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Neuroprotective effect of Cistus laurifolius on hydrogen peroxide-induced neurodegeneration in differentiated SH-SY5Y cells

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Abstract

Neurodegeneration is an important finding of various neurological diseases such as Alzheimer's Disease and Parkinson's Disease. MAP2 and Rbfox3/NeuN genes are also two important neuronal markers. In this study, our aim is to investigate the neuroprotective effect of *Cistus laurifolius L.* extract in neurodegeneration caused by hydrogen peroxide (H_2O_2) treatment in differentiated SH-SY5Y (f-SH-SY5Y) cells.

In the study, the effects of H_2O_2 (62.5-1000µM) and *Cistus laurifolius* (15.62-1000 mg/ml) doses on cell viability were determined by the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrasodium bromide (MTT) method. The effect of *Cistus laurifolius* in d-SH-SY5Y cells followed by H_2O_2 treatment on cell viability was determined by the MTT test. The effect of the extract on MAP2 and Rbfox3/NeuN gene expressions in 24-hour periods was evaluated by the Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) method.

According to our findings, *Cistus laurifolius* extract significantly reduced cell viability in SH-SY5Y cells at 24 h and 48 h ($p<0.05$). H_2O_2 caused a decrease in d-SH-SY5Y cell viability at all doses ($p<0.05$). We showed that *Cistus laurifolius* extract applied for 24 hours and 48 hours before toxicity significantly increased cell viability at doses of 31.25 and 62.5 mg/ml in 24 hours compared to the H_2O_2 group. In d-SH-SY5Y cells, MAP2 gene expression in the H_2O_2 group was significantly decreased compared to the control group, however, it was upregulated in *Cistus laurifolius* groups at 31.25 and 62.5 mg/ml administered before toxicity compared to the H_2O_2 group ($p<0.05$). However, there was no significant difference between the groups in terms of NeuN gene expression ($p>0.05$).

The neuroprotective effect of *Cistus laurifolius* in the in-vitro neurodegeneration model has been extensively investigated for the first time. According to the data we obtained, *Cistus laurifolius* increased the cell viability and MAP2 gene expression and showed a neuroprotective effect. However, further studies are still needed to confirm the therapeutic value of the extract.

Keywords: SH-SY5Y, *Cistus laurifolius*, neuroprotective effect, MAP2, NeuN, cytotoxicity[*]

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Cistus laurifolius'un farklılaşmış SH-SY5Y hücrelerinde hidrojen peroksit kaynaklı nörodejenerasyon üzerindeki nöroprotektif etkisi

Özet

Nörodegenerasyon, Alzheimer ve Parkinson gibi çeşitli nörolojik hastalıkların önemli bir bulgusudur. MAP2 ve Rbfox3/NeuN genleri de çeşitli nörodejeneratif hastalıkların patogenezinde rol alan iki önemli nöronal belirteçtir. Bu çalışmada amacımız olgun nöron benzeri hücreye farklılaşmış SH-SY5Y (f-SH-SY5Y) hücrelerinde hidrojen peroksit

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(H_2O_2) muamelesi kaynaklı gelişen nörodejenerasyonda *Cistus laurifolius* L. ekstresinin nöroprotektif etkisinin araştırılmasıdır.

Çalışmada H_2O_2 (62.5-1000uM) ve *Cistus laurifolius* (15.62-1000mg/ml) dozlarının hücre canlılığı üzerine etkileri MTT yöntemi ile belirlendi. f-SH-SY5Y hücrelerinde *Cistus laurifolius'*un, ardından H_2O_2 uygulamasının hücre canlılığı üzerindeki etkisi 3-[4,5-dimetiltiyazol-2-il]-2,5-difenil-tetrazolyum bromür (MTT) testi ile belirlendi. Ekstrenin 24 saatlik sürede MAP2 ve Rbfox3/NeuN gen ifadeleri üzerindeki etkisi Gerçek Zamanlı Kantitatif Revers Transkriptaz Polimeraz Zincir Reaksiyonu (RT-qPCR) yöntemi ile değerlendirildi.

Bulgularımıza göre *Cistus laurifolius* ekstresi uygulaması ile SH-SY5Y hücrelerinde 24 ve 48 saatte hücre canlılığı anlamlı düzeyde azalmıştır ($p<0.05$). H_2O_2 tüm dozlarda d-SH-SY5Y hücre canlılığında azalmaya neden olmuştur ($p<0.05$). Toksisite öncesi 24 saat ve 48 saatlik sürelerde uygulanan *Cistus laurifolius* ekstresinin 24 saatte 31.25 ve 62.5 mg/ml'lik dozlarda hücre canlılığını H_2O_2 grubuna göre anlamlı derecede artırdığını gösterdik. d-SH-SY5Y hücrelerinde, H_2O_2 grubunda MAP2 gen ekspresyonu kontrol grubuna göre anlamlı derecede azaldı, buna karşılık toksisite öncesinde uygulanan 31.25 ve 62.5 mg/ml'lik *Cistus laurifolius* gruplarında H_2O_2 grubuna göre MAP2 gen ekspresyonu upregüle edildi ($p<0.05$). Ancak gruplar arasında NeuN gen ekspresyonu açısından anlamlı bir fark görülmedi ($p>0.05$).

Cistus laurifolius'un *in vitro* nörodejenerasyon modelindeki nöroprotektif etkisi ilk kez kapsamlı bir şekilde araştırılmıştır. Elde ettiğimiz verilere göre *Cistus laurifolius* hücre canlılığı ve MAP2 gen ekspresyonunda artışa neden olmuş ve nöroprotektif etki göstermiştir. Bununla birlikte, *Cistus laurifolius* ekstresinin terapötik değerini doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: SH-SY5Y, *Cistus laurifolius*, nöroprotektif etki, MAP2, NeuN, sitotoksisite

1. Introduction

The most prominent feature of neurological diseases such as Alzheimer's disease (AD), and Parkinson's disease (PD) is the development of neurodegeneration in the brain tissue due to various reasons. As a result of neurodegeneration, pathophysiological changes occur in neurons such as morphological disorders, loss of function, microtubule dysfunction, and neuronal atrophy [1,2].

Microtubules (MT) are one of the main elements of the cytoskeleton, which has many structural and functional roles in cells [3]. In addition, Microtubule-associated proteins (MAPs) have been identified, which have various roles such as neuronal development and signaling, binding to MTs, and stabilizing them [1,3]. One of these proteins, MAP2, provides stabilization by binding to microtubules, is a regulator in forming dendritic structures, mediates MT and actin cross-linking, and plays a role in cell signal transmission. It has been shown that irregularities in MAP2 expression and immunoreactivity are associated with neurodegenerative diseases such as AD, Prion disease, and neurodegenerative diseases [1,4,5]. Neuronal nuclei (NeuN) is a specific neuronal marker found in nuclei of mature neurons [6]. Although the physiological roles of NeuN have not been fully elucidated, it has been used in many studies to directly assess neuronal death or loss [6-8]. A study by Kim et al. explained that Neun is the gene product of Rbfox-3 [9]. Based on the available information, it is understood that MAP2 and NeuN genes are good therapeutic targets in neurodegeneration models.

In subjects such as investigating the pathogenesis of neurodegenerative diseases or developing treatment strategies, SH-SY5Y neuroblastoma cells, from which neuron-like cell models are obtained by applying differentiation protocol, are often used. SH-SY5Y cells can be differentiated into morphologically and structurally neurons with inducers such as retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) [10-12]. In addition, since hydrogen peroxide (H_2O_2) causes neuron damage and increases Reactive oxygen species (ROS) in cells, it is often preferred to create an *in-vitro* neurotoxicity model [13]. Our study used the neurotoxicity model induced by H_2O_2 in differentiated SH-SY5Y (d-SH-SY5Y) cells.

Cistaceae is a plant family containing approximately 180 different species with antimicrobial, antiulcerogenic, antidiarrheal, antirheumatic, and vasodilator effects [14,15]. *Cistus laurifolius* ., one of the members of this family, is known to be used in traditional treatment methods for the treatment of rheumatic pain, high fever and urinary tract inflammation [16]. Studies have reported that *Cistus laurifolius* extracts are rich in flavonoids and have anti-inflammatory, antimicrobial, antioxidant, and anticarcinogenic effects [16-19]. In addition, the anti-cholinesterase and antioxidant effects of various Cistus species (*C. libanotis, C. creticus, C. Salvifolius*) were demonstrated in the AD model [20]. In another study, phytochemical compounds and antioxidant capacity of 5 different Cistus species were shown, according to the results obtained from the SH-SY5Y cells [21]. According to our research, limited reports in the literature describe the functional role of Cistus species in the prevention of neurodegeneration.

In this study, we aimed to examine the neuroprotective effect of the extract obtained from the leaves of *Cistus laurifolius* in the model of H_2O_2-induced neurodegeneration. In the study, the cytotoxic effects of *Cistus laurifolius* extract on d-SH-SY5Y cells were evaluated and the IC_{50} dose was calculated. Then, its protective effect on cell viability and its effects on MAP2 and NeuN gene expression were investigated in the neurodegeneration model.

2. Materials and methods

2.1. Plant material and extract preparation

Fresh leaves of *Cistus laurifolius* were collected in July 2013 by Prof. Dr. It was collected by Ersin YÜCEL. This sample was coded with ANES No. 15518 from Anadolu University Herbarium. The leaves of *Cistus laurifolius* were first dried in the sun for 3 days, then in an oven at 40-45 ºC for 48 hours, and then they were obtained in powder form. 40 g of powdered leaves were boiled in 800 ml of water for 60 minutes and the extract was evaporated in a vacuum evaporator and lyophilized. The final lyophilized form was stored at + 4°C until used in the tests [16].

2.2. Cell culture and neuron-like differentiation

A human SH-SY5Y neuroblastoma cell line was obtained from the Culture Collection Animal Cells, FMD Institute, Ankara, Turkey. The cells were grown in DMEM/F12 Hams (1:1) medium (Capricorn, China) supplemented with 10% v/v fetal bovine serum (FBS) (Cegrogen, Germany) and 1 units/ml penicillin-streptomycin (Cegrogen, Germany) at 37°C in an incubator containing 5% CO_2. The medium content was replaced every two days and cells were sub-cultured once they reached 70-80% confluence.

For cell differentiation, culture dishes were first coated with 50 mg/ml type I collagen (Gibco, USA). Next, cells were cultured as described above. After 24 hours of plating, the percentage of FBS in the culture medium was reduced to 1% and supplemented with 10 µM Retinoic acid (RA) (Bldpharm, China). The medium content was replaced per 2 days and differentiation was completed in 5 days [10]. Morphological changes and cell density were examined under inverted light microscopy (Zeiss Axio, Germany).

2.3. Cell Viability Assay

For cell viability tests, $1x10^3$ cells were added to each well in a 96-well plate and a differentiation protocol was applied. First, *Cistus laurifolius* extract (up to 15.62-1000 mg/ml) was applied to d-SH-SY5Y cells for 24 and 48 hours, and the cytotoxic effect of the extract on d-SH-SY5Y cells was evaluated. Then, H_2O_2 (up to 50-100µM) was treated to d-SH-SY5Y cells to determine the toxicity model, and the appropriate dose was determined. Finally the cells were treated with increasing concentration (up to 15.6-1000 mg/ml) of the extract for 24 and 48 hours. After incubation, cells were treated with a medium containing 250 µM H_2O_2 for 24 hours. After cell viability tests, protective doses of *Cistus laurifolius* extract on viability were determined. All experiments were repeated three times.

In all experiments, MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrasodium bromide) test was used to determine cell viability at the end of the incubation period. 100 µl of MTT solution was added to each well with a final 5 mg/ml concentration and incubated for 3 hours at 37 ^{0}C. After the incubation period, 100 µl of DMSO was added to each well and the tetrazolium salts formed were dissolved. Then, it was read spectrophotometrically in an ELISA Reader device (SynergyTM H1, Biotek, USA) at 630 nm and 570 nm absorbance. Cell viability was evaluated on a percentage basis compared to the control group [17].

2.4. Gene expression analysis

For further experiments, extract doses (31.25 mg/ml and 62.5 mg/ml) and time (24 hours) at which the highest viability was detected in the neurotoxicity model were determined. A total of four groups were formed for gene expression: control group, H_2O_2 group, extract doses of 31.25 mg/ml and 62.5 mg/ml. Cells were cultured at the specified doses and times and harvested at the end of the incubation periods. Gene expression analyses were performed by the Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) method.

2.4.1. RNA extraction and cDNA synthesis

For the evaluation of gene expression, firstly, total RNA isolation was performed from cell groups using the Total RNA Purification Isolation Kit (Jena Bioscience, Germany). The purity of isolated RNAs was measured with ELISA Plate Reader (260/280 nm= 1.8-2.1). RNAs were stored at -80 ^{0}C until further use.

In the second step, cDNA synthesis was performed from Totatl RNA using the cDNA synthesis kit (ABT, Turkey) in accordance with the manufacturer's instructions. cDNAs were stored at -20 ^{0}C until RT-qPCR studies.

2.4.2. Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) Analysis

MAP2 and NeuN gene expression in d-SHSY5Y cells treated with *Cistus laurifolius* extract was evaluated by RT-qPCR (LightCycler®96 Instrument - Roche Diagnostics). This test was performed using the SYBR-Green Master Mix (A.B. T™ 2X, Turkey) kit in accordance with the manufacturer's instructions. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) gene was used as housekeeping gene. The relative expression values were calculated using

the Cycle threshold (Ct) method according to the $2^{-\Delta\Delta Ct}$ formula and the fold change in mRNA expressions of target genes was determined [22].

2.5. Statistics

We used SPSS 21.0 for Windows software to analyze study data. The normality of data distributions was confirmed using the Shapiro–Wilk test. The groups showed normal distribution and the continuous variables were presented as means and standard error. One-way ANOVA and Tukey Test were used to test the differences between the groups. Other statistical analyses were calculated using GraphPad Prism (Version 7.04 for Windows, GraphPad Software, USA) software. A result of $p<0.05$ and $p<0.001$ was considered statistically significant.

3. Results

3.1. Cell Viability Analysis

To investigate the protective effects of *Cistus laurifolius* extract against H_2O_2-induced cytotoxicity in d-SH-SY5Y cells, cell viability was evaluated by MTT assay. First, to understand the optimum concentration range of *Cistus laurifolius* extract, SH-SY5Y cells were treated with the extract at logarithmically decreasing concentrations (15.62-1000 mg/ml) for 24 and 48 hours. According to our findings, there was a regular dose-dependent decrease in cell viability at 24 h exposure, and a statistically significant decrease in cell viability was observed at doses of 125 mg/ml and above ($p<0.05$). In 48-hour extract exposure, a significant decrease was observed at all doses compared to the control group ($p<0.05$). The 24 h and 48 h IC_{50} dose of *Cistus laurifolius* extract in SH-SY5Y cells was determined as 154.4 mg/ml and 122.4 mg/ml, respectively.

In order to determine the toxic dose of H_2O_2 in d-SH-SY5Y cells, doses of 62.5 µM, 125 µM, 250 µM, 500 µM and 1000 µM were tested in 24 hours (Figure 1. B). A significant decrease was observed in all doses compared to the control group ($p<0.05$) and the IC_{50} dose of H_2O_2 was determined as 212.7 µM. It was decided to use 250 µM as the neurotoxicity dose to be used in later experiments.

Finally, to evaluate the protective effect of *Cistus laurifolius* extract, d-SH-SY5Y cells were treated with increasing doses (15.62-1000 mg/ml) of the extract for 24 and 48 hours. The cells were then exposed to 250 µM H_2O_2 for 24 hours. After 24 hours of *Cistus laurifolius* treatment, a significant increase in cell viability was observed at concentrations of 31.25 and 62.5 mg/ml compared to the H_2O_2 toxicity group ($p<0.05$) (Figure 1.C.). At increasing doses, cell viability decreased with the effect of H_2O_2 and extract, and the toxicity rate increased significantly at concentrations of 500 and 1000 mg/ml ($p<0.05$). After 48 hours of *Cistus laurifolius* treatment, there was no significant increase in cell viability compared to the H_2O_2 toxicity group ($p>0.05$), on the contrary, the toxicity rate increased significantly at 500 and 1000 mg/ml concentration ($p<0.05$) (Figure 1.D.).

3.2. Analysis of gene expression in H_2O_2-induced d-SH-SY5Y cells pretreated with Cistus laurifolius

To evaluate the neuroprotective effect of *Cistus laurifolius* extract in H_2O_2-induced d-SH-SY5Y cells, mRNA expression levels of MAP2 and NeuN genes were evaluated by RT-qPCR method. According to our results, MAP2 gene expression level in the H_2O_2 toxicity group decreased 5.9-fold compared to the control group without any drug treatment ($p<0.001$). The MAP2 gene expression level in the 31.25 mg/ml and 62.5 mg/ml *Cistus laurifolius* extract group administered 24 hours before H_2O_2 treatment increased 2.2 and 2.4-fold, respectively, compared to the H_2O_2 toxicity group ($p<0.05$). MAP2 level in the d-SHSY5Y control group was still significantly higher than all groups ($p<0.05$; $p<0.001$) (Figure 2.A.).

On the other hand, NeuN gene expression level in the H_2O_2 toxicity group decreased 1.54-fold compared to the control group, but this was not statistically significant ($p>0.05$). In the treatment groups, the NeuN gene expression level in 31.25 mg/ml and 62.5 mg/ml *Cistus laurifolius* extract applied for 24 hours increased 1.2 and 1.1 fold, respectively, but these results were not statistically significant ($p>0.05$).

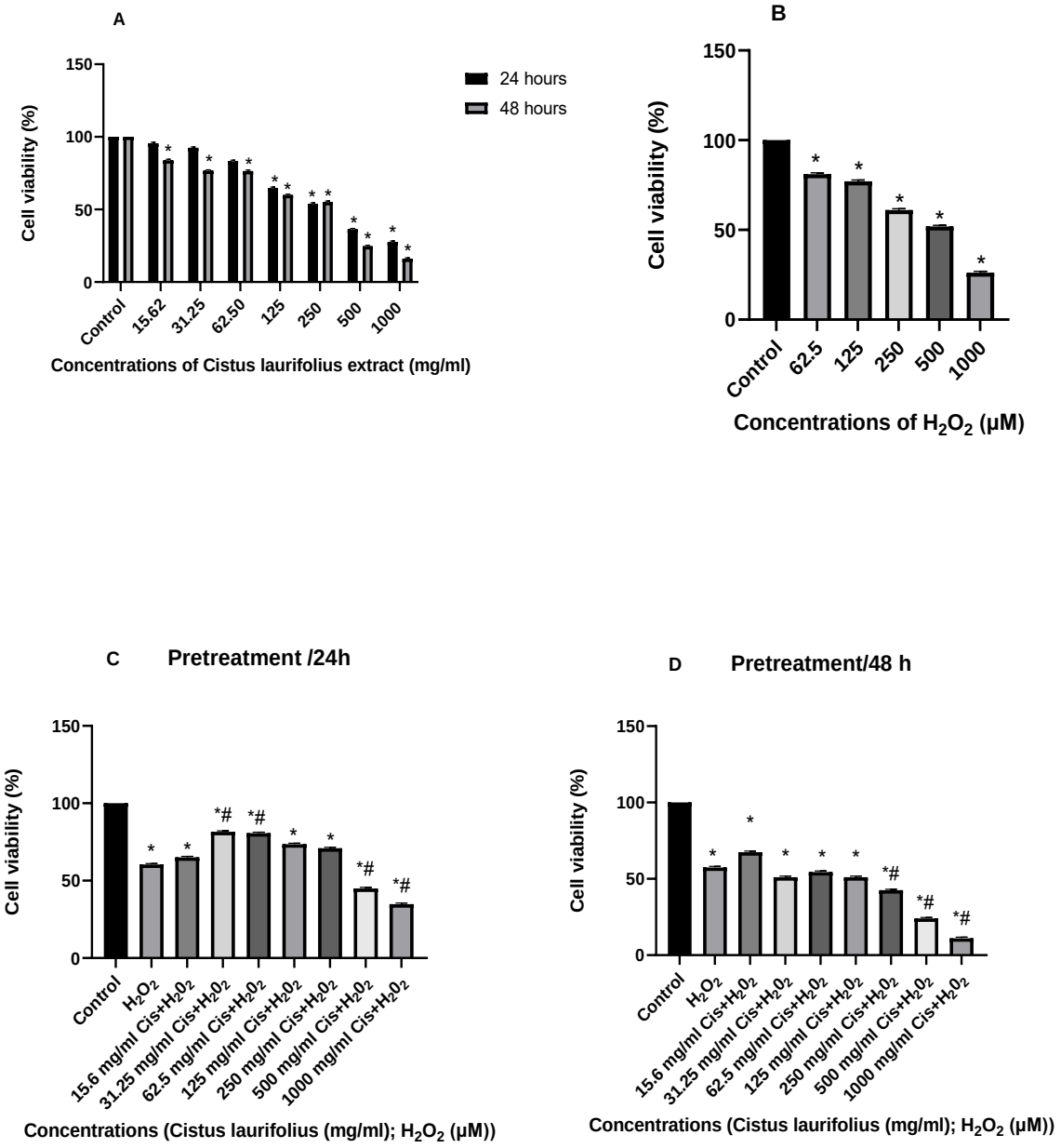

Figure 1. Evaluation of the effect of *Cistus laurifolius* extract on H_2O_2-induced cell death in d-SH-SY5Y cells by MTT test. A) Dose (up to 15.62-1000 mg/ml) and time (24h and 48 h)-dependent cytotoxic effect of *Cistus laurifolius* extract on SH-SY5Y cell viability. B) Dose-dependent cytotoxic effect of H_2O_2 for 24 h in d-SH-SY5Y cell. C) Effect of *Cistus laurifolius* extracts on d-SH-SY5Y cell viability against H_2O_2 (250 µM) neurotoxicity in 24-hour treatment. D) Effect of *Cistus laurifolius* extracts on d-SH-SY5Y cell viability against H_2O_2 (250 µM) neurotoxicity in 48-hour treatment. d-SH-SY5Y cells were pretreated with *Cistus laurifolius* before the toxicity of H_2O_2.) (Cis: *Cistus laurifolius*) Values are presented as mean±SEM. ∗p<0.05 compared to control group; #p<0.05 compared to H_2O_2 group. (Sample size of 4 per group; n=4).

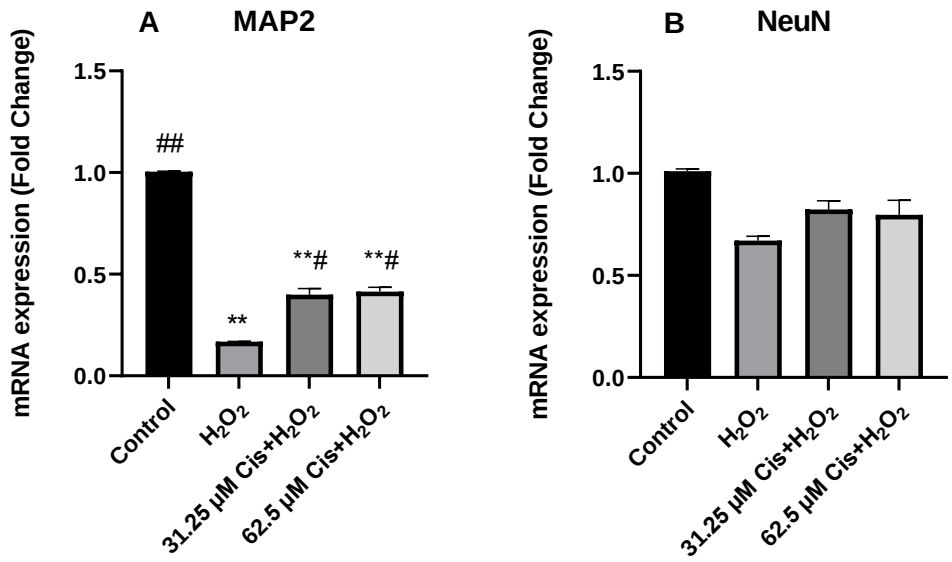

Figure 2. Effect of 24 h *Cistus laurifolius* treatment against H_2O_2 (24h) toxicity on the expression of MAP2 and NeuN genes in d-SH-SY5Y neuroblastoma cells. A) MAP2 gene expression B) NeuN gene expression analysis by RT-QPCR. (Cis: *Cistus laurifolius*) Values are presented as mean±SEM. **p<0.001 compared to control group; #p<0.05, ##p<0.05 compared to H_2O_2 group. (n=4).

4. Conclusions and discussion

In the pathogenesis of neurodegenerative diseases, cell damage, loss of function and even neuronal cell death are observed due to various causes such as oxidative stress, inflammation or accumulation of toxins [1,2]. Therefore, the discovery of potential neuroprotective agents that can reduce neurotoxicity is very important in reducing or treating the symptoms of neurodegenerative diseases [23]. In this study, we investigated the neuroprotective effect of *Cistus laurifolius* extract against H_2O_2-induced neurotoxicity in d-SH-SY5Y cells. In the study, we first evaluated the cytotoxic effect of *Cistus laurifolius* extract in SH-SY5Y cells by the MTT method. Then, we examined the cytotoxic effect of H_2O_2 at a certain dose range to determine the dose to be used to create a neurotoxicity model. Then, we evaluated the neuroprotective effect of pretreatment administration of *Cistus laurifolius* extract by the MTT method. We also evaluated the effect of *Cistus luurifolius* extract on the expression levels of MAP2 and NeuN genes in d-shSY5Y cells by RT-qPCR method.

H_2O_2 is a frequently used agent to create a neurodegeneration model *in vitro* studies. This agent causes oxidative stress and neuronal damage in cells [13,23]. In studies, an H_2O_2 dose of around 250 to 300 uM is preferred to induce neurotoxicity in SH-SY5Y cells [23, 24]. In this study, we examined the 24-hour cytotoxic effect of H_2O_2 at increasing doses up to 62.5-1000uM. A significant cytotoxic effect was observed at all doses compared to the control group. The IC_{50} dose of H_2O_2 was determined as 212.7 µM and 250 uM H_2O_2 dose was selected as the neurotoxicity dose to be used in further experiments. It is seen that the results we obtained are also compatible with the literature.

The cytotoxic effects of various Cistus species (such as *Cistus ladanifer, C. creticus, C. parviflorus*) have been studied in cancer types such as breast cancer, prostate cancer, cervical cancer, and neuroblastoma under *in vitro* conditions and reported to show antiproliferative effects [17, 21, 25]. SH-SY5Y cells, the IC_{50} dose of essential oils of *Cistus ladanifer* was 92.8 ppm [25], and the IC50 dose of C. parviflorus extract was 7.89 µg / ml [21]. The cytotoxic effects of *Cistus laurifolius* L. extract were examined in mouse fibrosarcoma cells (Wehi 164), human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD) cells, and it was reported that it showed significant antiproliferative effect at doses of 250 mg/ml and above in all these cell lines [17]. We found only one study evaluating the cytotoxic effect of *Cistus laurifolius* in SH-SY5Y cells. In this study conducted by Onal et al., the 24-hour IC_{50} dose of *Cistus laurifolius* in SH-SY5Y cells was determined as 45,73 mg/ml [21].

In our study, we firstly observed that *Cistus laurifolius* had a significant toxic effect on SH-SY5Y cell viability at doses of 125 mg and above in 24 and 48 hours applications, and we determined the IC_{50} doses as 154.4 mg/ml and 122.4 mg/ml for 24h and 48 hours, respectively. Our study results showed antiproliferative effect in cancer cells as in other species available in the literature. However, our main aim in this study was to investigate its protective effect against the neurotoxicity model in neuron-like d-SH-SY5Y cells. Therefore, we examined the protective effect of the treatment of the extract before toxicity on cells. The doses that increased cell viability the most in the pretreatment *Cistus laurifolius* treatment were 31.25 and 62.5 mg/ml for 24 hours. In these groups, cell viability increased significantly compared to the H_2O_2 group. However, at higher doses of *Cistus laurifolius*, cell viability decreased with

the combined effect of H_2O_2. It probably provides a protective effect with the effect of antioxidant activity at lower doses.

Anti-inflammatory and antioxidant properties of *Cistus laurifolius* have been shown in various studies [18, 21]. However, we found a limited number of studies in the literature on its neuroprotective effect. Akkol et al. showed that *Cistus* L. has acetylcholinesterase (AChE) butyrylcholinesterase (BChE) inhibitory effect and neuroprotective effect in the AD model [20]. In this study, we also examined the changes in MAP2 and NeuN gene expressions to see the effects of *Cistus laurifolius* extract on neuro markers. MAP2 and NeuN genes are among the important neuro markers in neurodegeneration models [1,6]. It has been shown that irregularities in MAP2 expression and its immunoreactivity are also associated with neurodegenerative diseases such as AD, Prion disease, or neuropsychiatric disorders such as schizophrenia and autism [1, 4, 5]. Although the physiological roles of NeuN have not been fully elucidated, it has been used in many studies to directly assess neuronal death or loss [6, 7, 8]. In this study, MAP2 gene expression of Cistus L. was upregulated against 250 μM H_2O_2 neurotoxicity at doses of 31.25 and 62.25 mg/ml, but no significant change was observed in NeuN gene expression. In fact, NeuN gene expression in the H_2O_2 group did not show a significant change compared to the control group. According to these results, we can say that Cistus has a positive effect on MAP2 gene against neuronal toxicity, while it is not effective for NeuN

In conclusion, the neuroprotective effect of *Cistus laurifolius* in the in-vitro neurodegeneration model has been extensively investigated for the first time. According to the data we obtained, *Cistus laurifolius* caused an increase in cell viability and MAP2 gene expression and showed a neuroprotective effect. In this context, our results are quite promising. However, further studies are still needed to confirm the therapeutic value of Cistus extracts.

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