

A research paper on the immunomodulatory and anti-inflammatory activities of olive tree (*Olea europaea* L.) leaf

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Abstract: Olive tree (*Olea europaea* L.) leaf is known to have a number of bioactive properties being antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, antifungal, antiviral and antimicrobial. In this study, the immunomodulatory roles of Olive tree (*Olea europaea* L.) leaf against oxidative damage caused by carbon tetrachloride (CCl₄) in *Saccharomyces cerevisiae* were investigated. In the study, four groups were formed; namely, (i) Control Group: Yeast only planted group; (ii) CCl₄ Group: Group given CCl₄ (15 mM); (iii) Olive Tree Leaf Group: The group given olive tree leaf (10%); and (iv) Olive Tree Leaf + CCl₄ Group: Olive tree leaf (10%) + CCl₄ (15 mM) given group. Cultures of *Saccharomyces cerevisiae* were grown at 30 °C for 1, 3, 5, and 24 hours. Malondialdehyde (MDA), glutathione levels (GSH), cell growth and catalase (CAT) activity measurements were determined by spectrophotometer. Total protein concentrations were determined by SDS-PAGE electrophoresis and the Bradford protein method. According to the results obtained; compared to the CCl₄ group, cell growth (1, 3, 5 and 24 hours), total protein synthesis, and GSH and CAT activities (24 hours) increased in olive tree leaf groups, while MDA level (24 hours) decreased. Thanks to its strong bioactive properties, olive tree leaf has been found to increase cell growth and total protein synthesis by decreasing CCl₄ induced oxidative stress in *Saccharomyces cerevisiae* culture. It has been concluded that if the olive tree leaf is used regularly, it will be beneficial in eliminating many health problems.

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1. INTRODUCTION

In recent years, epidemiological studies have proven that the consumption of polyphenol-rich food is important for human health. The olive tree (*Olea europaea* L.), which is widely grown in the Mediterranean region, has been used for years in the treatment of various diseases with both its fruit and leaves (Hashmi *et al.*, 2015). In addition, olive tree leaves represent an inexpensive raw material as a good source of bioactive compounds used in food, agricultural, and biomedical applications (Rocchetti *et al.*, 2022). Olive tree leaf contains high levels of fatty acids (98-99%), especially monounsaturated acids such as oleic acid, as well as worthy components such as phytosterols, phenolics, and tocopherols. Thanks to these valuable compounds, it has high antioxidant activity with its capacity to scavenge reactive oxygen

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species (ROS) and stabilize oxygen radicals with an intramolecular hydrogen bond. The most important phenolic component of olive tree leaf, which is among the plants rich in polyphenols, is oleuropein. This phenolic compound constitutes about 14% of the plant and has strong anti-inflammatory and antithrombotic biochemical properties (Romani *et al.*, 2019). Olive leaf is used a lot in traditional medicine as it is a source of bioactive compounds. The most dominant compound in olive leaf extract is known to be oleuropein (Romero-Marquez *et al.*, 2022). Moreover, oleuropein inhibits the expression of proinflammatory cytokine genes by eliminating the end products of lipid peroxidation of the phenolic compound. Thanks to these unique phenolic compounds, it is responsible for increasing body resistance by strengthening the immune system. Pharmacological studies of olive tree (*Olea europaea* L.) leaf show that it has anticancer, anti-inflammatory, antioxidant, antidiabetic, antimicrobial, antifungal, antitumor, antihypertensive, regulation of blood pressure, glycemia, neuroprotective, and cardioprotective activities in terms of phenolic compounds. It is known that extra virgin olive oil obtained from its fruits plays chemopreventive roles in the treatment of cardiovascular diseases (Romani *et al.*, 2019; Romero-Marquez *et al.*, 2022). *Saccharomyces cerevisiae* (*S. cerevisiae*) is a powerful model organism to examine the fundamental aspects of eukaryotic cell biology. *S. cerevisiae* has 16 chromosomes and its total genome contains 78.520 nucleotide pairs of mitochondrial DNA and approximately 13.117.000 nucleotide pairs. The density of protein-coding genes is about 50 times higher than the gene density in the human genome. Due to these genome features, it is thought to be approximately 23% similar to the human genome (Duina *et al.*, 2014). Accordingly, *S. cerevisiae* was used as a model organism in our study due to its similarity to human genome characteristics. In our study, the negative effects of carbon tetrachloride (CCl₄), which we used to damage *S. cerevisiae*, were determined by malondialdehyde (MDA), an oxidative stress marker, while the therapeutic activities of olive tree leaf were determined by the antioxidant defense enzyme system catalase (CAT). In addition, the protective effects of olive tree leaf against cell growth were investigated by biochemical and molecular biology analyses.

2. MATERIALS and METHOD

2.1. Herbal Materials

The olive tree leaves used as herbal materials in our study were obtained from Yurtbaşı region in the province of Elazığ, Turkey. Olive tree leaves were collected in October and November, the harvest time.

2.2. Experimental Groups

In the study, 4 groups were formed: (i) Control Group: Yeast only planted group; (ii) CCl₄ Group: Group given CCl₄ (15 mM); (iii) Olive Tree Leaf Group: The group given olive tree leaf (10%); (iv) Olive Tree Leaf + CCl₄ Group: Olive tree leaf (10%) + CCl₄ (15 mM) given group. After sterilization, olive tree leaf and CCl₄ were additional to the cultures of *S. cerevisiae* at certain concentrations. For *S. cerevisiae* growth medium, 1.5 g glucose, 1.5 g yeast extract, and 1.5 g tryptone per 50 mL were used. In order to ensure proliferation and growth of *S. cerevisiae*, olive tree leaf was additional in addition to YEPD and yeast cells were developed (Aslan *et al.*, 2019a; Beyaz *et al.*, 2020).

2.3. Olive Tree Leaf Extract and CCl₄ Chemical Application to *Saccharomyces cerevisiae* Culture

Olive Tree Leaf + CCl₄ was additional to cultures of *S. cerevisiae* and grown at 30°C. Olive Tree Leaf + CCl₄ Group: Olive tree leaf + CCl₄ was additional to the group (Gokce 2020). Preparation of 10% olive leaf extract: The leaves collected during the olive harvest were washed several times with distilled water. It was left to dry for three weeks at dark room temperature.

The raw dried leaves were ground and stored in the dark until the time of extraction. 10 grams of olive leaves were weighed and infused in 100 ml of boiling distilled water for 3-4 hours. It was then filtered through a sterile filter paper and made ready for cultivation. Immediately afterwards, carbon tetrachloride (CCl₄) and olive leaf plant extract were added to the other flasks that were removed from the oven, along with the burner flame (Mahyoob *et al.*, 2022).

2.4. *Saccharomyces cerevisiae* Cell Growth Measurements

S. cerevisiae cultures were developed at 30 °C at different time intervals (1, 3, 5, 24 hours) in a 600 nm wavelength spectrophotometer (OD₆₀₀) (Aslan *et al.*, 2019b).

2.5. *Saccharomyces cerevisiae* SDS- PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) Analysis

S. cerevisiae culture samples, which were homogenized, were analyzed by SDS PAGE and the protein band intensities between the groups were determined (Aslan *et al.*, 2019b; Beyaz *et al.*, 2020).

2.6. *Saccharomyces cerevisiae* Malondialdehyde Analysis (MDA)

The thiobarbituric acid (TBA) form of malondialdehyde, the end produce of lipid peroxidation, was determined according to its concentration. 200 ml of olive tree leaf extract was taken and put into a tube. 800 ml of phosphate buffer, 25 ml of butyl hydroxy toluene (BHT) solution, and 500 ml of 30% trichloric acid (TCA) were added. After mixing the tubes in vortex, the caps were closed and left in an ice bath for about 2 hours. After the tubes were brought to room temperature, the caps of the tubes were lifted and centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant obtained from the centrifuge was taken and transferred to other tubes. 75 µl of EDTA and 25 µl of TBA were additional to the filtrate, 1 ml of which was taken. After mixing the tubes in a vortex, they were kept in a hot water bath at 70 °C for 15 minutes. The samples were brought to room temperature and a pink color was formed with maximum absorption at a wavelength of 532 nm in the spectrophotometer. The measurement results were recorded as nmol/ml (Gutteridge, 1995; Mohsin, 2020).

2.7. *Saccharomyces cerevisiae* Catalase (CAT) Activity Determination

1.4 ml of 30 mM H₂O₂ was added to the blank tube and the tubes containing the culture samples, and 0.1 ml of phosphate buffer was added. 0.1 ml enzyme was added to the tubes containing only the culture samples and mixed with vortex. Absorbance values at 240 nm wavelength were read in the spectrophotometer at intervals of 30 seconds and the activity determination results were recorded as U/ml catalase activity (Aebi, 1974; Mohsin, 2020).

2.8. *Saccharomyces cerevisiae* Total Protein Density Measurements (Bradford)

Total protein changes in the groups were performed at 595 nm (OD₅₉₅) according to the Bradford method in spectrophotometer (Aslan *et al.*, 2019b). Bovine serum albumin (BSA) standards were plotted to measure total protein concentrations in cultures against this standard value (Bradford, 1976; Beyaz *et al.*, 2020).

2.9. Statistical Analysis

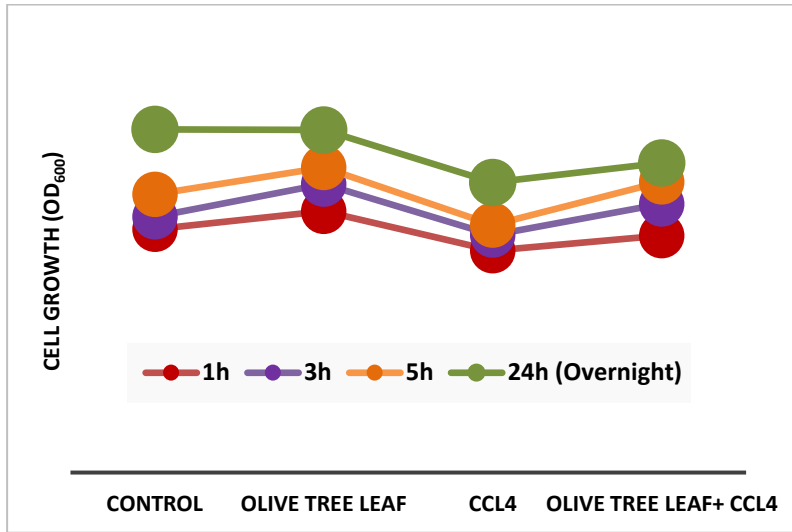
One Way Anova *Post Hoc* LSD test was used to determine the differences between the groups. SPSS 22 package program was used for statistical calculations.

3. RESULTS

As a result of the experimental analysis, it has been determined that the olive tree leaf has therapeutic effects in the treatment of many diseases thanks to its highly antioxidant effects. When the results are examined, it is observed that there is an important differentiation between the groups with distinct developmental times as can be seen in [Figure 1](#) ($p < 0.05$). It

was determined that the olive tree leaf transferred to the culture medium increased cell growth and reduced oxidative damage.

Figure 1. Development of *S. cerevisiae* at different time intervals.



When the total protein results given in Table 1, Table 2, Table 3, Figure 2, Figure 3 and Figure 4 are examined, we see that olive tree leaf promotes protein synthesis in *S. cerevisiae*. In addition, it was determined that total protein levels increased significantly in the Olive Tree Leaf (10%) + CCl₄ (15 mM) group compared to those in the CCl₄ group.

Table 1. *S. cerevisiae* supernatant protein density.

Groups (Supernatant)	Total protein levels (nmol/ml)
Control	1.25 ± 0.73 ^b
Olive Tree Leaf	1.37 ± 0.81 ^a
CCl ₄	0.81 ± 0.19 ^d
Olive Tree Leaf + CCl ₄	1.02 ± 0.64 ^c

a-d: There are statistical differences between groups ($p < 0.05$).

Table 2. *S. cerevisiae* pellet protein density.

Groups (Pellet)	Total protein levels (nmol/ml)
Control	2.55 ± 0.73 ^b
Olive Tree Leaf	2.80 ± 0.82 ^a
CCl ₄	1.97 ± 0.51 ^d
Olive Tree Leaf + CCl ₄	2.09 ± 0.69 ^c

a-d: There are statistical differences between groups ($p < 0.05$).

Table 3. Cell development of *S.cerevisiae* at 1h, 3h, 5h, and 24h time intervals.

Groups	1 hour	3 hours	5 hours	24 hours (Overnight)
Control	1.470 ± 0.81 ^d	1.544 ± 0.86 ^c	1.680 ± 0.89 ^b	2.072 ± 1.06 ^a
Olive Tree Leaf	1.578 ± 0.86 ^d	1.742 ± 0.88 ^c	1.842 ± 0,93 ^b	2.068 ± 1.08 ^a
CCl ₄	1.340 ± 0.63 ^d	1.434 ± 0.67 ^c	1.495 ± 0.70 ^b	1.752 ± 0,77 ^a
Olive Tree Leaf + CCl ₄	1.430 ± 0.77 ^d	1.622 ± 0.79 ^c	1.755 ± 0.81 ^b	1.869 ± 1.04 ^a

**a,b,c,d There are statistical differences between groups ($p < 0.05$).

Figure 2. *S. cerevisiae* BSA (bovine serum albumin) standard graph.

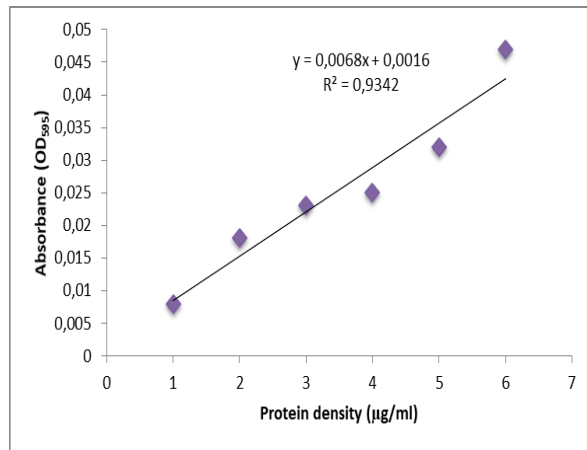


Figure 3. *S. cerevisiae* supernatant protein density.

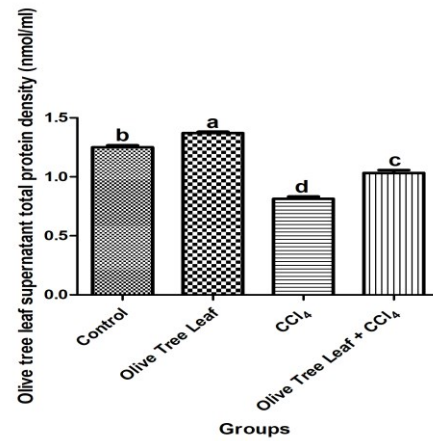
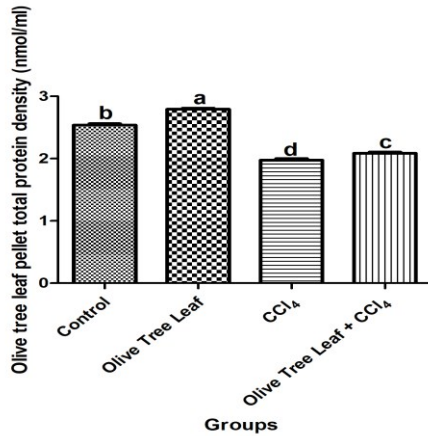


Figure 4. *S. cerevisiae* pellet protein density.



When we investigated the MDA levels as shown in Table 4, Figure 5 and Figure 6, it was determined that the MDA level was the highest in the CCl₄ group, while it was importantly reduced in the Olive Tree Leaf (10%) + CCl₄ (15 mM) group.

Table 4. *S. cerevisiae* MDA levels.

Groups	<i>S. cerevisiae</i> MDA levels (nmol/ml)
Control	2.93 ± 0.76 ^c
Olive Tree Leaf	2.96 ± 0,78 ^c
CCl ₄	6.79 ± 0.98 ^a
Olive Tree Leaf + CCl ₄	4.78 ± 0.81 ^b

a-c: There are statistical differences between groups ($p < 0.05$).

Figure 5. *S. cerevisiae* MDA standard graph.

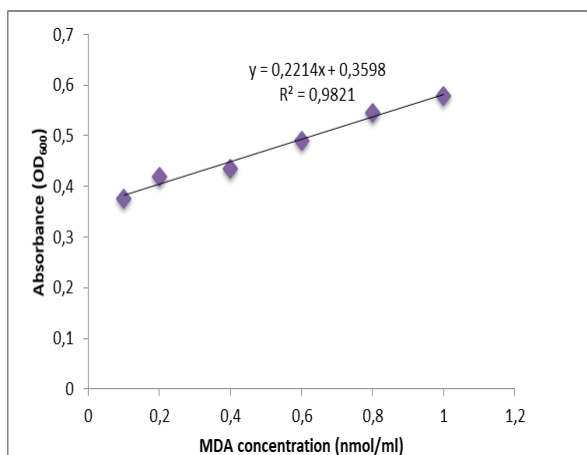
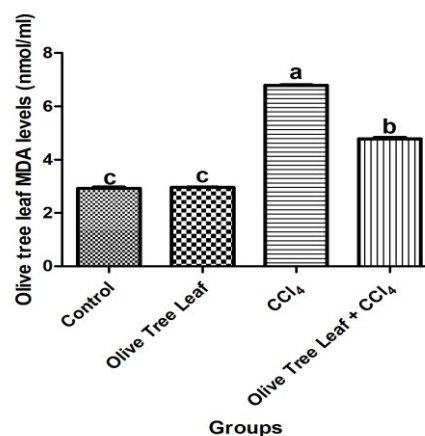


Figure 6. *S. cerevisiae* MDA levels.



When we investigated the CAT levels given in Table 5 and Figure 7, it was observed that CAT activity was the lowest in the CCl₄ group, while it was importantly decreased in the Olive Tree Leaf (10%) + CCl₄ (15 mM) group.

Table 5. *S. cerevisiae* catalase activity.

Groups	<i>S. cerevisiae</i> catalase activity (U/ml)
Control	5.05 ± 1.48 ^b
Olive Tree Leaf	5.84 ± 0.56 ^a
CCl ₄	2.77 ± 0.69 ^d
Olive Tree Leaf + CCl ₄	3.94 ± 1.37 ^c

a-d: There are statistical differences between groups ($p < 0.05$).

When we investigated the GSH levels given in Table 6 and Figure 8, it was observed that the GSH activity was the lowest in the CCl₄ group, while it was importantly reduced in the Olive Tree Leaf (10%) + CCl₄ (15 mM) group.

Table 6. *S. cerevisiae* GSH levels.

Groups	<i>S. cerevisiae</i> GSH levels (µg/ml)
Control	37.6 ± 2.84 ^b
Olive Tree Leaf	41.8 ± 2.70 ^a
CCl ₄	22.3 ± 1.65 ^d
Olive Tree Leaf + CCl ₄	30.2 ± 1.89 ^c

a-d: There are statistical differences between groups ($p < 0.05$).

Figure 7. *S. cerevisiae* catalase activity.

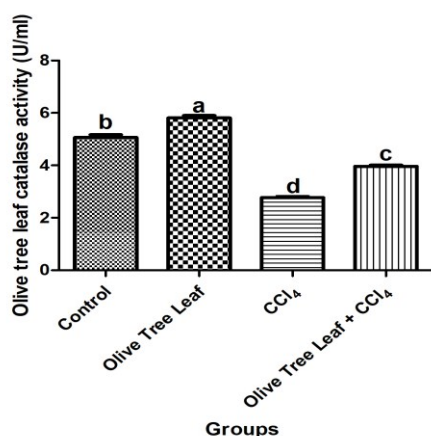
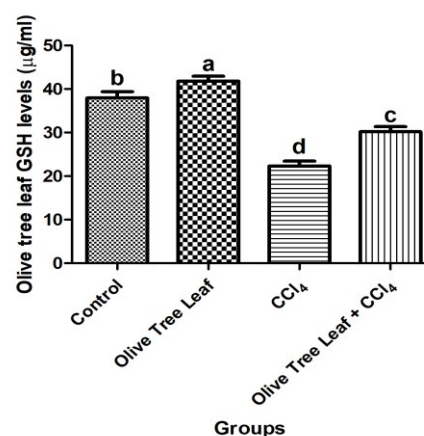


Figure 8. *S. cerevisiae* GSH levels.



In the SDS-PAGE pellet and supernatant gel images in Figure 9 and Figure 10, it was observed that protein density rose importantly in the Olive Tree Leaf (10%) and Olive Tree Leaf (10%) + CCl₄ (15 mM) groups compared to that in the CCl₄ group.

As a result of this study, it was determined that olive tree leaf increased the growth of *S. cerevisiae* by inhibiting the oxidative damage caused by CCl₄.

Figure 9. SDS- PAGE Supernatant Protein Bands. Bands; 1: Marker; 2: Control; 3: Olive Tree Leaf; 4: CCl₄; 5: Olive Tree Leaf + CCl₄.

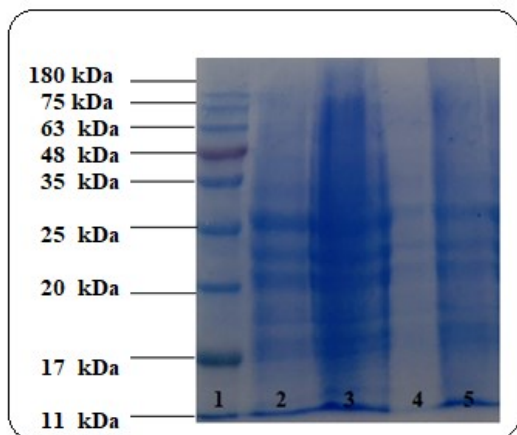
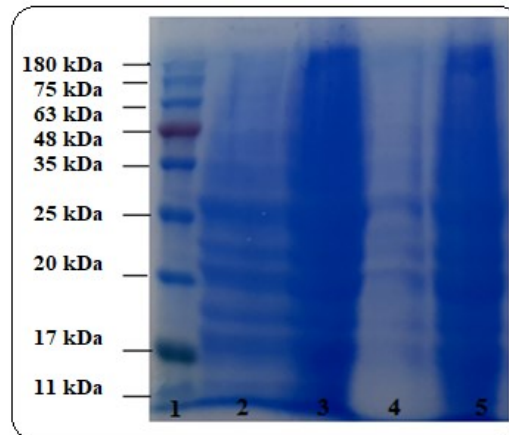


Figure 10. SDS- PAGE Pellet Protein Bands. Bands; 1: Marker; 2: Control; 3: Olive Tree Leaf; 4: CCl₄; 5: Olive Tree Leaf + CCl₄.



4. DISCUSSION and CONCLUSION

Natural plant polyphenols have many benefits on the human body. Bioactive polyphenols are natural compounds of various chemical structures and their main sources are fruits, vegetables, nuts, seeds, plant leaves, whole grain products, tea, and coffee. Studies have shown that polyphenols reduce morbidity and slow down the development of cancer as well as cardiovascular and neurodegenerative diseases. Moreover, the biological activity of polyphenols is strongly correlated with their antioxidant properties. In addition to scavenging free radicals and reactive oxygen species, they also have a tendency to neutralize potentially carcinogenic metabolites (Gorzynik-Debicka *et al.*, 2018). Olive tree leaf (*Olea europaea* L.), rich in plant polyphenols, is known to have such biological activities as antioxidant, anti-inflammatory, antiallergic, antiatherogenic, antithrombotic, and antimutagenic. Scientific studies have revealed that olive tree leaf has the ability to modulate the human immune system by influencing the production of cytokines or other factors involved in immunological defense in addition to proliferating white blood cells (Gorzynik-Debicka *et al.*, 2018; Borjan *et al.*, 2020).

Susalit *et al.* (2011) also investigated the hypolipidemic effects of olive tree leaf in hypertension patients and found that 500 mg/kg olive tree leaf application twice a day for 8 weeks was highly effective in lowering systolic and diastolic blood pressure. Gokce (2020) determined that pistachio (*Pistacia vera* L.) extract significantly increased GSH level and CAT activities by decreasing MDA and SOD levels against CCl₄-induced oxidative stress in *S. cerevisiae*. Lockyer *et al.* (2017) reported the effect of phenolic-rich olive tree leaf extract on blood pressure, plasma lipids, and inflammatory markers and stated that olive tree leaf application showed hypotensive and lipid-lowering effects. Bock *et al.* (2013) also found that olive tree leaf polyphenols increased insulin sensitivity in middle-aged and overweight men.

Somerville *et al.* (2019) concluded that the administration of olive tree leaf (100 mg/kg oleuropein) regulates the immune system and blood circulation in upper respiratory tract patients. Ferdousi *et al.* (2019) compared the effects of olive tree leaf tea and green tea on

hematological parameters and pinpointed the application of olive tree leaf tea has a preventive effect against anemia and other red blood cell disorders. Araki *et al.* (2019) evaluated the beneficial effects of olive tree leaf on dyslipidemia, type 2 diabetes, and obesity and found that olive tree leaf significantly reduced serum levels of triglycerides and low-density lipoprotein cholesterol. Wong *et al.* (2014) concluded that consuming a combination of olive tree leaf, green coffee bean, and beet extracts regularly has ameliorative effects on blood pressure, arterial compliance, blood lipids, blood sugar, and insulin sensitivity.

Markopoulos *et al.* (2009) evaluated the intraluminal stability of oleuropein, an important polyphenol of olive tree leaf, in human stomach and small intestine contents and stated that oleuropein application balances the pH of the environment by inhibiting reactive oxygen species in the environment. Ahmed *et al.* (2021) reported that olive leaf treatment against testicular tissue damage caused by lead acetate in rats provided a significant increase in GSH, SOD, and CAT activities as they concluded that treatment with lead acetate + olive leaf showed healing effects on oxidative stress activities and antioxidant parameters. Jamnik *et al.* (2007) investigated the protective effects of royal jelly treatment in *S. cerevisiae* and stated that royal jelly positively affects growth and metabolic energy activity in the cell in a growth phase-dependent manner by reducing intracellular oxidation in a dose-dependent manner. In addition, Jamnik *et al.* (2007) determined that royal jelly increased protein expression by acting as a scavenger of reactive oxygen species in the cell. Larussa *et al.* (2017) reported that the application of oleuropein, an olive tree leaf polyphenol, reduced the expression of cyclooxygenase-2 and interleukin-17 and inflammatory damage in ulcerative colitis patients. Cicco *et al.* (2020) reported that olive tree (*Olea europaea* L.) leaf extract reduced obesity-induced inflammation by stimulating it with high dose free fatty acid palmitate.

Kaiidi *et al.* (2019) investigated the effect of oleuropein, the major component of olive tree wool, on oxidative stress and inflammation against kidney damage caused by ureteral obstruction in rats and reported that oleuropein administration reduces oxidative stress by modulating inflammatory parameters. Moreover, Kaiidi *et al.* (2019) determined that oleuropein has antioxidative stress, antiapoptotic and anti-inflammatory effects as well as a renoprotective effect. Aslan (2021) stated that Goji berry extract increased the cell growth of *S. cerevisiae* by eliminating chromium-induced oxidative damage in *S. cerevisiae* culture. Perrinjaquet-Mocchetti *et al.* (2008) concluded that olive tree leaf extract has antihypertensive and cholesterol-lowering properties by regulating blood pressure in hypertensive patients. Oprea *et al.* (2014) determined that bilberry extract has a chemoprotective effect against cadmium-induced toxicity in *S. cerevisiae*.

Pereira *et al.* (2007) identified phenolic compounds by HPLC-DAD analysis of caffeic acid, verbascoside, oleuropein, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside, and luteolin 4'-O-glucoside of olive leaf aqueous extract. Quantification of phenolics in the aqueous extract revealed high amount of these compounds and determined that they were superior to the previously found values for hydromethanol extracts of the same and other olive leaf varieties. In addition, Pereira *et al.* (2007) reported that the aqueous extract, oleuropein, was the compound with the highest amount, unlike the hydromethanol extracts, where flavonoids were the main compounds and further stated that caffeic acid is approximately the corresponding minor compound. Kiruthik and Padma (2013) stated that *Zea mays* leaf extract has a strong antioxidant effect on *S. cerevisiae* against H₂O₂-induced oxidative stress.

Javadi *et al.* (2019) evaluated that olive tree leaf therapy significantly reduced inflammation, which is the main cause of hypertension, in hypertensive patients. Beyaz *et al.* (2021a) stated that *Curcumin* treatment increased GSH levels and decreased MDA levels compared to H₂O₂ group. Chen *et al.* (2019) stated that EGCG can facilitate glycolysis and redox balance of *S. cerevisiae* by attenuating the damage caused by ethanol on the cell wall and cell

membrane. Malfa *et al.* (2021) investigated that olive tree leaf has a very rapid and effective activity to improve gastrointestinal system symptoms. Gok *et al.* (2021a) found that persimmon leaf has a therapeutic effect by increasing *S. cerevisiae* cell growth and promoting protein synthesis. Elkarawy *et al.* (2020) stated that *Hibiscus sabdariffa* L. and olive tree leaves have very strong antihypertensive effects. Similarly, Beyaz *et al.* (2021b) found out that EGCG treatment showed biological activities such as antioxidant, antimicrobial and anti-inflammatory. Gok *et al.* (2021b) stated that ellagic acid reduces oxidative damage in yeasts, increases cell growth, and has a protective effect by promoting protein synthesis.

Historically, olive tree leaf have been used in the treatment of many diseases since ancient civilizations. Olive tree leaf, which are part of natural medicine, contain polyphenolic compounds with various bioactive properties, and their use in alternative medicine has become an increasing product. Both inflammatory and cancer cell models suggest that olive tree leaf polyphenols have anti-inflammatory roles against free radical initiated DNA damage. According to the results, it was determined that olive tree leaf significantly increased cell growth by promoting total protein synthesis in *S. cerevisiae*.

In our study, it was determined that CAT activity, which is one of the antioxidant defense mechanism markers, increased in the groups with olive tree leaf added compared to the groups in which CCl₄ was added, while the levels of MDA, which is a marker of oxidative stress in the cell, decreased significantly. Our results, which support the existing studies in the literature, make us think that olive tree leaf may be a reasonable drug for preventing the progression and development of various cancer types as well as many diseases.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Seda Beyaz: Investigation, resources and writing original draft. **Ozlem Gok:** Investigation and writing original draft. **Abdullah Aslan:** Reading and editing of article.

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