

Article - Human and Animal Health

A New Research on the Antioxidant Properties and Therapeutic Effects of Bee Pollen in *Saccharomyces cerevisiae*

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HIGHLIGHTS

- Bee pollen reduces oxidative damage and thus it can protect *S. cerevisiae* against to ROS.
- Bee pollen induces total protein synthesis.
- Bee pollen induces *S. cerevisiae* cell development and it reduces MDA levels and it increases

Abstract: Bee pollen is a valuable product with apitherapeutic properties, highly valued by natural medicine for its medicinal and nutritional properties. Bee pollen, one of the basic elements of alternative therapy, has various biological activities such as antifungal, antiviral, antimicrobial, anti-inflammatory and antioxidant. In this study, four groups were created to investigate whether bee pollen has a protective role against copper chloride (CuCl_2) damage in *Saccharomyces cerevisiae*. Study groups: (1) Control group: only yeast was cultivated; (2) CuCl_2 group: given CuCl_2 (30 mM); (3) Bee Pollen group: given bee pollen (10%); (4) Bee Pollen + CuCl_2 group: given bee pollen (10%) + CuCl_2 (30 mM). *Saccharomyces cerevisiae* cultures were grown at 30 °C for 1, 3, 5 and 24 hours. Cell growth, lipid peroxidation, MDA (malondialdehyde) analyzes, GSH (glutathione) levels and CAT (catalase) activities were determined by spectrophotometer. Total protein changes were determined by SDS-PAGE electrophoresis and Lowry protein method. In the results of working; when compared to the CuCl_2 group, cell development (1, 3, 5 and 24 hours), total protein synthesis and GSH level (24 hours) increased in bee pollen groups, while MDA level (24 hours) decreased. It has been determined that bee pollen has a role of promoting cell growth and total protein synthesis by reducing oxidative stress in *Saccharomyces cerevisiae* culture thanks to its antioxidant properties. Moreover, these natural products have been found to have a strong therapeutic effect in the treatment of many diseases.

Keywords: Bee pollen; CuCl_2 ; *Saccharomyces cerevisiae*; SDS-PAGE.

INTRODUCTION

Today, the increase in the number and variety of fatal diseases leads people to various organic and natural products. Among these natural products, also known as the healing store, there are bee products. Bee products, other than honey, it is known as beeswax, pollen, propolis, royal jelly, bee venom. Especially in recent years, "apitherapy", which is called treatment with bee products, which has developed rapidly all over the world, is of great importance. All bee products not only increase body resistance, but also increase the interest in their use by having protective effects on health. Bee pollen is a wrinkled, prickly, oily and sticky substance found in flowering plants, anthers and pollen sacs above the organs of flowers. They are dried flower powders collected by the honey bee and differ significantly from the plant. Honey bees add the sugar they take from nectar to the pollen to hold the pollen grains together and transfer the pollen to the colony by taking it to their hind legs. Pollen plays a very important role for the colony. Bees use pollen in their diets and to feed the larvae. The nutritional value of bee pollen varies depending on the variety of the flower it is collected, climatic conditions, geography, genetics of the plant species, and the application of different beekeeping techniques. The main ingredients that make up pollen are carbohydrates and these carbohydrates consist of insoluble polysaccharides, starch, fructose, glucose and sucrose. It contains pollen, protein and amino acids and it is the main protein source in bee nutrition. In addition, lipids, fatty acids, sterols are found in the structure of pollen as fat content, while trace minerals, vitamins and flavonoids are also present. Pollen, a nutrient-rich food, is used to increase body resistance, provide a balanced diet, and relieve mental and physical fatigue. It is generally used to prevent appetite loss and malnutrition in children. In addition, studies have reported that it has antioxidant, antiinflammatory, antibacterial and antifungal properties [1, 2] In some studies, it has been stated that it strengthens liver functions and that the use of pollen has a detox effect in heavy metal and pesticide poisoning [3]. It has been reported in various studies that it reduces the lipid level in blood plasma. It is also used to strengthen the immune system and heal burn wounds [4].

In studies, it has been stated that pollen is used in the treatment of anemia and helps the digestion of iron, calcium, phosphorus and magnesium. When pollen is taken together with chemotherapy it can treat the side effects of cancer [5]. It has been reported that pollen consumption can be used in the treatment of advanced prostate cancer [6]. In addition to these, it has been observed to have important effects against colon cancer [7]. *Saccharomyces cerevisiae* (*S. cerevisiae*) cells are immobile, relatively small oval spheres, approximately 10 μm long and 5 μm wide. However, their size may vary even within the same strain of a particular species due to environmental conditions. *S. cerevisiae* can be easily cultured in solid or liquid media containing essential nutrients. If the nutrients in the environment are sufficient, they can grow as fast as bacteria. *S. cerevisiae* has 16 chromosomes. The total genome contains 78,520 nucleotide pairs of mitochondrial DNA and approximately 13.117,000 nucleotide pairs. Due to these genome characteristics, it is thought to be approximately 23% similar to the human genome. Especially in health issues, *S. cerevisiae* is used as a biological control agent. Recombinant DNA technology is of interest for the production of therapeutic heterologous proteins and the use of yeast. The reason for this is that functional genome analysis of *S. cerevisiae* provides a better understanding of the human genome [8]. For this reason, *S. cerevisiae* is used as a model organism in our study. While the negative effects of copper chloride (CuCl_2), which we use to cause damage in our study, are determined by malondialdehyde (MDA), which is an oxidative stress indicator, the protective effects of bee pollen, which we use for treatment, are due to the antioxidant defense enzyme systems glutathione (GSH) and catalase (CAT) activity has been determined. Lipid peroxidation produces a variety of complex products, mostly reactive electrophiles. These products easily diffuse and cause damage to other parts of the cell. MDA is one of the end products of lipid peroxidation and is known as an oxidative stress marker. MDA, by forming cross-links with the DNA and proteins in the cell, it disrupts their functions, leading to a number of disorders.

Glutathione is a nonprotein thiol and serves in the antioxidant defense of the cell. It has many physiological roles and has important roles in the protective mechanisms of the cell. It has a protective effect against the transport of amino acids, the synthesis of proteins and nucleic acids, the continuity of active forms of enzymes, the regulation of the hexose mono phosphate pathway, exposure to radiation and endotoxins. Free radicals are constantly formed in the body in normal physiological metabolism and the body has protective antioxidant mechanisms against these harmful radicals. One of these antioxidant enzymes is catalase. It is responsible for forming the intracellular defense system by rendering free radicals harmless [9]. In this study, the protective effects of bee pollen was investigated against *S. cerevisiae* cell growth with biochemical and molecular biology analyzes.

MATERIAL AND METHODS

Experimental Groups

In the study, 4 groups were formed and the groups were as follows: (1) Control Group: only yeast was cultivated; (2) CuCl₂ Group: given CuCl₂ (30 mM); (3) Bee Pollen Group: given bee pollen (10%); (4) Bee Pollen + CuCl₂ Group: given bee pollen (10%) + CuCl₂ (30 mM). After sterilization, bee pollen and CuCl₂ were added to the cultures of *S. cerevisiae* at specific concentrations. Cultures developed at 30 °C for 1 hour, 3 hours, 5 hours and 24 hours (overnight). Bee pollen was added to the growth medium of *S. cerevisiae* (YEPD, for 50 mL; 1.5 g yeast extract, 1.5 g tryptone, 1.5 g glucose) and allowed to grow [10].

Application of Bee Pollen and CuCl₂ Chemical to Culture

Bee pollen (10%) and CuCl₂ (30 mM) was added to *S. cerevisiae* medium and developed at 30 °C.

Cell Growth Measurements

The cultures samples were grown at 30 °C for 1, 3, 5 and 24 hours (overnight) and were measured using a spectrophotometer at 600 nm wavelength. In summary, samples were taken from cultured yeast samples at the indicated time intervals. These samples were read in the spectrophotometer, the development of yeast cells was recorded, and the results were analyzed and evaluated [10].

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

Samples of *S. cerevisiae* cultures were prepared for SDS-PAGE. Protein samples were then analyzed by SDS PAGE. Protein bands were examined between groups by taking gel images [10]. Briefly, protein samples were treated with sample application buffer before electrophoresis. Gels were prepared for electrophoresis and previously prepared protein samples were loaded onto the prepared gel. The movement of the proteins in the gel was ensured by applying electric current, and then the gels were stained with coomassie brilliant blue, necessary analyzes were made, and their images were taken and used in the study [10].

Malondialdehyde (MDA) Analysis

The basic principle of MDA, which is the most important indicator of lipid peroxidation, is that it reacts with thiobarbuturic acid in an acid environment and forms a pink chromogen. The intensity of the pink color is directly proportional to the concentration of MDA in the sample. 1.15% KCl solution was added to the culture samples to form 10% homogenate and homogenized in ice for 1-2 minutes at 15000 rpm. The obtained homogenates were used in MDA analysis. After adding 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid, 0.8% 2-thiobarbuturic acid (TBA) and 2 mmol / L 1.1', 3.3' tetraethoxypropane the mixture were vortexed then incubated at 45 min. and cooled in a boiling water bath (95 °C). It was vortexed after the addition of 2 ml of n-butanol. Tubes were kept in boiling water (at least 95 degrees) for 1 hour, then cooled and centrifuged at 5000 rpm for 10 minutes. The spectrophotometer was set to blindly zero absorbance at 532 nm. The absorbances of the pink colored supernatants were read at 532 nm and the results were expressed in nmol/mL [9].

Glutathione (GSH) Analysis

Distilled water was added to 10% homogenate taken from the cultures and homogenized at 12000 rpm for 1-2 minutes on ice. Homogenates were centrifuged at 5000 rpm at +4 °C for 20 min. The obtained supernatant was mixed by adding TCA solution and centrifuged again at 3000 rpm at +4 °C for 20 min in order to precipitate the proteins. Test tubes were prepared using 10% trichloric acetic acid, 1% trisodium citrate, 0.4% 5.5'-dithiobis 2-nitrobenzoic acid, 0.3 M disodium hydrogen phosphate reactants. Tubes were vortexed to mix the solutions thoroughly. As a result of the color formed in the samples kept at room temperature for 5 min., the absorbance values at 410 nm wavelength were read in the spectrophotometer and the results were recorded as μmol/mL [9].

Catalase (CAT) Activity Analysis

For the determination of H₂O₂, 250 µL of supernatant was taken from the cultures and treated with 100 mM 250 µL Tris-HCl, 1 M 500 µL KI (potassium iodide) and incubated for 90 min. at room temperature in the dark. At the end of 90 min., 200 µL of the samples were taken and the measurements were made at 390 nm wavelength in a spectrophotometer against blank. By reading the absorbance change of H₂O₂ for 1 min, the results were recorded as U/mg catalase activity [11].

Total Protein Density Measurements

Total protein changes were carried out by measuring the spectrophotometer at 650 nm according to the Lowry protein method. Accordingly, the total amount of protein in *S. cerevisiae* groups corresponding to this standard value was calculated [9, 12, 13].

Statistical Analysis

The statistical analysis of this study was evaluated by analysis of variance in the SPSS 22 package program. One Way Anova *Post Hoc* Tukey and LSD tests were applied in at least 3 repetitions to ensure the reliability of the differences between groups.

RESULTS

S. cerevisiae Cell Development Measurement Results

According to Figure 1A, there is a significant difference between the groups at different development times ($p < 0.05$). Bee pollen increased cell growth in Bee pollen and Bee pollen + CuCl₂ groups in comparison to the CuCl₂ damage group.

S. cerevisiae Total Protein Density Measurements

When the total protein results given in Table 1, 2, 3, Figure 1B and Figure 1C examined, we can say that Bee pollen promotes protein synthesis in *S. cerevisiae*. Especially when compared with CuCl₂ group, it is seen that the protein synthesis increased at a high rate in the Bee pollen + CuCl₂ group.

Table 1. *Saccharomyces cerevisiae* cell growth in bee pollen

Groups	1h	3h	5h	24h (Overnight)
Control	1.21 ± 0.35 ^d	1.54 ± 0.40 ^c	1.80 ± 0.48 ^b	2.59 ± 0.51 ^a
Bee Pollen	2.42 ± 0.38 ^d	2.55 ± 0.41 ^c	2.57 ± 0.51 ^b	2.63 ± 0.56 ^a
Copper chloride (CuCl ₂)	1.04 ± 0.17 ^d	1.20 ± 0.21 ^c	2.14 ± 0.33 ^b	2.23 ± 0.39 ^a
Bee Pollen + Copper chloride (CuCl ₂)	2.66 ± 0.22 ^d	2.70 ± 0.34 ^c	2.78 ± 0.41 ^b	2.88 ± 0.46 ^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

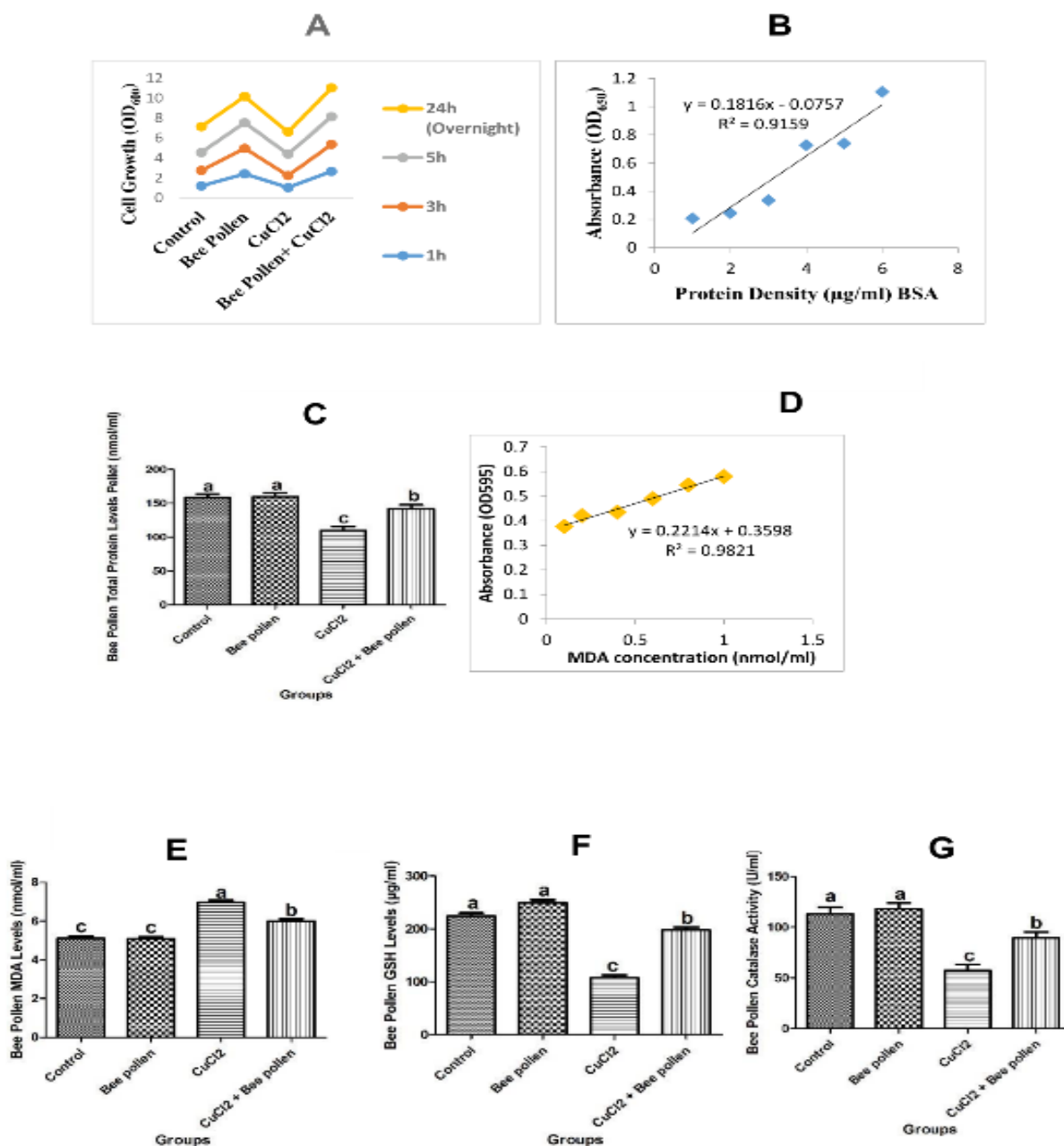


Figure 1. A: *S. cerevisiae* Cell development at different times, **B:** Standard protein of bovine serum albumin (BSA), **C:** Bee pollen total protein pellet density between groups, **D:** MDA standard, **E:** MDA level between groups, **F:** GSH level between groups, **G:** Catalase activity between groups.

Table 2. Pellet protein density

Groups (Pellet)	Total Protein Density (nmol/mL)
Control	158.23 ± 1.00 ^a
Bee Pollen	159.78 ± 1.04 ^a
Copper chloride (CuCl ₂)	110.02 ± 0.62 ^c
Bee Pollen + Copper chloride (CuCl ₂)	142.00 ± 0.80 ^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. Supernatant protein density

Groups (Supernatant)	Total Protein Density (nmol/mL)
Control	16.00 ± 1.03 ^a
Bee Pollen	15.75 ± 1.07 ^a
Copper chloride (CuCl ₂)	8.89 ± 0.86 ^c
Bee Pollen + Copper chloride (CuCl ₂)	14.03 ± 1.00 ^b

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

***S. cerevisiae* Malondialdehyde (MDA) Analysis Results**

Table 4, Figure 1D and Figure 1E reveals that the highest MDA levels were in CuCl₂ group and significantly decreased in Bee pollen + CuCl₂ group ($p < 0.05$).

Table 4. MDA levels

Groups	MDA Levels (nmol/mL)
Control	5.11 ± 0.55 ^c
Bee Pollen	5.09 ± 0.45 ^c
Copper chloride (CuCl₂)	6.97 ± 0.74 ^a
Bee Pollen + Copper chloride (CuCl₂)	6.00 ± 0.65 ^b

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

***S. cerevisiae* Glutathione (GSH) Analysis**

When we examine the GSH levels given in Table 5 and Figure 1F, the lowest GSH level was in the CuCl₂ group and decreased significantly in Bee pollen + CuCl₂ group ($p < 0.05$).

Table 5. GSH levels

Groups	GSH Levels (µg/mL)
Control	224.11 ± 1.03 ^a
Bee Pollen	249.11 ± 0.89 ^a
Copper chloride (CuCl₂)	181.47 ± 0.71 ^c
Bee Pollen+ Copper chloride (CuCl₂)	197.64 ± 0.74 ^b

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

***S. cerevisiae* Catalase (CAT) Activity Determination**

When we examine the CAT levels given in Table 6 and Figure 1G, the lowest CAT level was in the CuCl₂ group and decreased significantly in Bee pollen + CuCl₂ group ($p < 0.05$).

Table 6. CAT levels

Groups	CAT Activity (U/mL)
Control	112.88 ± 0.61 ^a
Bee Pollen	118.02 ± 0.62 ^a
Copper chloride (CuCl₂)	57.41 ± 0.57 ^c
Bee Pollen + Copper chloride (CuCl₂)	89.47 ± 0.60 ^b

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

***S. cerevisiae* SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) Analysis**

The SDS-PAGE gel image (Figure 2A, 2B, 2C, 2D) show that the protein concentration was significantly increased in Bee pollen + CuCl₂ group when compared to the CuCl₂ group. As a result of this study, it was concluded that Bee pollen increases the development of *S. cerevisiae* despite the negative effects of CuCl₂.

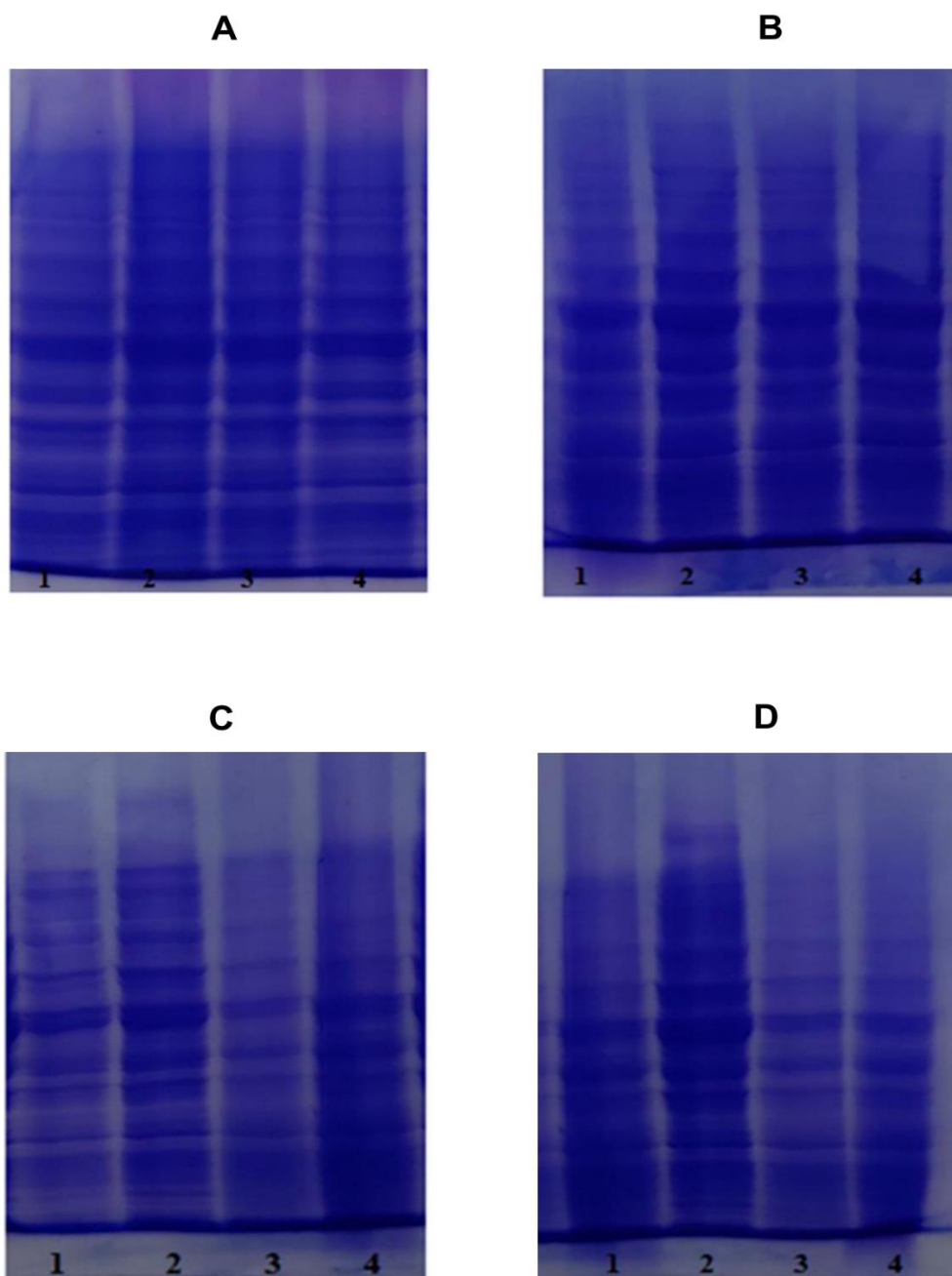


Figure 2. A: SDS-PAGE Pellet Protein Bands (1h). Bands 1: Control; 2: Bee pollen; 3: CuCl₂; 4: Bee pollen + CuCl₂, **B:** SDS-PAGE Pellet Protein Bands (3h). Bands 1: Control; 2: Bee pollen; 3: CuCl₂; 4: Bee pollen + CuCl₂, **C:** SDS-PAGE Pellet Protein Bands (5h). Bands 1: Control; 2: Bee pollen; 3: CuCl₂; 4: Bee pollen + CuCl₂, **D:** SDS-PAGE Pellet Protein Bands (24h). Bands 1: Control; 2: Bee pollen; 3: CuCl₂; 4: Bee pollen + CuCl₂.

DISCUSSION

Bee pollen improves memory, diabetes, cardiovascular disease, aging and resistance to metabolic syndrome. In addition, it has been reported to have many functional properties such as increasing the number of germ cells that facilitate wound healing, antibacterial, antiviral, antifungal, anti-inflammatory and antitumoral activity. [14, 15].

Gok and coauthors [10] investigated the protective roles of ellagic acid against H₂O₂-induced damage in *S. cerevisiae* and found that ellagic acid have a protective feature thanks to their antioxidant properties. Beyaz and coauthors [13] stated that curcumin reduces the oxidative stress damage caused by H₂O₂ in *S. cerevisiae* and has a protective role on the growth of *S. cerevisiae*. In addition, they found that the MDA level increased in the H₂O₂ groups. Beyaz and coauthors [16] investigated the protective effects of black mulberry (*Morus nigra* L.) and cranberry (*Cornus mas* L.) fruits on H₂O₂-induced oxidative stress in *S. cerevisiae* and

concluded that black mulberry and cranberry fruits have a very strong therapeutic effect against oxidative stress. In addition, they stated that MDA level decreased and total protein level increased significantly in the given black mulberry and cranberry extracts groups compared to the H₂O₂ group. Alugoju and coauthors [17] stated that quersetin protects *S. cerevisiae* from apoptotic cell death and increases cell viability. Aslan [18] emphasized that different juices and their combinations have a protective role in reducing oxidative damage and increasing cell growth in *S. cerevisiae*. Aslan and coauthors [12] stated that kiwi extract increases cell growth by reducing oxidative damage in *S. cerevisiae* thanks to its antioxidant properties. Rajkumari and coauthors [19] concluded that *Syzygium jambos* and *Terminalia citrina* plants have the ability to synthesize a wide variety of enzymatic and non-enzymatic antioxidants that can reduce ROS-induced oxidative damage in *S. cerevisiae*. Aslan [20] stated that mulberry extract increased cell growth by providing significant protection against H₂O₂ damage in *S. cerevisiae*. Ali and Kunugi [21] investigated the effects of Royal jelly and bee pollen on skeletal muscle dysfunction (sarcopenia) in older adults. They found that Royal jelly and bee pollen treatment provided effective protection by improving inflammation and oxidative damage in skeletal muscle. Münstedt and Mannle [22] investigated the effect of bee products in the prevention and treatment of oral mucositis due to cancer treatment. They found that bee products such as honey, Royal jelly, bee pollen healed mucositis formed in the mouth in patients receiving chemotherapy and radiotherapy.

Didaras and coauthors [23] investigated the effects of bee pollen on the human gut microbiome and prebiotic potentials and found that bee pollen treatment provides effective treatment opportunities for patients by providing high antimicrobial activity. Altunalmaz and Aksu [24] investigated the microbiological quality of bee pollen, especially used in children, elderly and patient nutrition, due to its biological usefulness. As a result of microbiological analysis, they found that bee pollen does not pose a health hazard and helps to eliminate the conditions that cause the development of molds and the formation of mycotoxins. Karlıdag and Keskin [25] found that bee pollen inhibits lipid peroxidation *in vitro*, scavenges many free radicals that have oxidant properties and are known to be carcinogenic, and that bacteria are antimicrobial. In addition, it has been reported that pollen is used as a supportive for the treatment of colds, osteoporosis, headache, nervous and ulcer ailments as well as its digestive, cell-regenerating, appetite-enhancing effects. Onbaslı [26] stated that bee pollen has antibacterial, antifungal and antioxidant and anticancer properties. Moreover, they found that bee pollen was effective in reducing the negative effects of cancer treatment. Yıldız [27] revealed that pollen has a healing effect against liver diseases. Although pollen is known to be allergic, studies have reported that it alleviates asthma and allergy symptoms by reducing histamine that causes allergic reactions in the body. Saral [28] found that MDA levels decreased and CAT activity increased in the groups given honey, pollen, propolis and Royal jelly compared to the CCl₄ applied groups. When the liver tissue histopathology analyzes were examined, they found that bee pollen provided hepatoprotective protection against CCl₄-induced liver damage.

Aslan and coauthors [29] investigated the therapeutic effect of ellagic acid (EA) against to pancreatic tissue damage caused by carbon tetrachloride (CCl₄). They stated that MDA level, Bcl-2 and NF-κB protein expressions decreased, GSH level and CAT activity as well as caspase-3 and Nrf-2 protein expressions increased significantly in CCl₄ applied group compared to the EA applied group. Colak [30] investigated the effect of bee pollen and propolis on the expression of voltage-gated sodium channels in metastatic human prostate cancer cell lines. He stated that pollen treatment has antimetastatic activity by suppressing the development of prostate cancer cells. Aslan and coauthors [31] evaluated the effects of EA on anti-oxidative and anti-inflammation pathways against CCl₄-induced kidney damage in rats. It was concluded that it significantly increased caspase-3 and Nrf-2 protein expressions by decreasing NF-κB, VEGF, COX-2 and TNF-alpha protein expressions as well as renal tissue damage in EA-treated groups.

When examined in terms of biochemical analysis, they stated that there was a decrease in MDA levels and a significant increase in GSH level and CAT activities in the EA groups.

Laaroussi and coauthors [32] investigated the effect of antioxidant-rich propolis and bee pollen extracts against D-glucose-induced type 2 diabetes in rats. They concluded that propolis and bee pollen can be used as a protective natural product against dyslipidemia and hepato-kidney damage due to diabetes. Fratini and coauthors [33] found that Royal jelly and bee pollen prevent the growth of harmful microorganisms thanks to their powerful antimicrobial effects.

Saral [34] investigated the bioactive properties of apitropic bee products (honey, pollen, propolis and Royal jelly) and their roles in preventing liver damage against carbontetra chloride (CCl₄) induced liver damage. He stated that MDA levels increased and CAT activities decreased in CCl₄ applied groups compared to the bee products applied groups. It has been determined that the treatment of bee products such as honey, pollen, propolis and Royal jelly provides effective protection against damage by reducing the effect of toxic

agents in the liver. Aslan and Can [35] stated that in *S. cerevisiae* culture, lemon juice provides strong protection against chromium-induced oxidative stress and increases cell growth by reducing oxidative damage. Gok and coauthors [36] indicated that persimmon leaf have a protective effect against oxidative damage caused by chromium in *Saccharomyces cerevisiae*. Aslan and coauthors [37] point out that Milk thistle induces apoptosis in development of carbontetrachloride-induced liver DNA damage in rats and Aslan and coauthors [38] emphasized that Black cummin may be a drug for lung damage in rats.

CONCLUSION

From the past to the present, bee products have been used in the treatment of many diseases and new medicines have been developed from the chemical components of the products, providing different treatment possibilities. It has been determined by the studies that bee pollen, one of these products with high antioxidant capacity, has protective and therapeutic roles as well as prolonging the life span of people. When the results of our study are evaluated, it has been determined that bee pollen promotes total protein synthesis in *S. cerevisiae* and increases cell growth.

Moreover, it was determined that GSH levels and CAT activities increased significantly in the bee pollen added groups compared to the other groups and MDA levels, which are an oxidative stress marker in the cell, decreased significantly. The lack of information about the parameters investigated in the literature review reveals the importance of the study and it is thought that it will provide new medical opportunities in the treatment of many diseases in the future by contributing to the literature (Figure 3).

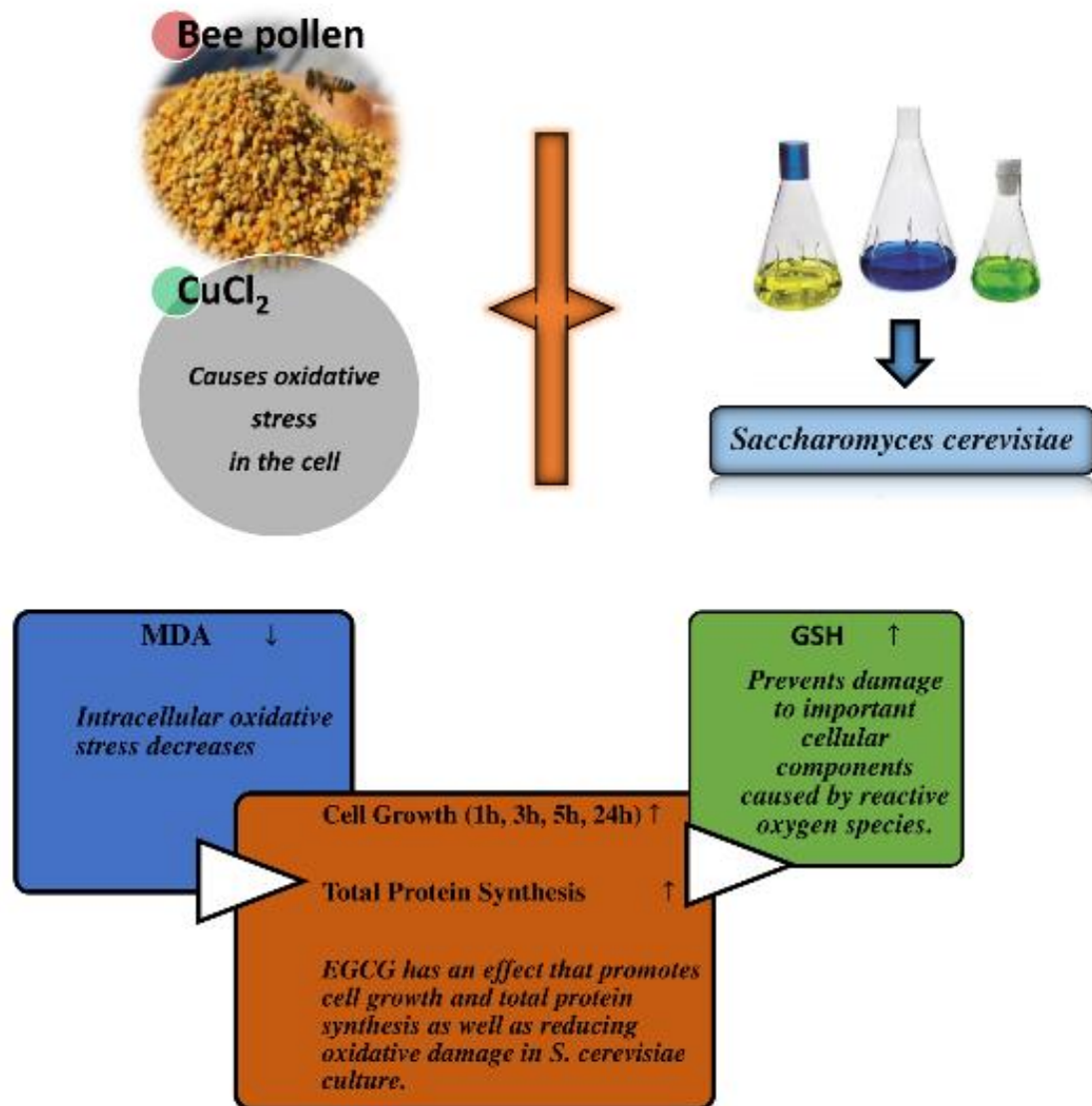


Figure 3. The effect of bee pollen on *Saccharomyces cerevisiae* cell growth.

Conflicts of interest: There is no conflicts of interest between the authors.

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