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Original article

Does persimmon leaf have a protective effect against oxidative damage caused by chromium in Saccharomyces cerevisiae?

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Summary. In this study, 4 groups were formed. Groups; (i) Control group; (ii) Persimmon Leaf Group (10%); (iii) Chromium ($K_2Cr_2O_7$) Group (10 mM); (iv) Persimmon Leaf (10%) + Chromium ($K_2Cr_2O_7$) (10 mM) Group. *Saccharomyces cerevisiae* (*S. cerevisiae*) cultures were grown at 30 °C for 1 hour, 3 hours, 5 hours and 24 hours. Cell growth, lipid peroxidation, MDA (malondialdehyde) analyzes, glutathione (GSH) levels and catalase activities were determined by spectrophotometer. Total protein changes were detected by SDS-PAGE electrophoresis and calculated by the Bradford method. According to the results obtained; cell growth (1, 3, 5 and 24 hours), total protein synthesis (24 hours), GSH levels (24 hours) and catalase activities (24 hours) in the Persimmon Leaf + Chromium group compared to the Chromium group while increasing, MDA level (24 hours) decreased. These results show that persimmon leaf reduces oxidative damage, enhances cell growth and it has a protective effect to promote protein synthesis in *S. cerevisiae* culture.

Key words: Cell development, oxidative damage, persimmon leaf, *Saccharomyces cerevisiae*, SDS-PAGE

Introduction

Diospyros kaki Ebenales order, also known as persimmon or paradise palm, is included in the family Ebenaceae, *Diospyros* genus. Although it is a subtropical climate fruit, it is a species that has adapted to hot temperate climate conditions. It is more resistant to cold compared to other subtropic plants because it shed leaves in winters. Persimmon fruits are very rich in ascorbic acid and phenolic compounds. This fruit has high antioxidant activity due to its rich carbohydrate and tannin content in vitamins A and E (1,2). It is an important fruit variety in terms of nutrition and at the same time, it is a fruit that can respond to the search for change in people's taste buds with its color, appearance and bitter taste. It is a fruit preferred by the consumer with its unique shape, color, taste and bioactive components in its content (3).

Although the palm fruit is eaten fresh or dried, its leaves are generally used for tea due to its functional properties. In Japan, palm leaves are brewed with hot water, and are drunk as green tea (kakinoha - cha) for its healing effects in freezing, paralysis, burns and bleeding-stopping conditions. Persimmon leaf contain numerous bioactive compounds such as phenols, tannins, flavonoid oligomers, ascorbic acid, caffeine and chlorophyll. In particular, flavonoids in persimmon leaf, including kaempferol, quercetin and catechin, exhibit antioxidant activities and are beneficial in balancing blood pressure. In addition, quercetin exhibits pharmacological activities such as anti-cancer, anti-allergic and anti-inflammatory. Kaempferol shows preventive activity against Alzheimer's disease**.** Persimmon leaf have become popular not only in medicinal use but also in the cosmetic, pharmaceutical and food industries due to their effects on the skin. East Asian people incorporated persimmon leaf into their rice cake, cookies, athlete's foot socks and soaps, and also used it as an ingredient for sushi because of its anti-microbial properties (4-6).

In this study, 4 different groups were formed to examine the oxidative damage caused by the $K_2Cr_2O_7$ oxidant substance. The effects of persimmon leaf on cellular development and protein expression in *S. cerevisiae* were studied. We believe that the results we obtained in this study will make an important contribution to the current literature.

Materials and Methods

Research groups

In this study, the protective effect of persimmon leaf was investigated against the damage caused by chromium in *S. cerevisiae*. 4 groups were formed in the study. Groups; (i) Control group; (ii) Persimmon Leaf Group (10%); (iii) Chromium ($K_2Cr_2O_7$) Group (10 mM); (iv) Persimmon Leaf (10%) + Chromium ($K_2Cr_2O_7$) (10 mM) Group.

Application of persimmon leaf and $K_2Cr_2O_7$ to the culture

The development environment of *S. cerevisiae*: YEPD (for 250 ml; 7.5 g yeast extract, 7.5 g tryptone, 7.5 g glucose) was prepared for the growth and spread of the yeast. Then 5 flasks were taken and 50 ml of 250 ml medium were added to each flask. After waiting at 121^0C in the autoclave for 1 hour, it was removed and cooled. In addition to the burner flame, 800 µl of yeast was planted in each flask. After waiting in the oven for 20 minutes, blind measurement was made. For the preparation of 10% persimmon leaf; 10 g persimmon leaf were weighed. It was brewed in 100 ml of boiling distilled water for 10-15 minutes. It was then used by filtration through a sterile cheesecloth. Then, $K_2Cr_2O_7$ and the persimmon leaf were added to the other cones removed from the oven. According to the content of the groups, 10 ml of persimmon leaf and 0.30 grams of $K_2Cr_2O_7$ were added and developed at 30°C (7).

Cell development measurements

Culture samples were developed at 30 °C for 1 hour, 3 hours, 5 hours and 24 hours (overnight) and measured using a spectrophotometer at 600 nm (OD_{600}) wavelength.

Protein isolation for SDS-PAGE (Sodium dodecyl sulfate - polyacrylamide gel electrophoresis)

After taking 1 ml of the culture samples and centrifuging at 13000 rpm for 5 min. the pellets were dissolved in 500 µl TEA (Tris EDTA Acetic acid) (pH: 7.5). The cells were kept in ice for 5 min. after being disintegrated twice at power 2 for 10 seconds with a sonicator (Bandelin Sonopuls, Germany). Then it was centrifuged at 13000 rpm for 10 min. and pellets were removed. For SDS-PAGE studies, an equal amount of sample was mixed with the staining solution and made ready to use for electrophoresis (8).

SDS-PAGE analysis

Protein samples of *S. cerevisiae* cultures were boiled for 5 min. after the addition of an equal amount of SDS-PAGE SAB (Sample amplification buffer) dye before loading into the wells. 1 x tank buffer was used for electrophoresis and a current of 30 mA was applied until the blue band of the dye (bromophenol blue), which allows the movement of proteins in the gel to be monitored, reached the end of the gel. Then, the protein bands in the gel were washed with a dye remover solution until the protein bands became visible and the protein bands between the groups were examined by taking the gel images (8,9).

Total protein density measurements (Bradford)

BSA (bovine serum albumin) protein standards were obtained at different concentrations using BSA protein. The total amount of protein in the *S. cerevisiae* groups (24 hours) corresponding to this standard value was calculated. Total protein density (pellet and supernatant) were determined using spectrophotometer at 595 nm (OD_{595}) according to the bradford method (7).

MDA (malondialdehyde) analysis

MDA analysis, 0.5 µl sample was added to test test tube and 0.5 ml distilled water was added to blind tube and then 2.5 ml of 20 % trichloroacetic acid and 1 ml of TBA (Thiobarbituric Acid) were added to all test tubes. Then, after waiting 30 min. in a boiling water bath at 90 °C, it was cooled. After adding 4 ml of

Table 1. *S. cerevisiae* cell growth in persimmon leaf

Groups	1h	3h	5h	24h (Overnight)
Control	1.00 ± 0.02[d]	1.22 ± 0.02[c]	1.52 ± 0.02[b]	1.90 ± 0.03[a]
Persimmon **Leaf**	1.44 ± 0.02[d]	1.27 ± 0.02[c]	1.77 ± 0.02[b]	2.09 ± 0.02[a]
Chromium ($K_2Cr_2O_7$)	1.10 ± 0.02[d]	1.20 ± 0.02[c]	1.35 ± 0.02[b]	1.57 ± 0.02[a]
Persimmon **Leaf + Chromium ($K_2Cr_2O_7$)**	1.63 ± 0.02[d]	1.74 ± 0.02[c]	1.85 ± 0.02[b]	2.10 ± 0.02[a]

a-d: among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

n-butanol-pyridine mixture and vortexing, it was centrifuged at 4000 rpm for 15 min. At the end of this process, the upper phase part was taken and 532 nm wave measurement was made in the spectrophotometer and the results were recorded as nmol/ml (10,11).

Catalase activity determination

For catalase activity, two tubes were taken and 1.4 ml of 30 mM H_2O_2 (hydrogen peroxide) was added to the blank tube and 0.1 ml of phosphate buffer was added on it. 1.4 ml of 30 mM H_2O_2 and 0.1 ml of enzyme were added to the sample tube and mixed with vortex. Absorbances was read at 240 nm twice at 30 seconds intervals and thus the activity was determined (12).

GSH (Glutathione) levels measurement

0.1 ml of culture sample was taken. 0.4 ml of 10 % trichloroacetic acid solution was added and vortexed. After centrifuging at 3000 rpm for 5 min., the supernatant was taken and the pellet discarded. 0.1 ml of supernatant was taken into a clean tube and 0.9 ml of distilled water, 2 ml of 0.4 M pH: 8.9 Tris buffer and 0.1 ml of DTNB (5,5′-dithiobis (2-nitrobenzoic acid) solution were added. The resulting yellow color was read against distilled water at a wavelength of 412 nm in the spectrophotometer (13).

Statistical analysis

The statistical analysis of the data we obtained as a result of our studies was evaluated with the analysis of variance in the SPSS 22 package program. One Way Anova Post Hoc Tukey LSD tests were used to determine differences between groups. In terms of the reliability of the statistical analysis of our studies, all measurements were made in 3 repetitions.

Results

Cell growth measurement results in S. cerevisiae

In Table 1 and Figure 1A, there is a significant difference between the groups depending on the development times at different times Cell growth increased in the Persimmon Leaf + Chromium and Persimmon Leaf groups compared to the Chromium damage group ($p < 0.05$).

Total protein density (Bradford) measurements

When the total protein results are given in Table 2,3, Figure 1B, Figure 1C and Figure 1D examined, we can say that the persimmon leaf increases protein synthesis in *S. cerevisiae*. Especially when compared with the chromium group, it is seen that protein synthesis increases in the Persimmon Leaf + Chromium group ($p < 0.05$).

S. cerevisiae MDA analysis results

When Table 4, Figure 1E MDA levels were examined, it was revealed that the highest MDA levels were in the chromium group and that it was significantly decreased in the Persimmon Leaf + Chromium group ($p < 0.05$).*S. cerevisiae GSH analysis results*

When we examine the GSH levels given in Table 5 and Figure 1F is seen that the lowest GSH level in was in the chromium group and it was significantly decreased in the Persimmon leaf + Chromium group ($p < 0.05$).

S. cerevisiae Catalase activity analysis results

When we examine the catalase activity levels given in Table 6 and Figure 1G It is seen that the lowest

catalase level in 1G was in the chromium group and it was significantly decreased in the Persimmon Leaf + Chromium group ($p < 0.05$).

S. cerevisiae SDS-PAGE analysis

SDS-PAGE gel images show that the protein concentration increased significantly in the Persimmon Leaf + Chromium group compared to the chromium group. As a result of this study, it was concluded that the persimmon leaf increased the development of *S. cerevisiae* despite the negative effects of chromium (Figure 2).

Discussion

We anticipate that the results obtained in this study, in which the protective effect of persimmon leaf is investigated will be a reference for future studies

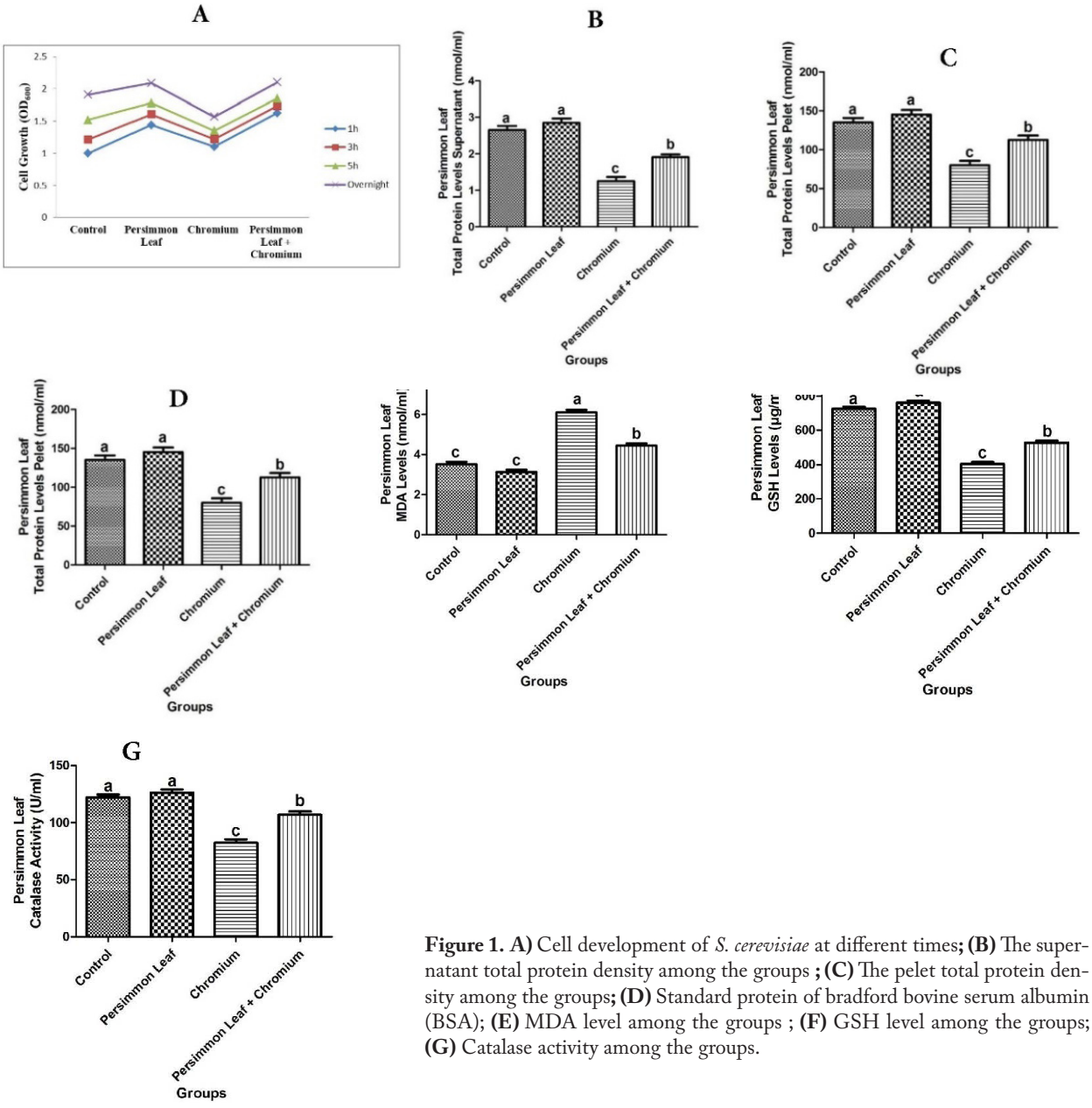

Figure 1. A) Cell development of *S. cerevisiae* at different times**; (B)** The supernatant total protein density among the groups **; (C)** The pelet total protein density among the groups**; (D)** Standard protein of bradford bovine serum albumin (BSA); **(E)** MDA level among the groups ; **(F)** GSH level among the groups; **(G)** Catalase activity among the groups.

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

Groups (Pellet)	*S. cerevisiae* Total Protein Density (nmol/ml)
Control	135.20 ± 2.00[a]
Persimmon Leaf	145.32 ± 2.00[a]
Chromium ($K_2Cr_2O_7$)	80.05 ± 2.00[c]
Persimmon Leaf + Chromium ($K_2Cr_2O_7$)	112.55 ± 2.00[b]

a-c: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Table 3. *S. cerevisiae* bradford supernatant protein density

Groups (Supernatant)	*S. cerevisiae* Total Protein Density (nmol/ml)
Control	2.65 ± 2.00[a]
Persimmon Leaf	2.85 ± 2.00[a]
Chromium ($K_2Cr_2O_7$)	1.25 ± 2.00[c]
Persimmon Leaf + Chromium ($K_2Cr_2O_7$)	1.96 ± 2.00[b]

a-c: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

Table 4. *S. cerevisiae* MDA Levels

Groups	S. cerevisiae MDA Levels (nmol/ml)
Control	3.52 ± 2.00[c]
Persimmon Leaf	3.13 ± 2.00[c]
Chromium ($K_2Cr_2O_7$)	6.11 ± 2.00[a]
Persimmon Leaf + Chromium ($K_2Cr_2O_7$)	4.44 ± 2.00[b]

a-c: Among the groups which bearing of different letter are significant (p<0.05). One-way Anova Post Hoc LSD test

Table 5. *S. cerevisiae* GSH Levels

Groups	*S. cerevisiae* GSH Levels (µg/ml)
Control	725.88 ± 2.00[a]
Persimmon Leaf	761.47 ± 2.00[a]
Chromium ($K_2Cr_2O_7$)	403.52 ± 2.00[c]
Persimmon Leaf + Chromium ($K_2Cr_2O_7$)	528.23 ± 2.00[b]

a-c: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

and will make important contributions to the current literature. In addition, the limited use of persimmon leaf on *S. cerevisiae* is important for the originality of the study. Persimmon is widely grown in East Asian countries, including Japan, China and Korea. Persimmon fruits are often used as a variety of foods, while its leaves are used in traditional Chinese medicine to quench chronic ulcers, hemostasis, cough and thirst. The dried leaves are also consumed as green tea in many communities. Persimmon leaf are abundant in medicinal compounds such as carotenoids, terpenoids, tannins, vitamins, sterols, organic acids, fatty acids and flavonoids. These phytochemicals show many pharmacological effects such as anti-oxidant, anti-cancer, anti-hyperlipidemia, anti-inflammatory, anti-bacterial, anti-hypertensive, anti-arthritis and myocardial protective (14,15).

Table 6. *S. cerevisiae* CAT Levels

Groups	*S. cerevisiae* CAT Activity (U/ml)
Control	122.85 ±2.00[a]
Persimmon Leaf	126.23 ±2.00[a]
Chromium ($K_2Cr_2O_7$)	81.97 ± 2.00[c]
Persimmon Leaf + Chromium ($K_2Cr_2O_7$)	106.82 ±2.00[b]

a-c: Among the groups which bearing of different letter are significant (p<0.05). One way Anova Post Hoc LSD test

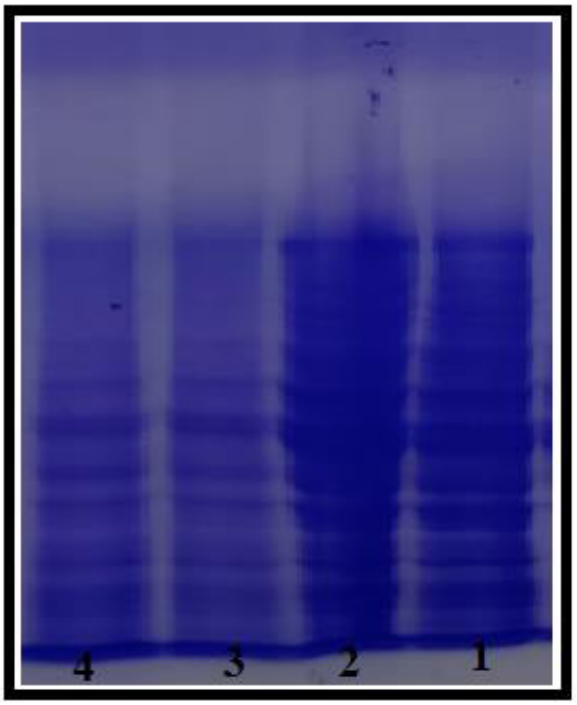

Figure 2. SDS-PAGE pelet total protein bands profiles for development at 30 °C. Lanes 1: Control 2:Persimmon Leaf 3:Chromium 4: Persimmon Leaf + Chromium

As a result of this study, it was determined that persimmon leaf have protective effects against oxidative damage. In our study using grape seed to previous *S. cerevisiae* cultures, we can say that the grape seed extract transferred to the culture medium increases cell growth against the negative effect of hydrogen peroxide (8). In another study, when we examine the total protein results of tomato extract against chromium damage created in *S. cerevisiae* we can state that tomato extract increases protein synthesis in *S. cerevisiae* (16). In another study we conducted using pomegranate juice, we can say that the harmful effects of hydrogen peroxide oxidant minimized with pomegranate juice and that pomegranate juice has a protective role in reducing the oxidative damage in *S. cerevisiae* (17). As a result of this study, it was concluded that persimmon leaf also increase protein synthesis and cell development in *S. cerevisiae*.

Beyaz et al. (18) they compared the protective effect of black mulberry (*Morus nigra* L.) and cranberry (*Cornus mas* L.) in terms of molecular biological and biochemical parameters. They stated that MDA level decreased while cell development and total protein synthesis increased in the Black Mulberry + Cranberry + H_2O_2 group (1, 3, 5 and 24 hours) compared to the H_2O_2 group. Kim et al. (19) investigated the inhibitory effect of the persimmon on mast cell mediated hypersensitivity and the underlying mechanism of action. They stated that the concentration of *Diospyros kaki*, through the reduction of intracellular calcium in mast cells, suppressed histamine release dependently and decreased the expression of Nf-ϰB, tumor necrosis factor-α and IL-4. Liu et al. (20) investigated the effect of Curcumin and Astragalus administration in diabetic rats, they stated that it increased GSH levels by decreasing oxidative stress and MDA levels. Aslan et al. (7) stated that the Kiwi extract reduce oxidative damage in *S. cerevisiae* culture and it has a protective role to increase cell growth and protein synthesis. Aslan (21) indicated that the mulberry extract increased cell growth in *S. cerevisiae* by providing significant protection against hydrogen peroxide damage. In our results, we obtained similar results to these studies. It was concluded that persimmon leaf increase the GSH level and catalase activity. Kim et al. (22) stated that *Diospyros kaki* leaves cause cancer cell death and prevent their reproduction. Aslan et al. (23) investigated the potential effect of ellagic acid (EA) in the treatment of pancreatic injury. They found that Nrf-2 and caspase-3 protein expressions, catalase activities and GSH levels increased, TNF-α, NF-ϰB, Bcl-2, VEGF and Akt protein expressions and MDA levels decreased in the EA + CCl_4 group. Sun et al. (4) evaluated the antioxidant activity of total flavonoid extract obtained from persimmon leaf. In their results, they stated that the extract has a strong total antioxidant activity and it scavenges superoxide anion and hydroxyl radicals. Matsumoto et al. (24) investigated the hypolipidemic effects and bile acid binding properties of persimmon fruit. They noted that persimmon intake increased bile acid excretion and reduced the concentration of hepatic lipids and plasma cholesterol. In one study, the protective role of ellagic acid in rats with CCl_4-induced lung injury was investigated. As a results, TNF-α, NF-ϰB and bcl-2 expression levels increased, caspase-3, Nrf-2 and COX-2 expression levels decreased significantly in CCl_4 groups compared to EA-treated groups ($p < 0.05$) (25).

In another study they found that TNF-α, NF-ϰB, COX-2 and bcl-2 protein expression decreased MDA levels and increased GSH levels and catalase activities in the group given ellagic acid compared to CCl_4 group (26). Choi et al. (14) studied the role of persimmon leaf in protecting diabetes-related kidney damage in mice with type-2 diabetes. They stated that the persimmon leaf significantly lowers hydrogen peroxide and lipid peroxide levels in the kidney and the it activates of superoxide dismutase, catalase and glutathione peroxidase and their related genes increase mRNA expression. Zhou et al. (27) stated that ellagic acid has potential preventive and therapeutic effects on cardiovascular, neurodegenerative diseases and chronic diseases. Aslan et al. (10) the protective role of ellagic acid in rats with liver damage with carbon tetrachloride has been investigated. They found that ellagic acid reduced MDA levels. The protective role of ellagic acid has been investigated in rats with liver damage exposed with carbon tetrachloride in another study. They found that ellagic acid reduced MDA levels. Miao et al. (28) investigated that the persimmon leaf flavonoid decreased inflammatory reactions, vascular endothelial damage and increased ischemic tolerance, while high-

dose palm leaves had the same effect as flavonoid and ginatone. These results found that persimmon leaf increased brain ischemic tolerance in mice. Kalra et al. (29) investigated the nephroprotective effect of *Terminalia chebula* on cisplatin-induced nephrotoxicity in rats. They stated that TNF-α levels showed a dose-dependent decrease as a result of treatment with *Terminalia chebula* compared to the cisplatin. Babele et al. (30) found that zinc oxide nanoparticles induced toxicity in *S. cerevisiae* by affecting cell wall integrity and lipid homeostasis. Aslan et al. (31) studied the neuroprotective effect of EA against brain damage caused by CCl_4 in rats. They found that Nrf-2 and caspase-3 protein expression increased GSH levels and catalase activities in the group treated with ellagic acid. Vazquez et al. (32) stated that melatonin reduced oxidative stress damage caused by hydrogen peroxide in *S. cerevisiae.* Guan et al. (33) concluded that selastrol, which has antioxidant and anti-inflammatory effects, reduces the oxidative stress in skeletal muscle that occurs in diabetic rats. Aslan et al. (13) evaluated the effects of ellagic acid on antioxidative and anti-enflammation pathways in kidney damage. They found that EA significantly reduced lipid peroxidation, increased glutathione levels and catalase activity significantly. Ozsahin et al. (34) indicated that different sugar sources has positive effects on *S. cerevisiae* cell growth, additionally Aslan et al. (35) indicated that milk thistle has positive effect on rat liver damage and Aslan et al. (36) point out that black cumin has inhibition effects against lung damage in rats. On the other hand Aslan (37) incated that strawberry and banana juices have protective effects against H_2O_2 damage in *S. cerevisiae*.

Conclusion

Persimmon leaf provides effective protection against oxidative stress and it has been concluded that this compound suppresses lipid peroxidation products by reducing oxidative stress. The results of this study reveal that the persimmon leaf supports total protein synthesis and increases the cell. In addition, it was found that in groups treated with persimmon leaf, GSH levels, cell growth, total protein density and protein synthesis increased but MDA levels were significantly decreased relative to the chromium group. The results of this study are thought to contribute to the existing studies in the literature on the effect of persimmon leaf on *S. cerevisiae* growth and will be guide future studies.

Acknowledgments

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Conflicts of interest

There is no conflicts of interest between the authors.

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