely detectable. Being in the light or dark did not change the age-1 expression levels. It was lithium exposure that affected the levels significantly. In Figures 4.4.1.D and 4.4.1.E, it is evident that the decrease in gene expression significantly increases with prolonged treatment duration in both dark and light conditions. The impact of light exposure on the age-1 gene in comparison to the control groups is not conclusively determined until 89 h. However, as depicted in Figure 4.4.1.E, at 89 h, increased exposure leads to greater age-1 expression in the light-exposed groups as opposed to the dark condition. This disparity is particularly notable in the light-exposed group when contrasted with the control groups, demonstrating a more noticeable expression (P-value ≤ 0.001).

In the groups subjected to light and treated with LiCl, there is a marginal increase in gene expression compared to the dark group, although lacking statistical significance. Notably, the slightly higher expression observed in LiCl-treated light groups compared to the dark group equalizes the same expression levels at later response stages (65h, 73h, and 89h).

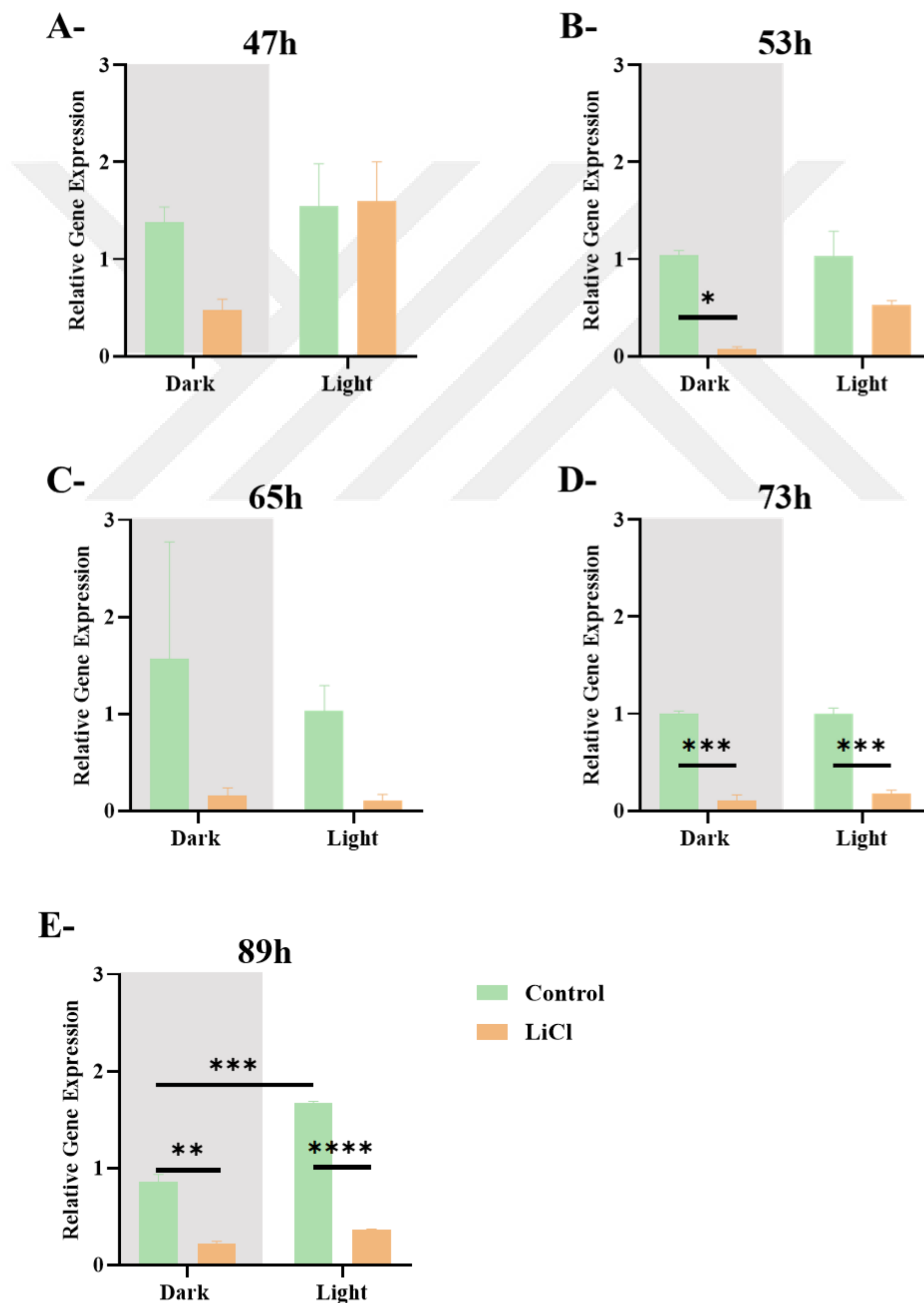

Figure 4.4.1: The relative gene expression of age-1 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and dark as well as light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

4.4.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: daf-16 Expression

The daf-16 gene serves as a key regulatory element in governing the lifespan and ability to withstand stress in *C. elegans*. It primarily operates downstream within the insulin/IGF-1 signaling pathway. The gene's expression is vital for facilitating the impact of diverse interventions that promote longevity and exposure to environmental stressors [42][63]. As a downstream effector of the insulin/IGF-1 signaling pathway, DAF-16 becomes activated upon pathway inhibition, leading to the translocation of DAF-16 into the nucleus where it regulates numerous gene expressions. The human FOXO family of transcription factors serve as the functional ortholog of DAF-16, which play similar roles (Table 1). The importance of daf-16 expression is underscored by its significant influence on an organism's capacity to adapt to environmental stressors and its association with enhanced longevity [42], [92].

Starting from 47h and 53h, lithium causes a reduction in daf-16 levels, this reduction becomes significant after 65h. Light or dark conditions did not have a significant effect on daf-16 expression, however, in 73h, lithium treatment resulted in more reduction when combined with the light. In 89h, although not statistically significant, lithium exerts its effect in diminishing gene expression.

After the administration of LiCl, it is observed that there is a decrease in the expression levels of daf-16 in both dark and light groups. This mirrors the findings related to the age-1 gene (Figure 4.4.2). As evidenced in Figures 4.4.2.C and 4.4.2.D, the decrease demonstrates a more significant statistical impact with extended treatment duration. In contrast to age-1, the presence of light does not lead to a notable alteration in the expression levels in either the control groups or the LiCl-treated groups. However, under light variation and LiCl treatment, there is a significant decrease in daf-16 expression in the light-exposed LiCl-treated groups compared to the dark-exposed groups, especially up to 73h. As illustrated in Figure 4.4.2.D, this reduced expression shows a significant decrease. However, by 89h, this trend is reversed, signifying an elevation in daf-16 expression within the light-exposed groups when compared to earlier time points.

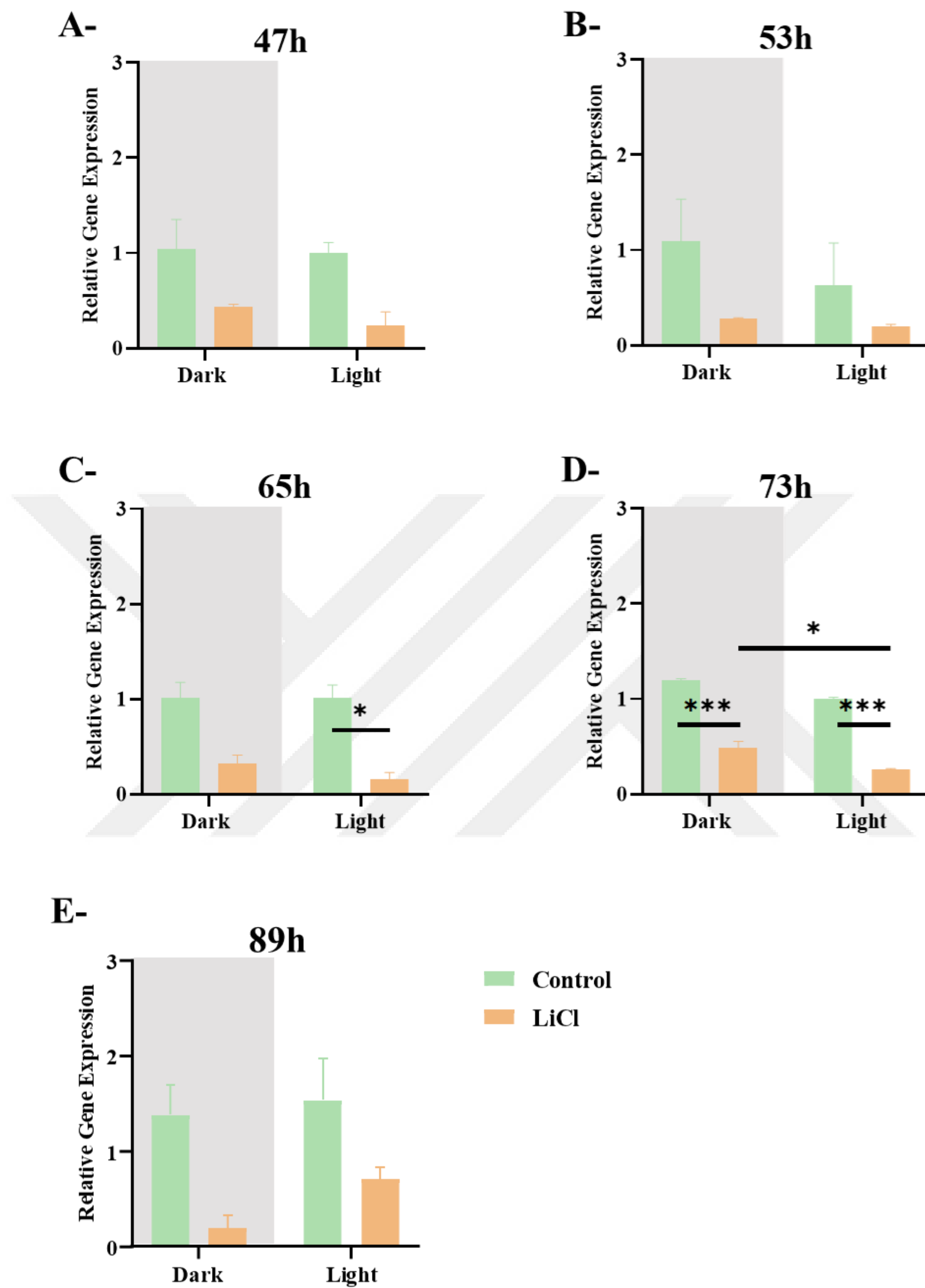

Figure 4.4.2: The relative gene expression of daf-16 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05, *** P<0.001.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

4.5. LiCl Treatment and Light-Induced Circadian Rhythm Affects the Fertility Efficiency of *C. elegans*

The relation between the efficiency of fertility and longevity is another critical characteristic of improving health span. The rate of reproduction and sustaining this capability to produce offspring means being fertile. This is a highly crucial topic for the continuation of living organisms and the opportunity for conveying genetic materials to generations. *C. elegans* is one the animals that has this advantage with high reproduction capacity as explained in section 1. 1. Longevity and health span concepts are effectors of reproduction efficiency. Fundamentally, the longevity regulator pathway, IIS is also a key regulator element for controlling fertility by affecting the reproduction and growth decision mechanism. Studies revealed that there is a direct association between the lifespan extension of *C. elegans* and the reduction in germline. For this prominent effect is facilitated through the modification of the nuclear localization of the DAF-16 transcription factor, a downstream element of the IIS pathway. This modification shares an essential regulatory effect for longevity. Studies assumed that the occurrence of germline reduction could be one of the significant players in lifespan extension. The ablation or genetic disruption occurs in the germline, and the translocation of DAF-16 happens to the nucleus. Ought to this essential alteration, essential genes' activity which are involved in stress resistance, metabolism, and longevity. This dynamic regulation in the IIS pathway highlights its significant role in maintaining the stability between reproductive activity and lifespan. Therefore, the analysis of fertility provides insights into the activity of daf-16 [93], [94], [95], [96]. In this part, the profound effect of changes in the lifespan and normal living conditions of *C. elegans* on reproduction was examined

to understand the change in daf-16. Similarly, the light-affected change in circadian rhythm and LiCl treatment were examined for the assessment of reproduction efficiency.

In the constant dark condition, control groups produced higher brood size than LiCl-treated groups (Figure 4.5.1.B). When the daily reproduction efficiency was investigated, the control group started the progeny production on Day 2. On the other hand, the LiCl-treated worms began to reproduce on Day 3 with a lower number and finished one day later than the control group. As depicted in Figure 4.5.1.A, the daily brood size did not exceed the control groups.

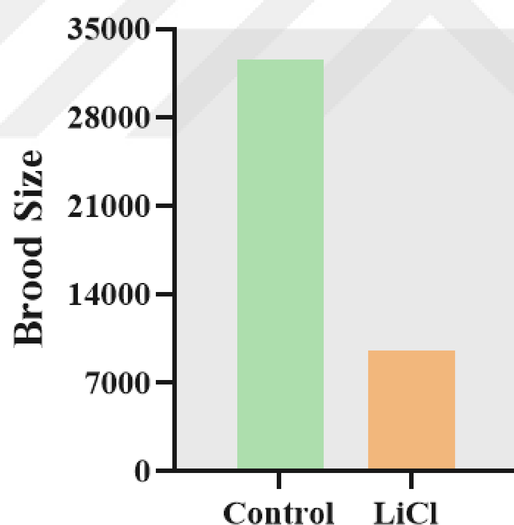

Figure 4.5.1: The total counts of brood size comparison between control and LiCl-treated groups at dark conditions. The green bars represent the control group that received no treatment and the orange bars are the LiCl-treated groups at constant dark.

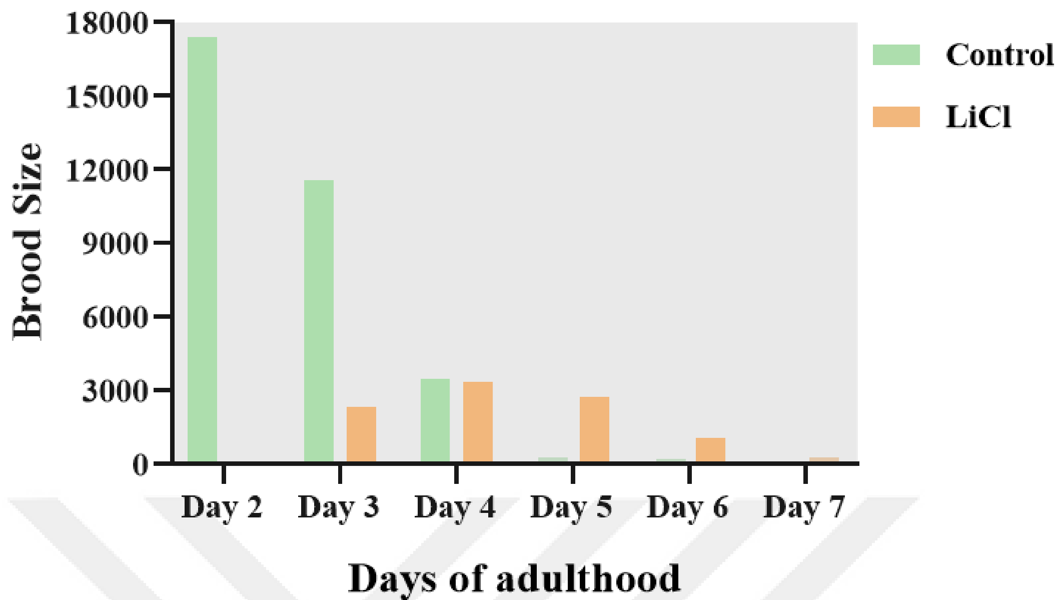

Figure 4.5.2: The effect of LiCl administration on brood size of *C. elegans* in a daily manner. The green bars signify the control group that received no treatment and the orange bars are the LiCl-treated groups at constant dark.

Similar to dark condition, constant light triggered the reduction in LiCl-treated groups when compared to controls (Figure 4.5.3). Additionally, the reproduction started on Day 2 for control groups and for LiCl-treated groups, it started on Day 3. On a daily basis, the produced brood size of LiCl-administered *C. elegans* did not exceed the number of brood sizes in control groups (Figure 4.5.4).

Meaningfully, in order to assign the effect of circadian rhythm alterations by changing light, the dark and light conditions were compared (Figure 4.5.5). As illuminated, the control group at constant dark condition produce more brood size than other groups. At both conditions, LiCl treatment caused a reduction in brood size when compared to controls. This reduction is slightly lower at constant light.

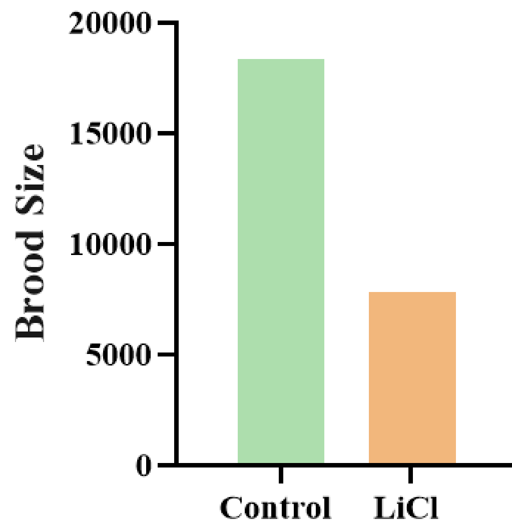

Figure 4.5.3: The total counts of the brood size comparison between control and LiCl-treated groups at light conditions. The green bars represent the control group that received no treatment and the orange bars are the LiCl-treated groups at constant light.

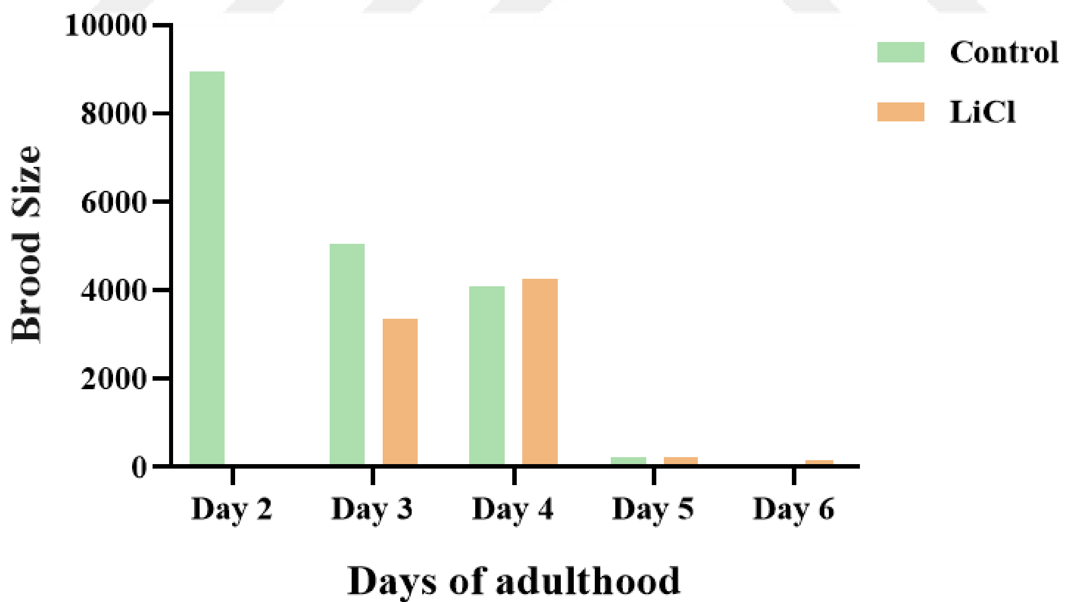

Figure 4.5.4: The effect of LiCl administration on brood size of *C. elegans* in a daily manner at light conditions. The green bars signify the control group that received no treatment and the orange bars are the LiCl-treated groups at constant light.

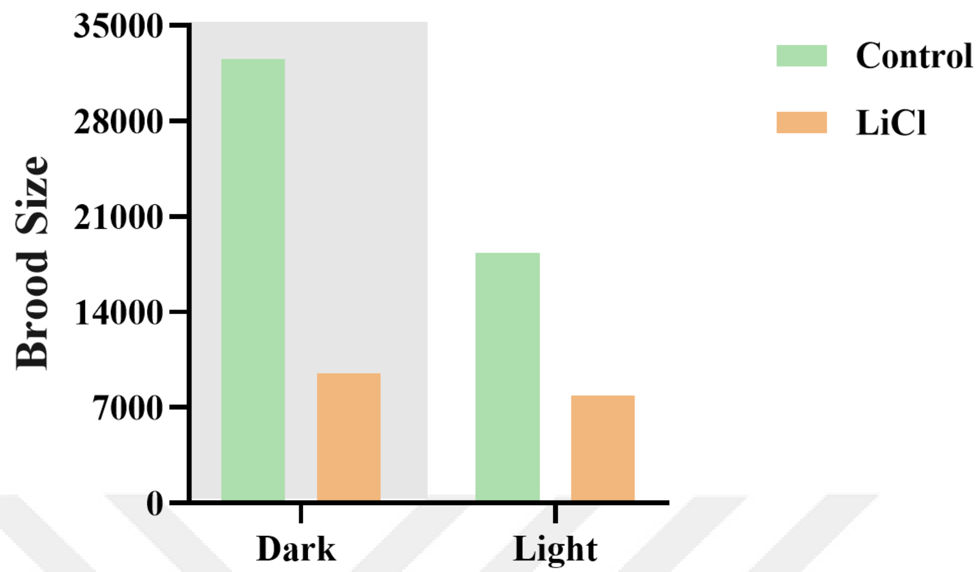

Figure 4.5.5: The comparison of *C. elegans*' brood size at different light conditions and LiCl treatment. Green bars are the control groups and orange bars are the LiCl-treated groups.

CHAPTER 5

5. RESPONSE TO ENVIRONMENTAL CHANGES

5.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: lite-1 Expression

This section focused on investigating the pattern of the lite-1 gene expression to ascertain its capability in detecting and responding to light stimuli. The lite-1 gene encodes a G-protein-coupled receptor-like protein that exhibits high sensitivity to ultraviolet (UV) and blue light, thereby facilitating critical phototransduction processes necessary for the organism's survival and adaptation to its environment. Its pivotal role in phototransduction enables the organism to perceive and react to light stimuli, significantly influencing behavioral responses to changes in light conditions, which are essential for circadian rhythm regulation [64].

Significantly, according to the current literature, there is no direct human ortholog of the lite-1 gene. However, the presence of light-sensitive proteins and photoreceptors in humans allows for correlating how lite-1 gene activity reflects in the photosensation of

C. elegans. For this study, understanding the differentiation pattern of lite-1 is crucial for assessing the impact of light condition changes and the presence of LiCl on *C. elegans*.

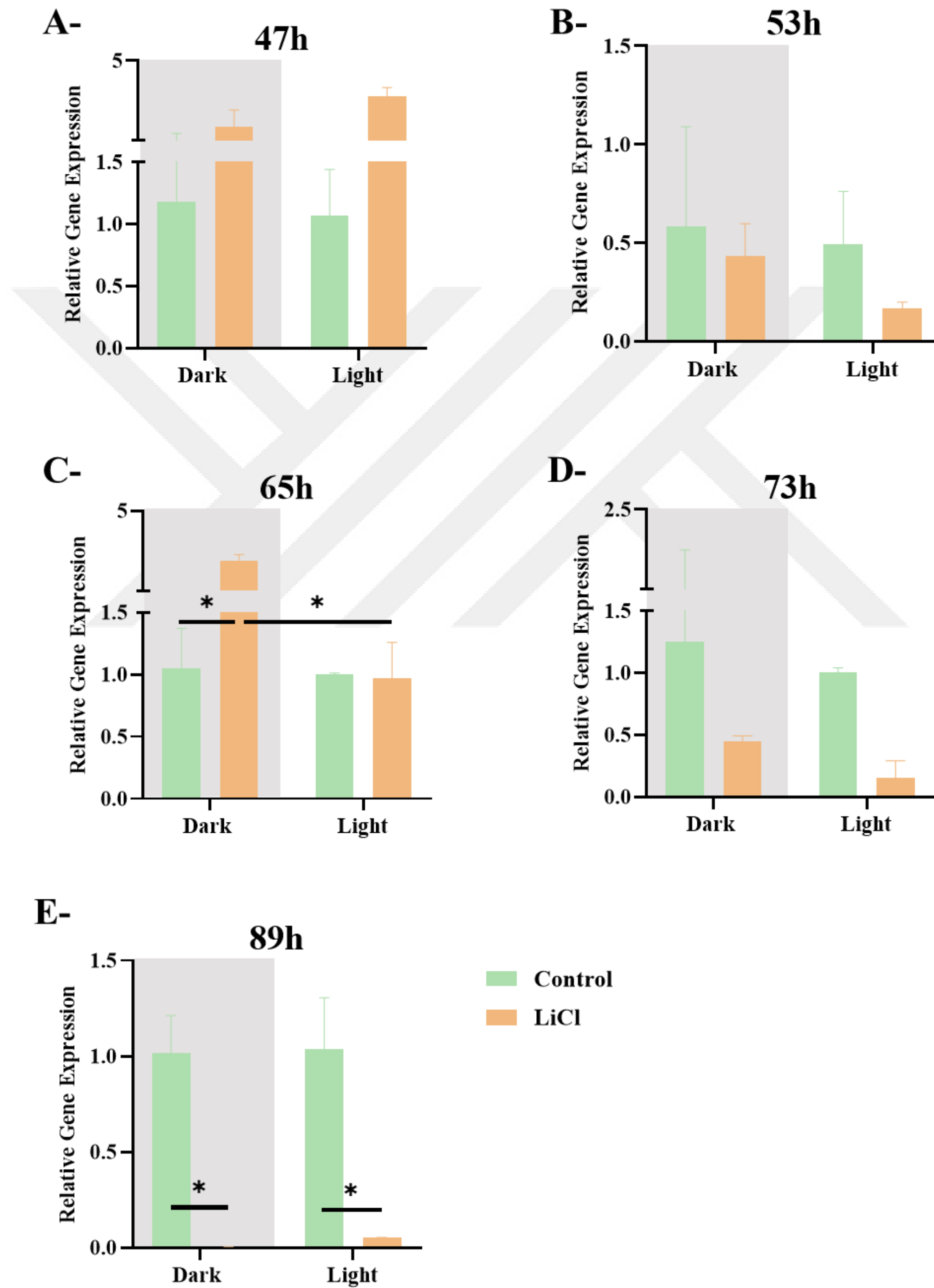

Figure 5.1.1: The relative gene expression of the lite-1 expression in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light

conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * $P<0.05$.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

The expression changes of lite-1, a gene responsible for detecting changes in light perception and producing appropriate responses, exhibit a distinct pattern compared to longevity genes. Notably, no significant difference in lite-1 expression was observed between the control groups under light and dark conditions. However, the introduction of LiCl treatment altered this expression pattern. As depicted in Figure 5.1.1.A, there was an increase in lite-1 expression in both the light and dark groups at 47 h following LiCl treatment as early response of lite-1. From this 47-hour time point onward, in the light groups, lite-1 expression decreased compared to the control groups, and this reduction became statistically significant, as represented in Figures 5.1.1.C and 5.1.1.E with a P-value lower than 0.05. At 89h, although the control groups have regular lite-1 expression, lithium treatment diminishes expression drastically.

5.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tax-2 Expression

In *C. elegans*, the tax-2 gene holds considerable implications due to its essential role in sensory perception and behavioral responses. tax-2 encodes a CNG ion channel subunit that is predominantly expressed in the sensory neurons of *C. elegans*. These neurons are essential for detecting environmental cues. Exploring tax-2 provides valuable insights

into the molecular mechanisms involved in sensory processing and neuronal signaling. Therefore, this enhances our understanding of neurobiology. This property makes tax-2 as a promising target for pharmacological interventions aimed at modulating sensory perception and behavior. Investigating tax-2 in *C. elegans* facilitates the screening of novel compounds and the evaluation of their effects on neuronal function, offering potential avenues for drug discovery and development. In this study, LiCl administration was employed as a pharmacological intervention to elucidate its impact on sensory perception at the molecular level [97], [98], [99].

In addition to its chemo-sensation property, tax-2 plays a key role in mediating behavioral responses to sensory stimuli, particularly in mechanosensation and photosensation. The study of tax-2 is instrumental in understanding the transduction of sensory information into behavioral responses, thereby shedding light on the mechanisms that govern behavioral plasticity and adaptation. This provides valuable insight into how changes in circadian rhythm can lead to alterations in the sensory perception of *C. elegans* [65], [66], [100].

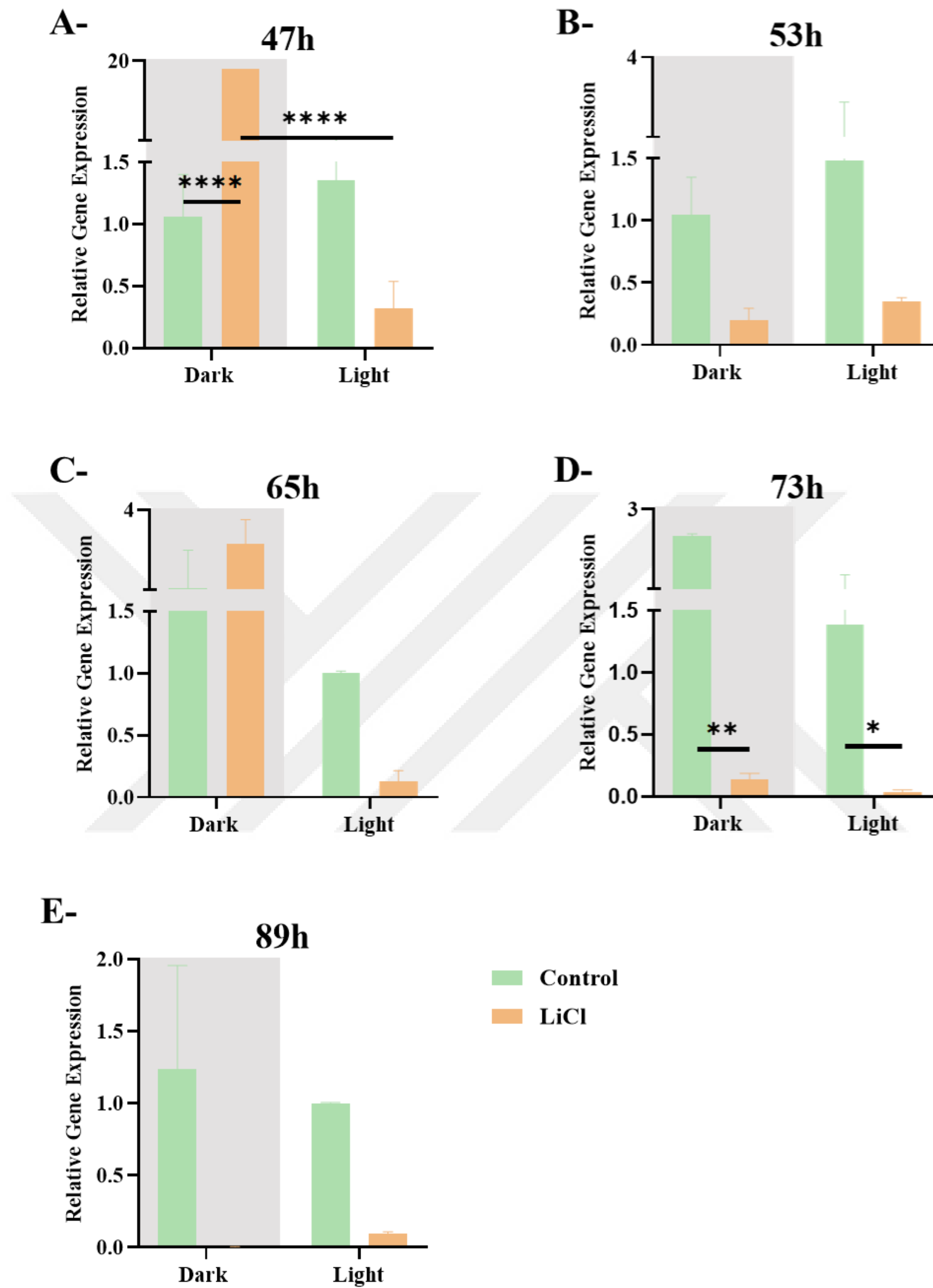

Figure 5.2.1: The relative gene expression of the tax-2 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05, ** P<0.01, **** P<0.0001.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

At 47h, lithium has contrasting effects on tax-2 gene expression; it increases the expression under dark conditions, whereas it decreases under light conditions. In the dark, the difference between lithium and control is significant, as well as the expression levels between light and dark, under the influence of lithium. This disparity is lost in 56h and emerges back in 65h; reminiscent of a circadian curve. Then strikingly, after 73h, lithium causes a suppression of the gene levels up to a barely detectable level. Apparently, light or dark conditions do not affect the tax-2 levels, but lithium does, significantly.

In the early-stage response (at 47 and 53 h), the expression in the light control groups is higher compared to the dark groups. However, as the exposure duration increases, this pattern reverses, with the dark control groups exhibiting higher expression levels. When examining the effect of light exposure on LiCl treatment, it is observed that at all times, there is less tax-2 expression in the light control groups compared to the dark control groups. At 73 h, this reduced expression value decreases significantly. In contrast to the dark condition, while higher expression values are observed in the control groups at 47 and 65 h, decreases are observed at other time points. However, at 73 h, this expression change occurs significantly.

The effect of LiCl treatment on the tax-2 gene, on the other hand, generally results in lower expression compared to the control groups. As shown in Figure 5.2.1.D, this decrease is significant at 73 h. However, at 47 h, there is notably higher expression in the dark compared to the control, and importantly, the effect of LiCl in the dark group is statistically significantly higher compared to the light group. A similar trend is also observed at 65 h (Figure 5.2.1.C).

5.3. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tax-4 Expression

tax-4 is even responsible for the regulation of sensory perception and behavioral response when environmental conditions are altered with chemicals, temperature and the presence of light. Similar to tax-2, this is essential for encoding a subunit of a CNG ion channel and shares functional similarities with human genes involved in sensory transduction, such as those CNG channels (Table 1). These channels are involved in the sensory systems such as vision and olfaction in humans. Therefore, monitoring of tax-4 gene alteration gives insights into the impact on sensation ability which is similar to sensory and signaling mechanisms in humans [99].

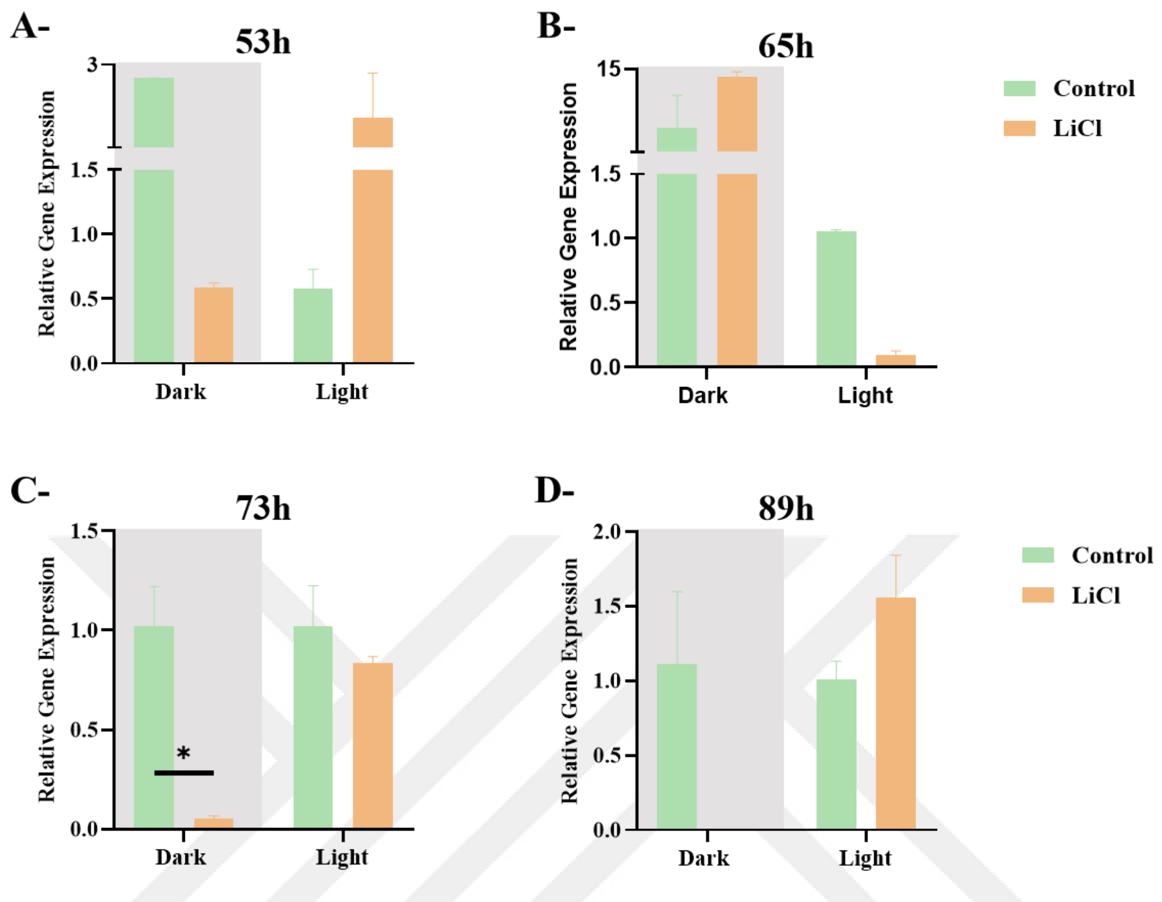

Figure 5.3.1: The relative gene expression of the tax-4 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

When comparing the control groups under light and dark conditions, it is observed that at 53 h and 65 h, there is a higher expression of the gene in the dark conditions. However, by the 73-hour and 89-hour time points, this difference in gene expression between the light and dark conditions becomes nearly equal.

In the presence of LiCl treatment, the pattern of gene expression did not represent a distinct trend. Specifically, except for the 65-hour time point, the expression levels in the dark-treated groups are generally lower compared to the control groups. As illustrated in Figure 5.3.1.C, this decrease in expression becomes statistically significant at 73 h, highlighting a notable reduction in gene activity due to LiCl treatment under dark conditions. Conversely, under light conditions, LiCl treatment does not produce a statistically significant difference in gene expression when compared to the control groups. However, it is noted that higher expression levels are observed in the LiCl groups than in dark groups at 53 h and 89 h, despite the absence of statistical significance.

CHAPTER 6

6. CIRCADIAN RHYTHM

6.1. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: lin-42 Expression

As a result of the manipulation of light conditions, controlling circadian rhythmicity is a key aspect of understanding its molecular behavior. For this assignment, lin-42 gene expression is the critical element in the biological clock of *C. elegans*, because it is the ortholog of the period (per) gene in humans. This is the main regulator for developmental timing and the circadian rhythm which helps the *C. elegans* synchronize its physiological processes with environmental cycles [101].

The differential gene expression pattern of lin-42, which plays a critical role in regulating circadian rhythms, exhibited similar expression levels in the control groups under both light and dark conditions. However, the influence of LiCl treatment on lin-42 expression varied significantly depending on the specific time points of exposure.

Initially, at 47 h, there were no significant differences in lin-42 expression between the control and LiCl-treated groups, indicating the minimal early effects of LiCl treatment. At the subsequent time points of 53 and 65 h, a marked change was observed. There was a statistically significant increase in lin-42 expression in the LiCl-treated groups compared to the control groups at these time points. This increase was apparent under both dark and light conditions, highlighting that the upregulation of lin-42 by LiCl is robust across different environmental lighting conditions. Specifically, at 53 h, the increase in lin-42 expression in the light-presence group had a P-value lower than 0.05, indicating statistical significance. At 65 h, the dark-treated group showed a P-value lower than 0.01, and the light group showed an even more significant P-value lower than 0.001. However, this trend reversed with further prolonged exposure. From 73 h onward, lin-42 gene expression began to decrease in the LiCl-treated groups compared to the control groups. This reduction in expression continued and became statistically significant by 89 h. As shown in Figure 6.1.1.E, at 89 h, the decrease in lin-42 expression was significant, with a P-value lower than 0.05.

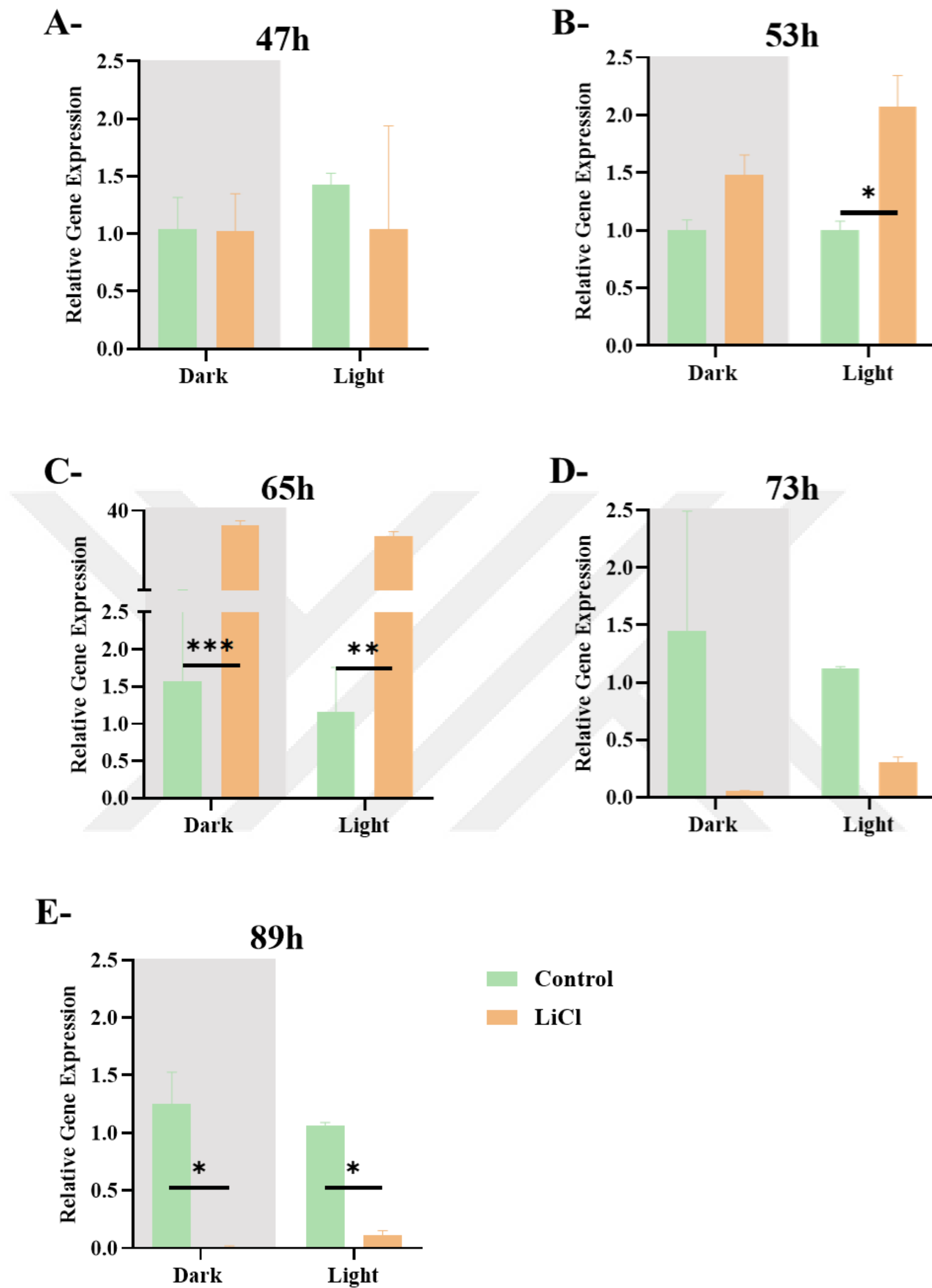

Figure 6.1.1: The relative gene expression of the lin-42 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05 ** P<0.01, *** P<0.001.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

6.2. Impact of Modification Following LiCl Treatment and Changes in Circadian Rhythm: tim-1 Expression

The tim-1 gene in *C. elegans* is another vital component of the circadian rhythm regulatory system. This gene is homologous to the timeless (tim) gene in humans which plays a fundamental role in maintaining and regulating circadian rhythms. Similar to the lin-42 gene, the elucidation of these genes' function helps to offer new insights into potential therapeutic targets, developmental biology and also, and improving health conditions [102].

Similar to lin-42, another gene integral to circadian rhythm regulation, tim-1, did not exhibit a significant difference in expression levels between the dark and light control groups. This suggests that, under normal conditions without a treatment, the expression of tim-1 remains consistent regardless of the environmental lighting conditions. However, the influence of LiCl treatment on tim-1 expression presented a distinct pattern. At 47 h, tim-1 expression did not show significant changes in the LiCl-treated groups compared to the controls, indicating that the initial response to LiCl is not immediate for tim-1. This absence of a significant effect at 47 h implies that tim-1's reaction to LiCl treatment requires more extended exposure to become apparent.

Beyond the 47 h, LiCl treatment resulted in a consistent decrease in tim-1 expression at subsequent time points. This downregulation of tim-1 was significant, as illustrated in Figures 6.2.1.B and 6.2.1.C. The data indicate that at various later stages of exposure,

LiCl exerts a suppressive effect on tim-1 expression. This suppression suggests that LiCl has a potent inhibitory effect on the gene's activity over time. Additionally, when the effects of LiCl treatment were examined separately in the dark and light groups, an interesting pattern emerged. Although the overall trend was a decrease with LiCl treatment, tim-1 expression was slightly higher in the light-present groups compared to the dark groups.

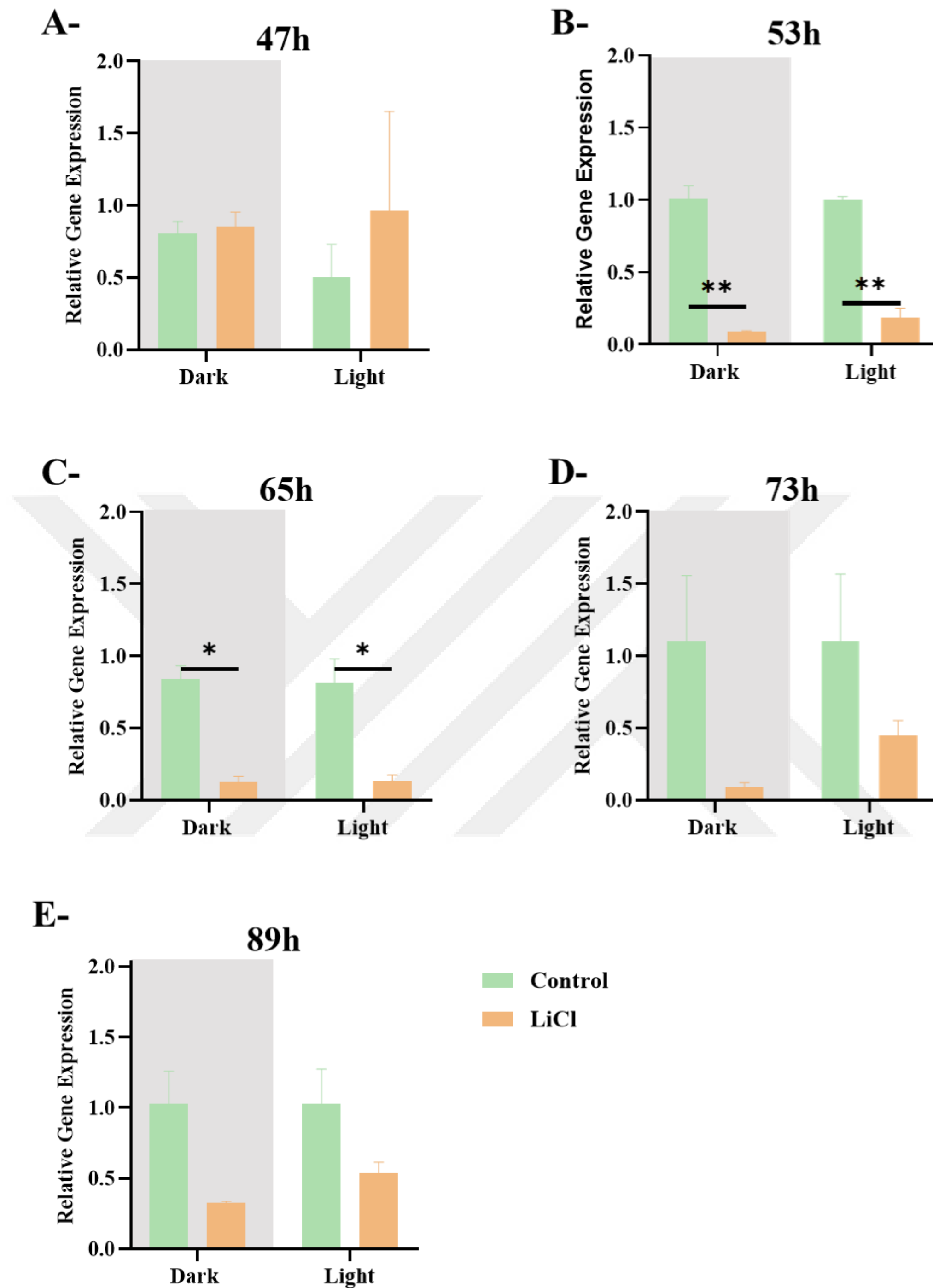

Figure 6.2.1: The relative gene expression of the tim-1 in a *C. elegans* model. Quantifications were performed according to LiCl treatment and different light conditions. Green bars: control, Orange bars: lithium, shaded area: dark, unshaded area: light. * P<0.05 ** P<0.01.

A- at 47 h, **B-** at 53 h, **C-** at 65 h, **D-** at 73 h and **E-** at 89 h.

CHAPTER 7

7. CONCLUSION AND FUTURE PROSPECTS

7.1. Conclusions

This study was initiated with curiosity about how environmental and biological variance influences organisms' lifespan, which is one of the popular research areas. To address this question, LiCl was used as a targeted drug, the potential effects of which have been shown but not fully understood. One of the significant references in this study is the work of McCol and colleagues, which demonstrated a correlation between intake of a specific lithium dose and increased lifespan [49]. These studies suggested that LiCl treatment increases the response to stress factors such as oxidative and hypoxia-induced in *C. elegans*. For instance, LiCl could be targeted SKN-1 which is the transcription factor and is required for response to oxidative stress. SKN-1 accumulated with the case of oxidative stress and LiCl acts as a depressor to activate the response [49], [103]. Moreover, critical chemosensory response is also found to link with LiCl treatment. One of these is the hypoxia response. The administration of LiCl triggers the adaptation to hypoxia conditions by mediating HIF-1 which is essential for the regulation of hydroxylation and chemosensory response [104]. Therefore, LiCl is an essential target for longevity studies.

Most commonly, LiCl is used for regulating unstable mood fluctuations in bipolar patients, providing an important treatment opportunity. How does the widespread use of LiCl in bipolar disorder treatment trigger these effects? Remarkably, research addressing this question points to the disruption of circadian rhythms in bipolar patients as a target [70], [105]. The circadian rhythm represents our biological clock; hence it plays a role in regulating many biological mechanisms and pathways, such as aging and metabolic activity regulation. Studies imply that disrupted circadian rhythms in bipolar patients must be considered regarding irregular neurological activity [106], [107]. Although the fundamental mechanism of this effect is not fully elucidated, it is known that LiCl interacts with clock genes to regulate this rhythm [107]. This interaction helps stabilize the disrupted circadian rhythm. As explained in section 1.3, research has primarily shown that the effects of LiCl occur primarily through GSK3β and are associated with cellular processes, homeostasis, and various pathways. These effects include important factors affecting lifespan. For example, the Wnt signaling, mammalian target of rapamycin (mTOR) and IIS pathways interact to potentially alter lifespan. Furthermore, research indicated that LiCl affects development, reproduction and maturation [108], [109]. As these studies indicate, the effect of LiCl requires further investigation to understand the main effectors and their relationships. Accordingly, this study aims to understand how LiCl induces this change in circadian rhythm and how this effect combines to affect lifespan.

The thesis was conducted utilizing the advantages of the *C. elegans* model organism. The study was advanced by examining three main areas: aging, response to manipulations, and circadian rhythm in *C. elegans* model organism. Primarily, manipulation of both

LiCl application and light changes triggered circadian rhythm disruption was attempted to understand their effects on lifespan. The experimental results show an increase in lifespan by 33% in darkness and 27% in light conditions with LiCl intake. This indicates that LiCl application affects lifespan both under normal conditions and when circadian rhythms are disrupted. The effect of circadian rhythm on lifespan resulted in an 8% decrease when manipulated with light (Table 2, Figure 4.1.1 and 4.1.2). Notably, these results indicate the presence of an associated mechanism of action between LiCl and circadian rhythm and their effect on lifespan.

Furthermore, the developmental effect of this relationship was investigated by examining early (47 h and 53 h) and late responses (65 h, 73 h, and 89 h). The developmental effect was monitored starting from the L4 stage. When measuring the accumulated autofluorescence signal associated with aging, an increase in signal intensity was observed with the duration of both light and dark conditions at late stages. Outstandingly, this elevation started at 65 h for darkness (Figure 4.2.1), although, it started at 53 h for constant light (Figure 4.2.2). However, this natural increase in intensity was reduced with LiCl treatment as consistent with the findings of lifespan experiments.

In order to better display this hour difference, confocal imaging was carried out (Chapter 4.3). Nuclear integrity loss, especially in the intestine is a sign of aging was tried to determine. The results illuminated that at 53 h, the dark groups showed similar integrity, while at 65 h, this integrity was disrupted for control groups. For light conditions, the lost integrity was displayed at 53 h and continued at 65 h. These results were matched

with the autofluorescence measurement that suggested early disruption when *C. elegans*' circadian rhythm was manipulated with light.

Further, to deepen the impact on longevity, the differential gene expression pattern was determined with longevity genes: age-1 (Figure 4.4.1) and daf-16 genes (Figure 4.4.2). Studies suggest that the decrease in the age-1 gene affects the decrease in the activation of the PI3K pathway and inhibits the IIS pathway. This inhibition allows the DAF-16 transcription factor to remain in the nucleus and activate genes that extend lifespan [31], [110]. The relationship between the effect of LiCl on the age-1 gene and daf-16 was examined, and the results showed a reduction in both age-1 and daf-16 gene expression with LiCl treatment. This result is noteworthy because a reduction in age-1 gene expression is associated with longevity while a decrease in daf-16 gene expression is associated with shortened lifespan. To better understand the obtained results, changes in reproduction were examined. The primary reason for this is that studies suggest if reproductive health is damaged, the DAF-16 gene is displaced to the nucleus, similar to when there is a reduction in the age-1 gene. This relocation leads to an increase in daf-16 expression, which positively impacts lifespan [41], [92]. Figure 4.5.5 shows a significant decrease in reproduction with LiCl treatment that needs to trigger the daf-16 gene expression. Therefore, this reduction indicates that the extension of lifespan caused by LiCl is not related to daf-16 but has a stronger association with age-1. Therefore, the decrease in reproduction with LiCl treatment might be indirectly connected to the age-1 gene.

Additionally, when examining the effect of circadian rhythm on these genes, it was found that there is no significant effect on the daf-16 gene. Conversely, for the age-1 gene, the impact of circadian rhythm is not evident up to 89 hours. At this advanced time point, it is seen that the light-manipulated circadian rhythm groups are more expressed than the dark control groups. This could explain the observed 8% reduction in lifespan in a meaningful way (Table 2). As related to the influence of LiCl treatment, the light-induced circadian rhythm effect on longevity could be through the age-1 gene.

For circadian rhythm, which was determined as another area of the study, clock genes: lin-42 and tim-1 were targeted and the effect of LiCl was tried to be investigated. Previous research suggests that lin-42 is correlated with improved health and regulation in the developmental timing of *C. elegans* by regulating circadian rhythms [102], [111], [112], [113]. However, the interaction between the tim-1 and lin-42 genes still requires investigation. In the current study, a noteworthy trend was observed in the expression of the lin-42 gene when exposed to LiCl. Initially, up to 73 hours, there was a significant increase in expression in the groups treated with LiCl. However, at 73 and 89 hours, a notable decrease in expression was observed in the LiCl-treated groups, indicating the effect on life cycle changes (Figure 6.1.1). As for the tim-1 gene, this effect became evident at 53 hours and this change continued (Figure 6.2.1). In summary, the influence of LiCl on circadian rhythm genes could be substantial. It suggests that exposure to LiCl triggers mechanisms that are effective for lifespan and generation cycles. However, this depends on the exposure time and has specific hourly thresholds relevant to circadian rhythm genes.

Finally, the study is designed to comprehend the effects of responses to these manipulations. For this purpose, popular response genes lite-1, tax-2, and tax-4 were examined. These genes play a crucial role in understanding responses to environmental stimuli. The lite-1 gene encodes a photoreceptor protein that provides sensitivity to light. It helps detect light signals and transmits them to cellular response pathways. This aids in enabling the organism to avoid harmful light sources.

tax-2 and tax-4 are involved in the detection of chemical and circadian rhythm changes in *C. elegans* and facilitate responses to these stimuli. These genes regulate signal pathways in neuro-sensory cells, forming appropriate behavioral responses to environmental stimuli. This enables the organism to quickly adapt to environmental changes. The molecular mechanisms regulated by these genes play a critical role in developing adaptive responses to environmental stress factors, enhancing survival, and potentially extending lifespan.

In the current study, lite-1 gene expression was generally high up to 73 hours in the dark groups treated with LiCl, followed by a decrease after 73 hours. This change in lite-1 gene expression indicates that adaptation to the chemical stimulus of LiCl in the dark was rapid, but as exposure time increased, this response could no longer be compensated. Similar results were observed for the tax-2 and tax-4 genes. Thus, it was found that the chemical stimulus in the dark was detected and responded to, but the response was negatively affected over time. In the presence of light, the results for tax-4 did not show a significant trend, while the results for lite-1 and tax-2 were similar to those in the dark,

except that the disrupted response rhythm began earlier. These findings indicate that *C. elegans* responded to the circadian rhythm change induced by light.

7.2. Future Perspectives

Within the scope of this study, the hypothesis that LiCl treatment can alter the adverse effects of circadian rhythm disruptions and reverse the aging process of *C. elegans* was tested. The most important impact of the study is that it brings together LiCl treatment and the circadian rhythm effect, which are two important perspectives for lifespan studies. In this way, a significant insight has been achieved by creating a model to answer many questions in this field. Similar studies will be continued with the mutants of these targeted genes whose effects are promising in the future of the study. Thanks to these studies, the effect of LiCl application on circadian rhythm and lifespan will be elucidated and the mechanism of action will be determined. In this manner, the effects of the changes in our circadian rhythm and the use of LiCl on the quality of life can be revealed and lead to the creation of personal treatments or changes for these effects.

Bibliography

[1] B. Goldstein, “Sydney brenner on the genetics of caenorhabditis elegans,” *Genetics*, vol. 204, no. 1. 2016. doi: 10.1534/genetics.116.194084.

[2] S. Brenner, “THE GENETICS OF CAENORHABDZTZS ELEGANS.” [Online]. Available: https://academic.oup.com/genetics/article/77/1/71/5991065

[3] Nobelprize.org, “The Nobel Prize in Physiology or Medicine for 2002 - Press Release,” *Nobelprize.org*. 2002.

[4] Nobelprize.org, “The 2006 Nobel Prize in Physiology or Medicine - Press Release,” *Nobel Media AB 2014.Web. 22 Jul 2018.* 2006.

[5] Nobel.org, “The Nobel Prize in Chemistry 2008: ‘for the discovery and development of the green fluorescent protein, GFP,’” *Nobelprize.org*, vol. 38, no. 10, 2008.

[6] F. Athar and N. M. Templeman, “C. elegans as a model organism to study female reproductive health,” *Comp Biochem Physiol A Mol Integr Physiol*, vol. 266, 2022, doi: 10.1016/j.cbpa.2022.111152.

[7] M. Markaki and N. Tavernarakis, “Caenorhabditis elegans as a model system for human diseases,” *Current Opinion in Biotechnology*, vol. 63. 2020. doi: 10.1016/j.copbio.2019.12.011.

[8] O. Sin, H. Michels, and E. A. A. Nollen, “Genetic screens in Caenorhabditis elegans models for neurodegenerative diseases,” *Biochimica et Biophysica Acta - Molecular Basis of Disease*, vol. 1842, no. 10. 2014. doi: 10.1016/j.bbadis.2014.01.015.

[9] "Genome sequence of the nematode C. elegans: A platform for investigating biology," *Science*, vol. 282, no. 5396. 1998. doi: 10.1126/science.282.5396.2012.

[10] R. K. Wilson, "How the worm was won: The C. elegans genome sequencing project," *Trends in Genetics*, vol. 15, no. 2. 1999. doi: 10.1016/S0168-9525(98)01666-7.

[11] Y. Kim, Y. Park, J. Hwang, and K. Kwack, "Comparative genomic analysis of the human and nematode caenorhabditis elegans uncovers potential reproductive genes and disease associations in humans," *Physiol Genomics*, vol. 50, no. 11, 2018, doi: 10.1152/physiolgenomics.00063.2018.

[12] A. Fire, S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello, "Potent and specific genetic interference by double-stranded RNA in caenorhabditis elegans," *Nature*, vol. 391, no. 6669, 1998, doi: 10.1038/35888.

[13] S. Zhang, F. Li, T. Zhou, G. Wang, and Z. Li, "Caenorhabditis elegans as a Useful Model for Studying Aging Mutations," *Front Endocrinol (Lausanne)*, vol. 11, no. October, pp. 1–9, 2020, doi: 10.3389/fendo.2020.554994.

[14] O. Hobert, "Neurogenesis in the nematode Caenorhabditis elegans.," *WormBook : the online review of C. elegans biology*. 2010. doi: 10.1895/wormbook.1.12.2.

[15] G. Rapti, "A perspective on C. elegans neurodevelopment: from early visionaries to a booming neuroscience research," *J Neurogenet*, vol. 34, no. 3–4, 2020, doi: 10.1080/01677063.2020.1837799.

[16] M. Raices, H. Maruyama, A. Dillin, and J. Kariseder, “Uncoupling of longevity and telomere length in C, elegans,” *PLoS Genet*, vol. 1, no. 3, pp. 295–301, 2005, doi: 10.1371/journal.pgen.0010030.

[17] R. B. McDonald and R. C. Ruhe, “Aging and longevity: Why knowing the difference is important to nutrition research,” *Nutrients*, vol. 3, no. 3. 2011. doi: 10.3390/nu3030274.

[18] J. Guo *et al.*, “Aging and aging-related diseases: from molecular mechanisms to interventions and treatments,” *Signal Transduction and Targeted Therapy*, vol. 7, no. 1. 2022. doi: 10.1038/s41392-022-01251-0.

[19] H. Adwan-Shekhidem and G. Atzmon, “The Epigenetic Regulation of Telomere Maintenance in Aging,” in *Epigenetics of Aging and Longevity: Translational Epigenetics vol 4*, 2018. doi: 10.1016/B978-0-12-811060-7.00005-X.

[20] M. Takasugi, Y. Yoshida, Y. Nonaka, and N. Ohtani, “Gene expressions associated with longer lifespan and aging exhibit similarity in mammals,” *Nucleic Acids Res*, vol. 51, no. 14, 2023, doi: 10.1093/nar/gkad544.

[21] M. R. Klass, “Aging in the nematode Caenorhabditis elegans: Major biological and environmental factors influencing life span,” *Mech Ageing Dev*, vol. 6, no. C, 1977, doi: 10.1016/0047-6374(77)90043-4.

[22] C. C. Kern *et al.*, “C. elegans ageing is accelerated by a self-destructive reproductive programme,” *Nat Commun*, vol. 14, no. 1, 2023, doi: 10.1038/s41467-023-40088-1.

[23] X. Wang *et al.*, " Ageing induces tissue-specific transcriptomic changes in Caenorhabditis elegans ," *EMBO J*, vol. 41, no. 8, 2022, doi: 10.15252/embj.2021109633.

[24] N. M. Ha, S. H. Tran, Y. H. Shim, and K. Kang, "Caenorhabditis elegans as a powerful tool in natural product bioactivity research," *Applied Biological Chemistry*, vol. 65, no. 1. 2022. doi: 10.1186/s13765-022-00685-y.

[25] Y. L. Chew *et al.*, "Recordings of Caenorhabditis elegans locomotor behaviour following targeted ablation of single motorneurons," *Sci Data*, vol. 4, 2017, doi: 10.1038/sdata.2017.156.

[26] A. Scharf, F. Pohl, B. M. Egan, Z. Kocsisova, and K. Kornfeld, "Reproductive Aging in Caenorhabditis elegans: From Molecules to Ecology," *Frontiers in Cell and Developmental Biology*, vol. 9. 2021. doi: 10.3389/fcell.2021.718522.

[27] M. A. Churgin, S. K. Jung, C. C. Yu, X. Chen, D. M. Raizen, and C. Fang-Yen, "Longitudinal imaging of caenorhabditis elegans in a microfabricated device reveals variation in behavioral decline during aging," *Elife*, vol. 6, 2017, doi: 10.7554/eLife.26652.

[28] T. Komura, M. Yamanaka, K. Nishimura, K. Hara, and Y. Nishikawa, "Autofluorescence as a noninvasive biomarker of senescence and advanced glycation end products in Caenorhabditis elegans," *NPJ Aging Mech Dis*, vol. 7, no. 1, 2021, doi: 10.1038/s41514-021-00061-y.

[29] X. Ni *et al.*, "A description of the relationship in healthy longevity and aging-related disease: from gene to protein," *Immunity and Ageing*, vol. 18, no. 1. 2021. doi: 10.1186/s12979-021-00241-0.

[30] N. Tavernarakis and M. Driscoll, “Caloric restriction and lifespan: A role for protein turnover?,” in *Mechanisms of Ageing and Development*, 2002. doi: 10.1016/S0047-6374(01)00341-4.

[31] K. I. Zhou, Z. Pincus, and F. J. Slack, “Longevity and stress in Caenorhabditis elegans,” *Aging*, vol. 3, no. 8. 2011. doi: 10.18632/aging.100367.

[32] J. Z. Morris, H. A. Tissenbaum, and G. Ruvkun, “A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in Caenorhabditis elegans,” *Nature*, vol. 382, no. 6591, 1996, doi: 10.1038/382536a0.

[33] D. Heras-Sandoval, E. Avila-Muñoz, and C. Arias, “The phosphatidylinositol 3-Kinase/mTor pathway as a therapeutic target for brain aging and neurodegeneration,” *Pharmaceuticals*, vol. 4, no. 8. 2011. doi: 10.3390/ph4081070.

[34] S. Ayyadevara, R. Alla, J. J. Thaden, and R. J. Shmookler Reis, “Remarkable longevity and stress resistance of nematode PI3K-null mutants,” *Aging Cell*, vol. 7, no. 1, 2008, doi: 10.1111/j.1474-9726.2007.00348.x.

[35] D. B. Friedman and T. E. Johnson, “A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility,” *Genetics*, vol. 118, no. 1, 1988, doi: 10.1093/genetics/118.1.75.

[36] S. Du and H. Zheng, “Role of FoxO transcription factors in aging and age-related metabolic and neurodegenerative diseases,” *Cell and Bioscience*, vol. 11, no. 1. 2021. doi: 10.1186/s13578-021-00700-7.

[37] R. Martins, G. J. Lithgow, and W. Link, “Long live FOXO: Unraveling the role of FOXO proteins in aging and longevity,” *Aging Cell*, vol. 15, no. 2. 2016. doi: 10.1111/acel.12427.

[38] G. Murtaza, A. K. Khan, R. Rashid, S. Muneer, S. M. F. Hasan, and J. Chen, “FOXO Transcriptional Factors and Long-Term Living,” *Oxidative Medicine and Cellular Longevity*, vol. 2017. 2017. doi: 10.1155/2017/3494289.

[39] M. Uno and E. Nishida, “Lifespan-regulating genes in c. Elegans,” *NPJ Aging Mech Dis*, vol. 2, no. 1, 2016, doi: 10.1038/npjamd.2016.10.

[40] D. Weinkove, J. R. Halstead, D. Gems, and N. Divecha, “Long-term starvation and ageing induce AGE-1/PI 3-kinase-dependent translocation of DAF-16/FOXO to the cytoplasm,” *BMC Biol*, vol. 4, 2006, doi: 10.1186/1741-7007-4-1.

[41] A. Mukhopadhyay and H. A. Tissenbaum, “Reproduction and longevity: secrets revealed by C. elegans,” *Trends in Cell Biology*, vol. 17, no. 2. 2007. doi: 10.1016/j.tcb.2006.12.004.

[42] K. Lin, H. Hsin, N. Libina, and C. Kenyon, “Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling,” *Nat Genet*, vol. 28, no. 2, 2001, doi: 10.1038/88850.

[43] C. A. Lazzara and Y. H. Kim, “Potential application of lithium in Parkinson’s and other neurodegenerative diseases,” *Frontiers in Neuroscience*, vol. 9, no. OCT. 2015. doi: 10.3389/fnins.2015.00403.

[44] K. M. Jacobs, S. R. Bhave, D. J. Ferraro, J. J. Jaboin, D. E. Hallahan, and D. Thotala, “GSK-3β: A bifunctional role in cell death pathways,” *International Journal of Cell Biology*. 2012. doi: 10.1155/2012/930710.

[45] E. Y. Villegas-Vázquez *et al.*, "Lithium: A Promising Anticancer Agent," *Life*, vol. 13, no. 2. 2023. doi: 10.3390/life13020537.

[46] J. Liang, M. Yu, Y. Li, L. Zhao, and Q. Wei, "Glycogen synthase kinase-3: A potential immunotherapeutic target in tumor microenvironment," *Biomedicine and Pharmacotherapy*, vol. 173. 2024. doi: 10.1016/j.biopha.2024.116377.

[47] R. Machado-Vieira, H. K. Manji, and C. A. Zarate, "The role of lithium in the treatment of bipolar disorder: Convergent evidence for neurotrophic effects as a unifying hypothesis," *Bipolar Disorders*, vol. 11, no. SUPPL. 2. 2009. doi: 10.1111/j.1399-5618.2009.00714.x.

[48] V. A. Fajardo, V. A. Fajardo, P. J. Leblanc, and R. E. K. Macpherson, "Examining the Relationship between Trace Lithium in Drinking Water and the Rising Rates of Age-Adjusted Alzheimer's Disease Mortality in Texas," *Journal of Alzheimer's Disease*, vol. 61, no. 1, pp. 425–434, 2018, doi: 10.3233/JAD-170744.

[49] G. McColl, D. W. Killilea, A. E. Hubbard, M. C. Vantipalli, S. Melov, and G. J. Lithgow, "Pharmacogenetic analysis of lithium-induced delayed aging in Caenorhabditis elegans," *Journal of Biological Chemistry*, vol. 283, no. 1, 2008, doi: 10.1074/jbc.M705028200.

[50] F. Coutts *et al.*, "The polygenic nature of telomere length and the anti-ageing properties of lithium," *Neuropsychopharmacology*, vol. 44, no. 4, pp. 757–765, 2019, doi: 10.1038/s41386-018-0289-0.

[51] Z. Y. Tam, J. Gruber, L. F. Ng, B. Halliwell, and R. Gunawan, "Effects of lithium on age-related decline in mitochondrial turnover and function in caenorhabditis

elegans," *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, vol. 69, no. 7, 2014, doi: 10.1093/gerona/glt210.

[52] T. Roenneberg and M. Merrow, "The circadian clock and human health," *Current Biology*, vol. 26, no. 10. 2016. doi: 10.1016/j.cub.2016.04.011.

[53] A. Patke, M. W. Young, and S. Axelrod, "Molecular mechanisms and physiological importance of circadian rhythms," *Nature Reviews Molecular Cell Biology*, vol. 21, no. 2. 2020. doi: 10.1038/s41580-019-0179-2.

[54] L. B. Vosshall, J. L. Price, A. Sehgal, L. Saez, and M. W. Young, "Block in nuclear localization of period protein by a second clock mutation, timeless," *Science (1979)*, vol. 263, no. 5153, 1994, doi: 10.1126/science.8128247.

[55] W. A. Zehring *et al.*, "P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic drosophila melanogaster," *Cell*, vol. 39, no. 2 PART 1, 1984, doi: 10.1016/0092-8674(84)90015-1.

[56] J. L. Price, J. Blau, A. Rothenfluh, M. Abodeely, B. Kloss, and M. W. Young, "double-time is a novel Drosophila clock gene that regulates period protein accumulation," *Cell*, vol. 94, no. 1, 1998, doi: 10.1016/S0092-8674(00)81224-6.

[57] H. T. McKenna, I. K. M. Reiss, and D. S. Martin, "The significance of circadian rhythms and dysrhythmias in critical illness," *Journal of the Intensive Care Society*, vol. 18, no. 2. 2017. doi: 10.1177/1751143717692603.

[58] F. Kippert, D. S. Saunders, and M. L. Blaxter, "Caenorhabditis elegans has a circadian clock [2]," *Current Biology*, vol. 12, no. 2. 2002. doi: 10.1016/S0960-9822(01)00670-4.

[59] K. Hasegawa, T. Saigusa, and Y. Tamai, “Caenorhabditis elegans opens up new insights into circadian clock mechanisms,” *Chronobiology International*, vol. 22, no. 1. 2005. doi: 10.1081/CBI-200038149.

[60] M. L. Migliori *et al.*, “Caenorhabditis elegans as a Promising Model Organism in Chronobiology,” *Journal of Biological Rhythms*, vol. 38, no. 2. 2023. doi: 10.1177/07487304221143483.

[61] M. E. Goya, A. Romanowski, C. S. Caldart, C. Y. Bénard, and D. A. Golombek, “Circadian rhythms identified in Caenorhabditis elegans by in vivo long-term monitoring of a bioluminescent reporter,” *Proc Natl Acad Sci U S A*, vol. 113, no. 48, 2016, doi: 10.1073/pnas.1605769113.

[62] D. Ramot, B. L. MacInnis, H. C. Lee, and M. B. Goodman, “Thermotaxis is a robust mechanism for thermoregulation in Caenorhabditis elegans nematodes,” *Journal of Neuroscience*, vol. 28, no. 47, 2008, doi: 10.1523/JNEUROSCI.2857-08.2008.

[63] P. Sengupta *et al.*, “Genome-wide analysis of light- and temperature-entrained circadian transcripts in Caenorhabditis elegans,” *PLoS Biol*, vol. 8, no. 10, 2010, doi: 10.1371/journal.pbio.1000503.

[64] J. Gong *et al.*, “The C. elegans Taste Receptor Homolog LITE-1 Is a Photoreceptor,” *Cell*, vol. 167, no. 5, 2016, doi: 10.1016/j.cell.2016.10.053.

[65] A. J. Iliff and X. Z. S. Xu, “C. elegans: a sensible model for sensory biology,” *J Neurogenet*, vol. 34, no. 3–4, 2020, doi: 10.1080/01677063.2020.1823386.

[66] C. D. De Magalhaes Filho, B. Henriquez, N. E. Seah, R. M. Evans, L. R. Lapierre, and A. Dillin, “Visible light reduces C. elegans longevity,” *Nat Commun*, vol. 9, no. 1, 2018, doi: 10.1038/s41467-018-02934-5.

[67] K. E. Cannon *et al.*, “LITE-1 mediates behavioral responses to X-rays in Caenorhabditis elegans,” *Front Neurosci*, vol. 17, 2023, doi: 10.3389/fnins.2023.1210138.

[68] S. M. Hanson *et al.*, “Structure-function analysis suggests that the photoreceptor LITE-1 is a light-activated ion channel,” *Current Biology*, vol. 33, no. 16, 2023, doi: 10.1016/j.cub.2023.07.008.

[69] M. B. Goodman and P. Sengupta, “How caenorhabditis elegans senses mechanical stress, temperature, and other physical stimuli,” *Genetics*, vol. 212, no. 1, 2019, doi: 10.1534/genetics.118.300241.

[70] H. R. Sanghani *et al.*, “Patient fibroblast circadian rhythms predict lithium sensitivity in bipolar disorder,” *Mol Psychiatry*, vol. 26, no. 9, 2021, doi: 10.1038/s41380-020-0769-6.

[71] A. Jagannath, S. N. Peirson, and R. G. Foster, “Sleep and circadian rhythm disruption in neuropsychiatric illness,” *Current Opinion in Neurobiology*, vol. 23, no. 5. 2013. doi: 10.1016/j.conb.2013.03.008.

[72] P. A. Reuter-Lorenz and D. C. Park, “Human neuroscience and the aging mind: A new look at old problems,” *Journals of Gerontology - Series B Psychological Sciences and Social Sciences*, vol. 65 B, no. 4, 2010, doi: 10.1093/geronb/gbq035.

[73] A. K. Gold and G. Kinrys, “Treating Circadian Rhythm Disruption in Bipolar Disorder,” *Current Psychiatry Reports*, vol. 21, no. 3. 2019. doi: 10.1007/s11920-019-1001-8.

[74] W. H. Walker, J. C. Walton, A. C. DeVries, and R. J. Nelson, "Circadian rhythm disruption and mental health," *Translational Psychiatry*, vol. 10, no. 1. 2020. doi: 10.1038/s41398-020-0694-0.

[75] M. Abe, E. D. Herzog, and G. D. Block, "Lithium lengthens the circadian period of individual suprachiasmatic nucleus neurons," *Neuroreport*, vol. 11, no. 14, 2000, doi: 10.1097/00001756-200009280-00042.

[76] S. A. Kaladchibachi, B. Doble, N. Anthopoulos, J. R. Woodgett, and A. S. Manoukian, "Glycogen synthase kinase 3, circadian rhythms, and bipolar disorder: A molecular link in the therapeutic action of lithium," *J Circadian Rhythms*, vol. 5, 2007, doi: 10.1186/1740-3391-5-3.

[77] E. Iwahana *et al.*, "Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei," *European Journal of Neuroscience*, vol. 19, no. 8, 2004, doi: 10.1111/j.0953-816X.2004.03322.x.

[78] E. Gómez-Orte *et al.*, "Effect of the diet type and temperature on the C. elegans transcriptome," *Oncotarget*, vol. 9, no. 11, 2018, doi: 10.18632/oncotarget.23563.

[79] S. R. Pandi-Perumal *et al.*, "Senescence, sleep, and circadian rhythms," *Ageing Research Reviews*, vol. 1, no. 3. 2002. doi: 10.1016/S1568-1637(02)00014-4.

[80] M. R. Green and J. Sambrook, "Total RNA extraction from caenorhabditis elegans," *Cold Spring Harbor Protocols*, vol. 2020, no. 9. 2020. doi: 10.1101/pdb.prot101683.

[81] M. Levi-Ferber *et al.*, “Neuronal regulated ire-1-dependent mRNA decay controls germline differentiation in Caenorhabditis elegans,” *Elife*, vol. 10, 2021, doi: 10.7554/eLife.65644.

[82] F. Sciarra *et al.*, “Disruption of circadian rhythms: A crucial factor in the etiology of infertility,” *International Journal of Molecular Sciences*, vol. 21, no. 11. 2020. doi: 10.3390/ijms21113943.

[83] A. Furtado, D. Costa, M. C. Lemos, J. E. Cavaco, C. R. A. Santos, and T. Quintela, “The impact of biological clock and sex hormones on the risk of disease,” in *Advances in Protein Chemistry and Structural Biology*, vol. 137, 2023. doi: 10.1016/bs.apcsb.2023.03.004.

[84] A. Borisuit, F. Linhart, J. L. Scartezzini, and M. Münch, “Effects of realistic office daylighting and electric lighting conditions on visual comfort, alertness and mood,” *Lighting Research and Technology*, vol. 47, no. 2, 2015, doi: 10.1177/1477153514531518.

[85] C. Blume, C. Garbazza, and M. Spitschan, “Effects of light on human circadian rhythms, sleep and mood,” *Somnologie*, vol. 23, no. 3. 2019. doi: 10.1007/s11818-019-00215-x.

[86] B. Erdem *et al.*, “Effects of lithium on locomotor activity and circadian rhythm of honey bees,” *Sci Rep*, vol. 13, no. 1, 2023, doi: 10.1038/s41598-023-46777-7.

[87] Z. Pincus, T. C. Mazer, and F. J. Slack, “Autofluorescence as a measure of senescence in C. elegans: Look to red, not blue or green,” *Aging*, vol. 8, no. 5, 2016, doi: 10.18632/aging.100936.

[88] M. D. McGee *et al.*, “Loss of intestinal nuclei and intestinal integrity in aging C. elegans,” *Aging Cell*, vol. 10, no. 4, 2011, doi: 10.1111/j.1474-9726.2011.00713.x.

[89] N. I. Dmitrieva and M. B. Burg, “Analysis of DNA breaks, DNA damage response, and apoptosis produced by high NaCl,” *Am J Physiol Renal Physiol*, vol. 295, no. 6, 2008, doi: 10.1152/ajprenal.90424.2008.

[90] C. Kenyon, “The first long-lived mutants: Discovery of the insulin/IGF-1 pathway for ageing,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 366, no. 1561. 2011. doi: 10.1098/rstb.2010.0276.

[91] O. Altintas, S. Park, and S. J. V. Lee, “The role of insulin/IGF-1 signaling in the longevity of model invertebrates, C. elegans and D. melanogaster,” *BMB Rep*, vol. 49, no. 2, 2016, doi: 10.5483/BMBRep.2016.49.2.261.

[92] A. Zečić and B. P. Braeckman, “DAF-16/FoxO in Caenorhabditis elegans and Its Role in Metabolic Remodeling,” *Cells*, vol. 9, no. 1. 2020. doi: 10.3390/cells9010109.

[93] J. R. Berman and C. Kenyon, “Germ-cell loss extends C. elegans life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling,” *Cell*, vol. 124, no. 5, 2006, doi: 10.1016/j.cell.2006.01.039.

[94] A. Ghazi, S. Henis-Korenblit, and C. Kenyon, “A transcription elongation factor that links signals from the reproductive system to lifespan extension in Caenorhabditis elegans,” *PLoS Genet*, vol. 5, no. 9, 2009, doi: 10.1371/journal.pgen.1000639.

[95] N. M. Templeman and C. T. Murphy, “Regulation of reproduction and longevity by nutrient-sensing pathways,” *Journal of Cell Biology*, vol. 217, no. 1. 2018. doi: 10.1083/jcb.201707168.

[96] A. Antebi, “Regulation of longevity by the reproductive system,” *Exp Gerontol*, vol. 48, no. 7, 2013, doi: 10.1016/j.exger.2012.09.009.

[97] M. Artan *et al.*, “Food-derived sensory cues modulate longevity via distinct neuroendocrine insulin-like peptides,” *Genes Dev*, vol. 30, no. 9, 2016, doi: 10.1101/gad.279448.116.

[98] C. M. Coburn and C. I. Bargmann, “A putative cyclic nucleotide-gated channel is required for sensory development and function in C. elegans,” *Neuron*, vol. 17, no. 4, 1996, doi: 10.1016/S0896-6273(00)80201-9.

[99] A. B. Hellman and K. Shen, “Sensory transduction channel subunits, tax-4 and tax-2, modify presynaptic molecular architecture in C. elegans,” *PLoS One*, vol. 6, no. 9, 2011, doi: 10.1371/journal.pone.0024562.

[100] A. Ward, J. Liu, Z. Feng, and X. Z. S. Xu, “Light-sensitive neurons and channels mediate phototaxis in C. elegans,” *Nat Neurosci*, vol. 11, no. 8, 2008, doi: 10.1038/nn.2155.

[101] R. Perales, D. M. King, C. Aguirre-Chen, and C. M. Hammell, “LIN-42, the Caenorhabditis elegans PERIOD homolog, Negatively Regulates MicroRNA Transcription,” *PLoS Genet*, vol. 10, no. 7, 2014, doi: 10.1371/journal.pgen.1004486.

[102] D. Banerjee, A. Kwok, S. Y. Lin, and F. J. Slack, “Developmental timing in C. elegans is regulated by kin-20 and tim-1, homologs of core circadian clock genes,” *Dev Cell*, vol. 8, no. 2, 2005, doi: 10.1016/j.devcel.2004.12.006.

[103] J. H. An *et al.*, "Regulation of the Caenorhabditis elegans oxidative stress defense protein SKN-1 by glycogen synthase kinase-3," *Proc Natl Acad Sci U S A*, vol. 102, no. 45, 2005, doi: 10.1073/pnas.0508105102.

[104] G. Macnee and M. Hejmadi, "Hypoxia signalling and regulation in chemosensory behaviour of Caenorhabditis elegans," *Bioscience Horizons*, vol. 8, 2015, doi: 10.1093/biohorizons/hzv003.

[105] N. Xu, K. Shinohara, K. E. A. Saunders, J. R. Geddes, and A. Cipriani, "Effect of lithium on circadian rhythm in bipolar disorder: A systematic review and meta-analysis," *Bipolar Disorders*, vol. 23, no. 5. 2021. doi: 10.1111/bdi.13070.

[106] A. K. Gold and G. Kinrys, "Treating Circadian Rhythm Disruption in Bipolar Disorder," *Current Psychiatry Reports*, vol. 21, no. 3. 2019. doi: 10.1007/s11920-019-1001-8.

[107] K. Liška *et al.*, "Lithium affects the circadian clock in the choroid plexus – A new role for an old mechanism," *Biomedicine and Pharmacotherapy*, vol. 159, 2023, doi: 10.1016/j.biopha.2023.114292.

[108] A. Inokuchi *et al.*, "Effects of lithium on growth, maturation, reproduction and gene expression in the nematode Caenorhabditis elegans," *Journal of Applied Toxicology*, vol. 35, no. 9, 2015, doi: 10.1002/jat.3058.

[109] P. R. Hunt, N. Olejnik, K. D. Bailey, C. A. Vaught, and R. L. Sprando, "C. elegans Development and Activity Test detects mammalian developmental neurotoxins," *Food and Chemical Toxicology*, vol. 121, 2018, doi: 10.1016/j.fct.2018.09.061.

[110] X. Sun, W. D. Chen, and Y. D. Wang, "DAF-16/FOXO transcription factor in aging and longevity," *Frontiers in Pharmacology*, vol. 8, no. AUG. 2017. doi: 10.3389/fphar.2017.00548.

[111] M. Prin, J. Bertazzo, L. A. Walker, B. Scott, and T. Eckle, "Enhancing circadian rhythms—the circadian MEGA bundle as novel approach to treat critical illness," *Ann Transl Med*, vol. 11, no. 9, 2023, doi: 10.21037/atm-22-5127.

[112] K. A. McCulloch and A. E. Rougvie, "Caenorhabditis elegans period homolog lin-42 regulates the timing of heterochronic miRNA expression," *Proc Natl Acad Sci U S A*, vol. 111, no. 43, 2014, doi: 10.1073/pnas.1414856111.

[113] G. C. Monsalve, C. Van Buskirk, and A. R. Frand, "LIN-42/PERIOD controls cyclical and developmental progression of C. elegans molts," *Current Biology*, vol. 21, no. 24, 2011, doi: 10.1016/j.cub.2011.10.054.