

Determination of The Promoting Role of Oleaster Fruit in Protein Synthesis by SDS-PAGE and Some Biochemical Analysis

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Abstract. *Background and aim:* Oleaster fruit (*Elaeagnus angustifolia*) have been used in traditional medicine to treat various diseases. In this study, the effect of oleaster fruit extract on oxidative damage in culture of *Saccharomyces cerevisiae* (*S. cerevisiae*) caused by copper chloride (CuCl₂) was investigated. *Methods:* In this study, 4 groups were formed. Groups; (i) Control group; (ii) Oleaster Fruit (8%) Group; (iii) CuCl₂ (30 mM) Group; (iv) Oleaster Fruit (8%) + CuCl₂ (30 mM) Group. *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 Lowry method. *Results:* According to the results obtained; oleaster fruit extract added to *S. cerevisiae* cultures showed cell growth (1, 3, 5 and 24 hours), total protein synthesis (1 hour, 3 hours, 5 hours and 24 hours), GSH levels (24 hours) and catalase activities (24 hours) and decreased the MDA level (24 hours). *Conclusions:* These results show that the oleaster fruit extract reduces oxidative damage, enhances cell growth and has a protective effect to promote protein synthesis in *S. cerevisiae* culture.

Key words: copper chloride, oleaster fruit, protein, *Saccharomyces cerevisiae*, SDS-PAGE

Introduction

Elaeagnus angustifolia is commonly found in Elaeagnaceae family and is known as Russian olive, wild olive or silver strawberry. Asia, Europe, Turkey and Central Asia as a large geographical area is grown widely. It is used as a food or herbal medicine because of its many medicinal properties. Especially its fruits and flowers are used in the treatment of common diseases such as cough, nausea, asthma, jaundice, fever and diarrhea (1,2). Also, the fruit is known to have various pharmacological effects such as anti-inflammatory, anti-oxidant, anti-tumor, anti-mutagenic, anti-fungal, anti-bacterial and gastroprotective effects (3).

Oleaster which grows rapidly up to 10 m in height and 30 cm in diameter has small reddish brown,

elliptical shaped fruits. It is a perennial tree that can tolerate a wide range of hard environmental conditions such as severe drought, stony, sandy and high salinity soils. Oleaster fruits in needles contain various functional components such as polysaccharides, amino acids, carotenoids, vitamins, phenolic acids and flavonoids. Different parts of the plant have been used as raw materials for functional food and new medicines in various medical formats, perfume industries, wood-working, musical instruments production (4,5). It is grown as an ornamental plant by people in Europe and America due to its low disease and insect problems, and as a fruit tree in Anatolia due to its sweet fruits in vineyards and gardens. At the same time, it is an ornamental plant especially preferred in gardens due to the very pleasant smell of its spring flowers (6).

S. cerevisiae is a microorganism widely used in beer and bread making as a cellular model to determine the antioxidant capacity of foods and beverages. It is used as a model organism to understand the molecular basis of eukaryotic cell functions. Because this model organism creates cellular response to oxidative stress and defense functions involved in this response (7,8). Since their genetic structure and cellular properties are well known, their use continues to spread every day and therefore it is one of the most preferred microorganisms in scientific studies (9,10).

The aim of this study is to investigate the protective effects of the oleaster fruit extract against copper chloride (CuCl_2) induced oxidative damage in *S. cerevisiae*. The effects of oleaster fruit extract on cellular development and protein expression in *S. cerevisiae* were investigated.

Materials and Methods

Research Groups

In this study, the oxidative effect of the oleaster fruit extract against the damage caused by copper chloride (CuCl_2) in *S. cerevisiae* was investigated. 4 groups were formed in the study. Groups; (i) Control group; (ii) Oleaster Fruit (8%) Group; (iii) CuCl_2 (30 mM) Group; (iv) Oleaster Fruit (8%) + CuCl_2 (30 mM) Group.

Application of Oleaster Fruit Extract and CuCl_2 to *S. cerevisiae* Cultures

For 250 ml for the development and reproduction of yeast; 7.5 g yeast extract, 7.5 g tryptone, 7.5 g glucose were weighed and prepared. Then 5 flasks were taken and 50 ml of 250 ml medium prepared were added to each flask. After waiting at 121 °C 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 8% oleaster fruit; 30 g of oleaster fruit was 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, CuCl_2 and oleaster fruit extract were added to the other flasks removed from the oven besides the burner flame. According to the content of the groups, 15 ml of oleaster fruit filtrate and 0.5 grams of CuCl_2 were added and developed at 30 °C (11,12).

S. Cerevisiae Cell Growth Measurements

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

Protein Isolation for SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)

SDS-PAGE protein isolation of *S. cerevisiae* culture samples developed for 1 hour, 3 hours, 5 hours and 24 hours was performed according to the method of Aslan et al. (10).

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) Analysis

Protein samples of *S. cerevisiae* cultures were boiled for 5 minutes after the addition of an equal amount of SDS-PAGE SAB dye before loading into the wells. 1X 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. After electrophoresis, the gel was stained with Coomassie blue for 30 min at room temperature. 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 (13, 14).

Total Protein Density Measurements with Lowry Protein

Lowry protein assay kit using total protein density was performed at 650 nm (OD_{650}) using spectrophotometer according to the kit method.

MDA (Malondialdehyde) Analysis

MDA analysis, 0.5 µl sample was added to 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 were added to all test tubes. Then, after waiting 30 minutes in a boiling water bath at 90 °C, it was cooled. After adding 4 ml of n-butanol-pyridine mixture and vortexing, it was centrifuged at 4000 rpm for 15 minutes. 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 (14, 15).

Catalase Activity Determination

Measure to catalase, two tubes are taken and 1.4 ml of 30 mM H₂O₂ is added to the blank tube and 0.1 ml of phosphate buffer is added on it. 1.4 ml of 30 mM H₂O₂ and 0.1 ml of enzyme are added to the sample tube and mixed with vortex. Absorbances at 240 nm are read twice at 30 second intervals and thus the activity is determined (16).

GSH Levels Measurement

0.1 ml of culture sample is taken. 0.4 ml of 10% trichloroacetic acid solution is added and vortexed. After centrifuging at 3000 rpm for 5 minutes, the supernatant is taken and the pellet is discarded. 0.1 ml of supernatant is 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 solution are added. The resulting yellow color is read against distilled water at a wavelength of 412 nm in the spectrophotometer (17).

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 applied to determine the differences within the groups. Our data were analyzed using GraphPad Prism 5.

Results and Discussion

When we look at the data in Table 1, Figure 1a and Figure 1b, it was seen that the MDA level decreased in the Oleaster Fruit + CuCl₂ groups and the MDA amount increased in the CuCl₂ groups (p <0.05). As a result of this study, it was observed that the catalase activity of the Oleaster Fruit + CuCl₂ group was higher than the CuCl₂ group. However, the difference between catalase activity was statistically significant in all other groups (p <0.05) (Figure 1c, Figure 1d and Table 2).

According to the results of our study, the GSH level was higher in the Oleaster Fruit + CuCl₂ group than the CuCl₂ group (Figure 1e and Table 3).

There was a statistically significant difference between the groups depending on the development times at different hours in Table 4 and Figure 1f (p <0.05). Compared to the CuCl₂ group it was determined that there was an increase in cell growth in the that received the Oleaster fruit groups. The lowest cell growth was determined in the CuCl₂ group.

When the total protein results were given in Table 5,6 when Figure 1g, Figure 1h and were examined, we can say that the Oleaster fruit increased protein synthesis in *S. cerevisiae*. Especially when compared with the CuCl₂ group, it is seen that the protein synthesis increased at a high rate in the Oleaster Fruit + CuCl₂ group.

When SDS-PAGE gel images were examined, it shows that the protein concentration increased significantly in the Oleaster Fruit + CuCl₂ group compared to the CuCl₂ group, depending on time (1 hour, 3 hours, 5 hours and 24 hours). As a result of this study, it was concluded that, despite the negative effects of CuCl₂ oleaster fruit increased the protein density and development of *S. cerevisiae* (Figure 2A, 2B, 2C and 2D).

Table 1. MDA Levels

Groups	MDA Levels (nmol/ml)
Control	4.18 ± 0.20 ^c
Oleaster Fruit	4.15 ± 0.20 ^c
CuCl ₂	5.24 ± 0.45 ^a
Oleaster Fruit + CuCl ₂	4.62 ± 0.35 ^b

^{a-c}: Among the groups which bearing of different letter are significant (p<0.05).

One way Anova Post Hoc LSD test

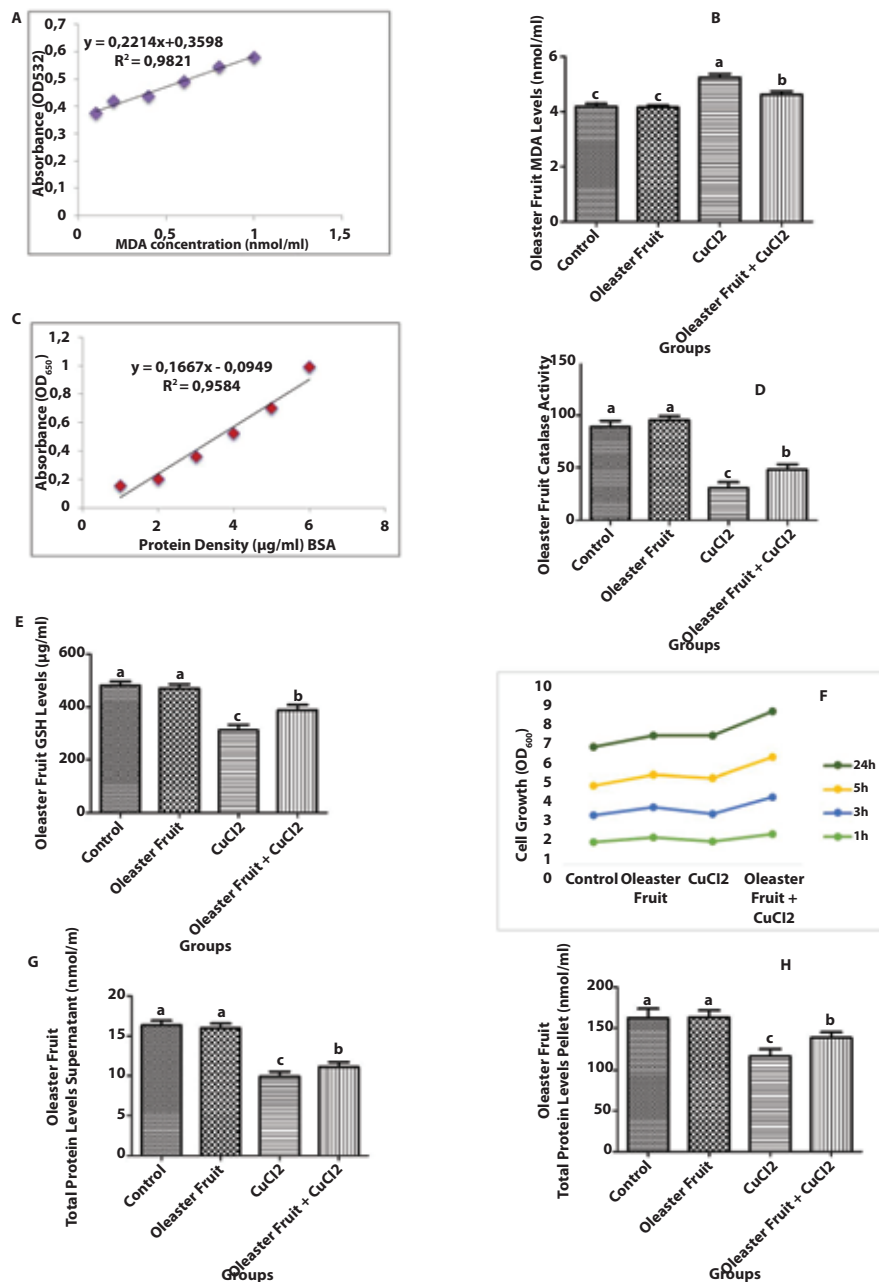


Figure 1. a) MDA standard; b) MDA level among groups; c) Standard protein of Bradford bovine serum albumin (BSA); d) Catalase activity among groups; e) GSH level among groups; f) Cell development of *S. cerevisiae* at different times; g) The supernatant total protein density among groups; h) The pellet total protein density among groups

Ameerah et al. (3) investigated the chemopreventive effect of *E. angustifolia* against hepatocellular carcinoma induced by diethylnitrosamine (DEN) in rats. They stated that *E. angustifolia* extract protected

against oxidative stress caused by DEN in the liver of rats, decreased MDA levels and increased GSH levels. Al-attar et al. (18), stated that *Olea oleaster* and *Juniperus procera* leaf extracts and their combination

Table 2. CAT Levels

Groups	CAT Activity (U/ml)
Control	88.88 ± 0.45 ^a
Oleaster Fruit	95.36 ± 0.45 ^a
CuCl ₂	30.35 ± 0.20 ^c
Oleaster Fruit + CuCl ₂	47.97 ± 0.37 ^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. GSH Levels

Groups	GSH Levels (µg/ml)
Control	481.52 ± 0.04 ^a
Oleaster Fruit	470.88 ± 0.04 ^a
CuCl ₂	311.95 ± 0.02 ^c
Oleaster Fruit + CuCl ₂	387.64 ± 0.03 ^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. *Saccharomyces cerevisiae* Cell Growth in Oleaster Fruit

Groups	1h	3h	5h	24h (Overnight)
Control	1.27 ± 0.03 ^d	1.55 ± 0.04 ^c	1.66 ± 0.03 ^b	2.14 ± 0.04 ^a
Oleaster Fruit	1.56 ± 0.03 ^d	1.70 ± 0.04 ^c	1.81 ± 0.04 ^b	2.20 ± 0.05 ^a
CuCl ₂	2.61 ± 0.02 ^d	2.62 ± 0.02 ^c	2.64 ± 0.01 ^b	2.72 ± 0.02 ^a
Oleaster Fruit + CuCl ₂	1.31 ± 0.03 ^d	1.56 ± 0.03 ^c	2.03 ± 0.03 ^b	2.36 ± 0.03 ^a

^{a,b,c,d} among the groups which bearing of different letter are significant (p<0.05).

One way Anova Post Hoc LSD test

had beneficial effects on thioacetamide-induced liver cirrhosis in mice. Beyaz et al. (19) compared the protective effect of black mulberry (*Morus nigra* L.) and cranberry (*Cornus mas* L.) fruits in terms of molecular biological and biochemical parameters. They stated that while cell development and total protein synthesis increased in the Black Mulberry + Cranberry + H₂O₂ group (1, 3, 5 and 24 hours) compared to the H₂O₂ group, the MDA level decreased. Aslan et al. (20) stated that *Nigella sativa* consumption has a protective role against damage to the lungs of rats treated with CCl₄. Amini et al. (21) investigated the effect of naringin (NAR) and trimetazidine (TMZ) on kidney

Table 5. *S. cerevisiae* Supernatant Protein Density

Groups (Supernatant)	Total Protein Density (nmol/ml)
Control	
Oleaster Fruit	16.32 ± 0.25 ^a
CuCl ₂	15.94 ± 0.25 ^a
Oleaster Fruit + CuCl ₂	9.95 ± 0.14 ^c
	11.14 ± 0.17 ^b

^{a-c}: Among the groups which bearing of different letter are significant (p<0.05).

One way Anova Post Hoc LSD test

Table 6. *S. cerevisiae* Pellet Protein Density

Groups (Pellet)	Total Protein Density (nmol/ml)
Control	
Oleaster Fruit	162.32 ± 0.41 ^a
CuCl ₂	163.13 ± 0.45 ^a
Oleaster Fruit + CuCl ₂	116.06 ± 0.21 ^c
	138.67 ± 0.33 ^b

^{a-c}: Among the groups which bearing of different letter are significant (p<0.05).

One way Anova Post Hoc LSD test

damage (IR) in rats. They stated that Nrf-2 expression decreased in the group induced by IR damage and NAR and TMZ increased Nrf-2 expression in the kidneys. Aslan et al. (22) 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 EA + CCl₄ group. In another study they performed on muscle tissue, 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

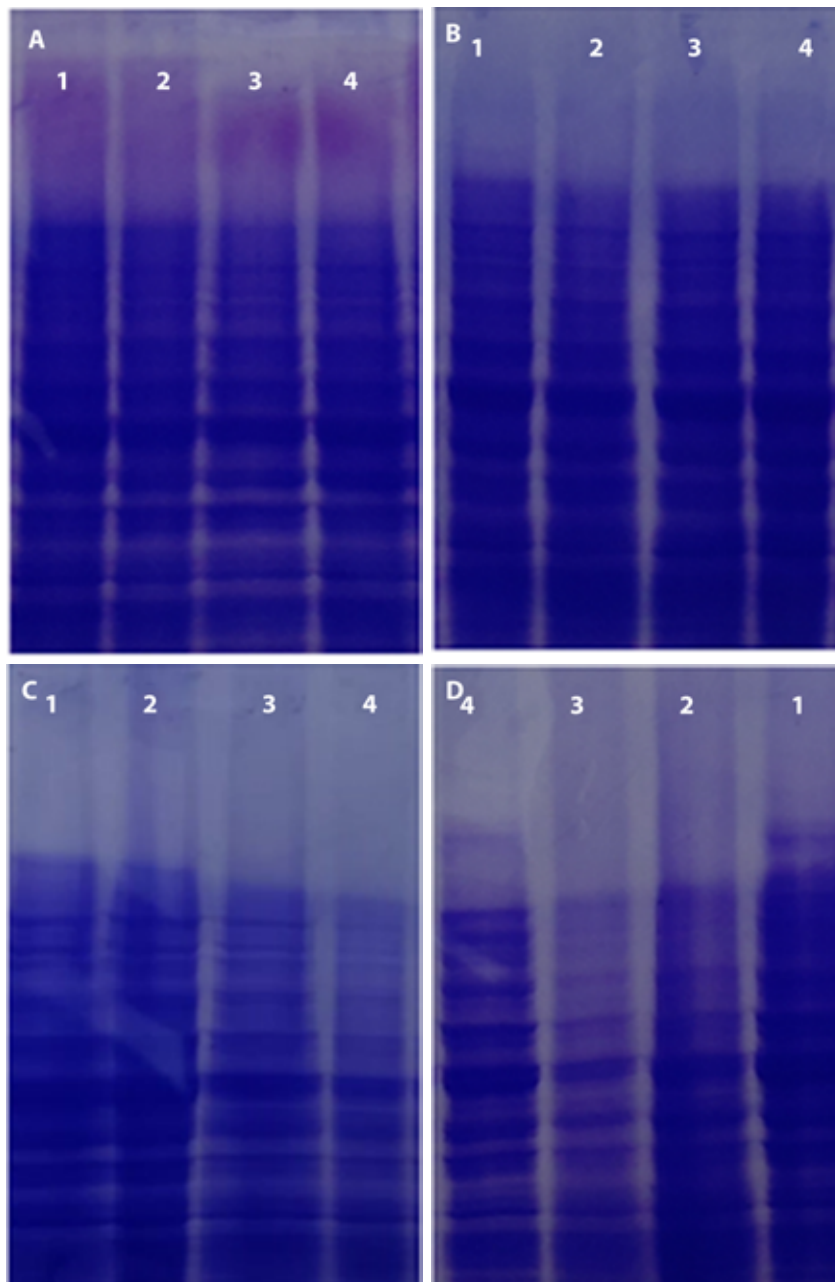


Figure 2. a) SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes (1h). Bands 1: Control; 2: Oleaster Fruit; 3:CuCl₂; 4: Oleaster fruit + CuCl₂; b) SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes (3h) 1: Control; 2: Oleaster Fruit; 3: CuCl₂; 4: Oleaster fruit + CuCl₂; c) SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes (5h). Bands 1: Control; 2: Oleaster Fruit; 3: Oleaster fruit + CuCl₂; 4: CuCl₂; d) SDS-PAGE pelet total protein bands profiles for development at 30°C. Lanes (24 h). Bands 1: Control; 2: Oleaster Fruit; 3: CuCl₂; 4: Oleaster fruit + CuCl₂

group given EA compared to the CCl₄ group (23). In their other study on kidney damage, they evaluated the effects of EA on antioxidative and anti-inflammation pathways (17).

Motevalian et al. (24) examined the effects of *Elaeagnus angustifolia* fruit extract on formalin-induced rat paw edema. They stated that *Elaeagnus angustifolia* fruit extract showed a significant anti-inflammatory effect in a dose-dependent manner. Dhingra and Jangra (25) stated that EA showed significant antiepileptic activity in mice with increasing brain gamma aminobutyric acid (GABA) levels. Guan et al. (26) found that selastrol, which has anti-oxidant and anti-inflammatory effects, protects against oxidative stress in skeletal muscle that occurs in diabetic rats. Luo et al. (27) found that epigallocatechin-3-gallate reduced the expression of bcl-2 and Nf-κB. Beyaz et al. (28) investigated whether Epigallocatechin-3-gallate (EGCG) has a protective role against H₂O₂-induced damage in *S. cerevisiae* and found that EGCG provides effective protection against oxidative stress and this compound suppresses lipid peroxidation products by reducing oxidative stress. have come to the conclusion. Aslan (29) emphasized that different fruit juices and their combinations have a protective role in increasing cell growth in *S. cerevisiae*. Aslan et al. (30) stated that pomegranate juice has a protective role on the growth of *S. cerevisiae*. Gok (31) stated that the harmful effects of lead solutions of plant hormones on the cambial activity of spindle steels significantly improved. Jilani et al. (32) investigated the effect of olive leaf polyphenols on antioxidant capacity in *S. cerevisiae* and concluded that olive leaf increased antioxidant activity. Gok et al. (33) investigated the protective role of sumac plant on the damage caused by copper chloride in *S. cerevisiae* culture. When they examined the cell growth for 1, 3, 5 and 24 hours, they determined that the sumac plant extract increased the cell growth and decreased the MDA level. Beyaz et al. (34) determined that royal jelly reduced oxidative stress, increased cell growth and total protein synthesis, thanks to its antioxidant properties in *S. cerevisiae* culture. Dinda et al. (35)

found that cranberry (*Cornus mas* L.) fruits and leaves respond positively to the treatment of diabetes, obesity, atherosclerosis and skin diseases. Beyaz et al. (36) investigated whether bee pollen has a protective role against copper chloride damage in *S. cerevisiae*. When compared with the copper chloride group, they found that total protein synthesis, cell growth and GSH levels increased, while MDA levels decreased in bee pollen groups. Hosseinzadeh et al. (37) found that *E. angustifolia* fruit seeds exhibit muscle relaxant activity due to flavonoid components. Aslan et al. (38) stated that EA has a neuroprotective effect against brain damage caused by CCl₄ in rats. Saleh et al. (39) investigated the effect of *Elaeagnus angustifolia* plant extract on oral cancer. They stated that *Elaeagnus angustifolia* extract prevented cell proliferation and colony formation. Beyaz (40) investigated the anti-inflammatory and antioxidant activities of clove plant extract (*Syzygium aromaticum* L.) against carbon tetrachloride (CCl₄)-induced oxidative damage in *S. cerevisiae*. Clove plant extract has been found to have a stimulating effect on cell growth and total protein synthesis by reducing oxidative stress thanks to its strong bioactive chemical components.

Conclusion

As a result of this study, it was concluded that, despite the negative effects of copper chloride, oleaster fruit extract increased the development of *S. cerevisiae*. In addition, it was concluded that the therapeutic effect provided by this fruit, which has anti-inflammatory and anti-oxidant properties is beneficial. As a result of the literature review, sources where this fruit was studied were found, but no studies on *S. cerevisiae* were found. For this reason, our study preserves its originality in terms of determining the protective effect of oleaster fruit (Figure 3).

Conflicts of Interest: There is no conflict of interest between the authors.

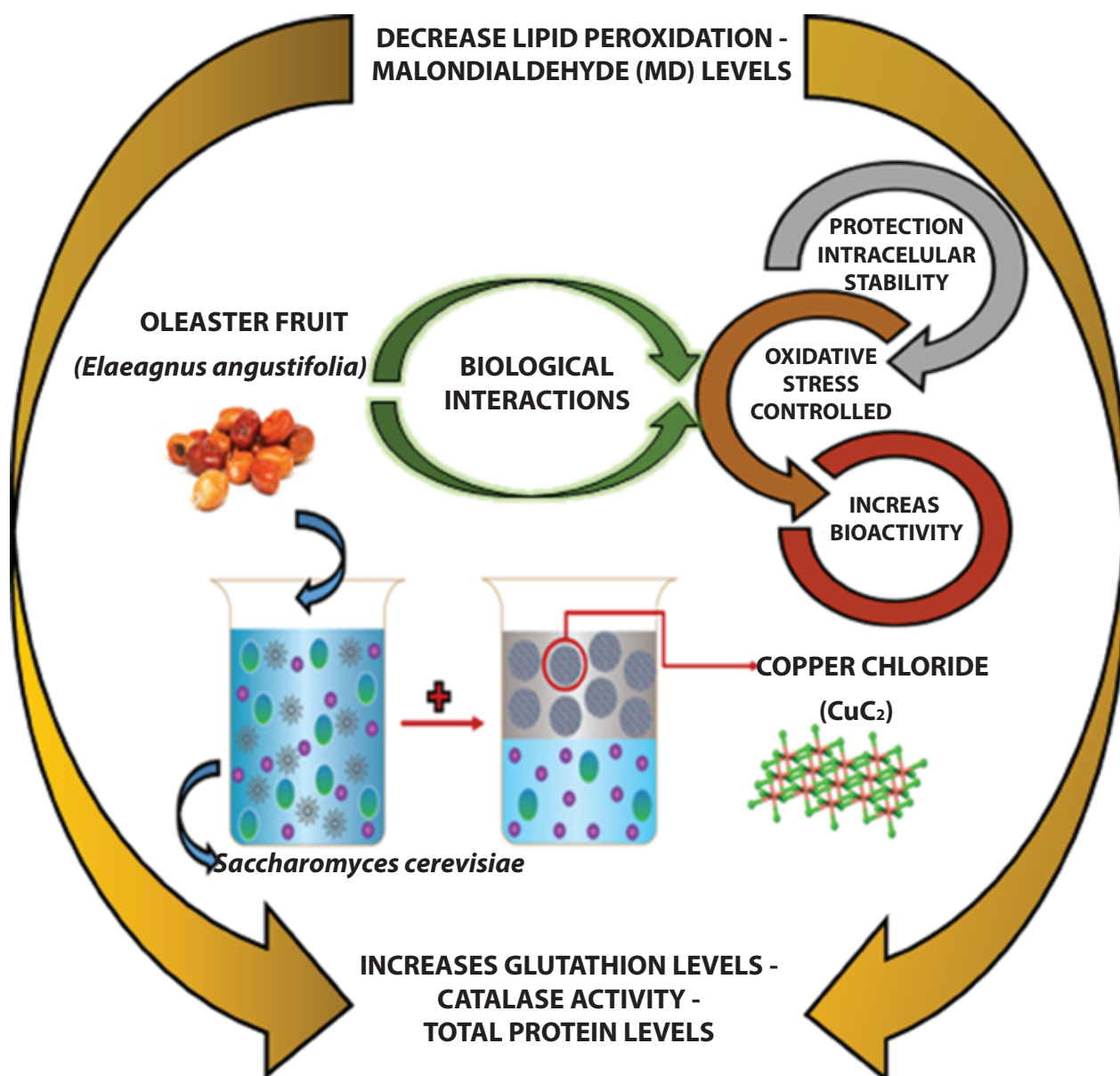


Figure 3. Oleaster Fruit (*Elaeagnus angustifolia*) Biological Interactions

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