

The determination of the effect of Curcumin on *Saccharomyces cerevisiae* totally protein expression changes and cell growth

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Summary. The use of Curcumin for the treatment of various diseases stems mainly from its active biological functions, namely anti-inflammatory, antioxidant, anti-microbial, anti-alzheimer, anti-tumor, anti-diabetic and anti-rheumatism activities. Curcumin is a hypoglycemic, hepatoprotective, nephroprotective, cardioprotective and neuroprotective molecule, and it is reported to suppress thrombosis and protect against myocardial infarction. In this study, four groups were formed to investigate whether Curcumin has a protective role against the damage caused by hydrogen peroxide (H₂O₂) in *Saccharomyces cerevisiae*. Groups: (i) Control Group: Yeast cultivated group only; (ii) Curcumin Group: Curcumin group (% 8); (iii) H₂O₂ Group: Group given H₂O₂ (15 mM); (iv) Curcumin + H₂O₂ Group: Group given Curcumin (% 8) + H₂O₂ (15 mM). *Saccharomyces cerevisiae* cultures were developed at 30 °C for 1, 3, 5 and 24 hours. Cell growth, lipid peroxidation MDA (malondialdehyde) analysis and GSH (glutathione) levels were determined by spectrophotometer. Total protein changes were detected by SDS-PAGE electrophoresis and calculated by Bradford method. According to the results obtained; Cell growth (1, 3, 5 and 24 hours), total protein synthesis and GSH levels (24 hours) increased in Curcumin groups, while MDA level decreased (24 hours) when compared with H₂O₂ group. As a result, it was determined that Curcumin *Saccharomyces cerevisiae* culture has an effect that promotes cell growth and total protein synthesis as well as reducing oxidative damage. *Practical applications:* Curcumin antiseptic, analgesic, anti-inflammatory, antioxidant, and is known to carry antimalarial properties. Antioxidant properties indicate that the therapeutic properties of many chronic diseases with inflammation plays a major role. It is also known to have preventive and therapeutic properties in various types of cancer.

Keywords: *Curcumin*, H₂O₂, oxidative damage, protein, *Saccharomyces cerevisiae*, SDS-PAGE

Introduction

Curcumin, popularly known as *Curcumin* is a yellow powdered spice from the roots of *Curcuma longa*. It is a short-stalked plant that can grow to a height of about 100 cm. It grows in the tropics of Asia, including India, China, Indonesia, Jamaica, Peru and Pakistan. In addition to cooking *Curcumin*, it is used as a coloring agent in herbal treatment and textile industry in various cultures, especially in India. *Curcumin*

contains 390 kcal energy per 100 g, 8.9 g fat, 69.9 g CHO, 8.5 g protein and 50 mg ascorbic acid [1]. *Curcuma*; It contains 6.3% crude protein, 5.1% crude oil, 69.4% crude oil, 13.1% water, 2.4-4% essential oil and 4.7-8.2% crude ash. In addition, *Curcumin* which has a natural pigment source is known as *Curcumin* but is called ancaik curciminoid and several phytochemicals active compounds [2]. Curcuminoids are the main components of *Curcumin* and constitute 3-5% of *Curcuma*. Curcuminoids are a non-volatile yellow phenolic

compound which is responsible for the therapeutic effect of *Curcumin* [1].

S. cerevisiae is a widely used microorganism in beer and bread making. *S. cerevisiae* are unicellular eukaryotic microorganisms found in the Ascomycota branch. Like complex eukaryotes, the chromosomes of the yeast strain of *S. cerevisiae* are also bound by histones and are located in the nucleus. The yeast genome contains 16 chromosomes and these chromosomes are reproduced from more than one origin during the S phase. It also contains 14 megabases double genomic DNA and about 6000 genes. When comparing all the potential protein coding genes of yeasts and mammalian protein sequences, it is known that there is a statistically about 31% similarity between them. Unlike high-build eukaryotes, *S. cerevisiae* grows fast, genes can be manipulated easily and cheaply. Thanks to these features, it provides an inexpensive, flexible and fast genetic system to investigate the events occurring in the cell. This greatly facilitates genetic analysis. Therefore, *S. cerevisiae* is used as a model organism to understand the molecular basis of eukaryotic cell functions [3]. Recently it has been widely used in the treatment of various diseases. It is also known to have protective effects such as antioxidant, antiinflammatory, antimicrobial, antialzheimer, antitumor, anti-diabetic and antirheumatism. In addition, it has been determined that *Curcumin* provides protection against hypoglycemic, hepatoprotective, nephroprotective, cardioprotective and neuroprotective and myocardial infarction. *Curcumin* is recognized and used in many different ways throughout the world to benefit health; used in curries in India, tea in Japan, cosmetics in Thailand, colorants in China, antiseptic in Malaysia, anti-inflammatory agent in Pakistan and mustard sauce, cheese, butter and chips in the United States as preservatives and coloring [4]. Reactive oxygen species (ROS); nucleic acid can affect protein, fat and carbohydrates. Oxidative damage is prevented by antioxidant defense. However, when the antioxidant defense system is insufficient, oxidative damage occurs in the cell. Cellular antioxidant defense mechanisms lead to oxidative stress when it cannot effectively eliminate ROS [5]. Hydrogen peroxide causes irreversible damage to lipids, proteins and DNA, causing dysfunction of cell organelles [6]. *Curcumin* anorexia, diabetes, sinusitis, bile, metabolic syndrome, rheumatism, Alzheimer's,

lung and liver disease has been found to provide strong protection against [7]. Since the genetic structure of *S. cerevisiae* is known, it is used as a model in scientific studies[5]. In this study, damage was created by applying hydrogen peroxide to *S. cerevisiae* culture and the effects of *Curcumin* in this living organism against cell growth were investigated. Recent studies have found that *Curcumin* provides antiviral protection. Human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), enterovirus 71 (EV71), ebola virus (EBOV), a large number of influenza A virus, including the virus and the intelligence function and the function in infertility has been found to inhibit [8].

Materials and Methods

Research groups

In this study, 4 groups were formed. Groups: (i) Control Group: Yeast cultivated group only; (ii) *Curcumin* Group: *Curcumin* group (% 8); (iii) H_2O_2 Group: Group given H_2O_2 (15 mM); (iv) *Curcumin* + H_2O_2 Group: Group given *Curcumin* (% 8) + H_2O_2 (15 mM). Immediately after sterilization, *Curcumin* (8 %) and H_2O_2 (15 mM) were added to *S. cerevisiae* cultures and the cultures were developed at 30 °C for 1 hour, 3 hours, 5 hours and 24 hours (overnight). Growth medium of *S.cerevisiae*: YEPD (for 50 mL, 1.5 g yeast extract, 1.5 g tripton, 1.5 g glucose) was added and developed *Curcumin* for growth and propagation of *S. cerevisiae* [9].

Curcumin extract and H_2O_2 chemical application to culture

Curcumin (8 %) and H_2O_2 (15 mM) were added to *S. cerevisiae* medium and developed at 30 °C. H_2O_2 ; H_2O_2 group (15 mM) and *Curcumin* (8 %) + H_2O_2 (15 mM) were added to the group [9].

S. cerevisiae cell development measurements

Culture samples were developed at 30 °C for 1, 3, 5 and 24 hours (overnight) and measured using a spectrophotometer at 600 nm [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. Gel bands were obtained and protein bands between the groups were examined [9,11].

Malondialdehyde (MDA) analysis

In the malondialdehyde analysis, the test tubes were taken and the sample was blinded. 0.5 ml of the supernatant was added to the sample tube and 0.5 ml of purified water was added to the blind tube. Subsequently, 2.5 ml of TCA and 1 ml of TBA were added to all of the test tubes. The test tubes were kept in a 90 °C water bath for 30 minutes and then cooled and 4 ml of n-butanol was added. Centrifugation was carried out at 3000 rpm for 10 minutes and the resulting supernatant layer was removed and transferred to a new test tube. The measurements were made in the spectrophotometer at 535 nm wavelength and the results were recorded as nmol / ml [12].

Glutathione (GSH) analysis

For GSH analysis; 0.4 ml of 10 % cell homogenate and 0.2 ml of 20 % TCA were mixed. The supernatant was removed by centrifugation at 3000 rpm for 15 minutes. For the blind; 0.2 ml of 150 mM KCl 0.2 ml of the standard solution of 0.2 ml of the supernatant was mixed by adding 1 ml of 0.3 M Na_2HPO_4 + 0.05 ml. After waiting for 5 minutes, the absorbance of the yellow color was measured in spectrophotometer at 412 nm wavelength [13].

Total protein density measurements (Bradford)

Total protein density was performed using a spectrophotometer at 595 nm (OD_{595}) according to the Bradford method. Using different concentrations of BSA protein, BSA protein standards were obtained. Accordingly, the total amount of protein in *S. cerevisiae* groups corresponding to this standard value was calculated [10].

Statistical analysis

SPSS 22 package program was evaluated by analysis of variance. One Way Anova *Post Hoc* LSD tests were used to determine intra-group differences. In order to ensure the reliability of the statistical analysis, the measurements were repeated at least 3 times.

Results

S. cerevisiae cell development measurement results

According to Figure 1A, there is a significant difference between the groups with different development times ($p < 0.05$). *Curcumin* increased cell growth in *Curcumin* and *Curcumin* + H_2O_2 groups in comparison to the H_2O_2 damage group.

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis

The SDS-PAGE gel image (Figure 2) show that the protein concentration was significantly increased in *Curcumin* + H_2O_2 group when compared to the H_2O_2 group. As a result of this study, it was concluded that *Curcumin* increases the development of *S. cerevisiae* despite the negative effects of H_2O_2 .

S. cerevisiae malondialdehyde (MDA) analysis results

Table 4, Figure 1E and Figure 1G reveals that the highest MDA levels were in H_2O_2 group and significantly decreased in *Curcumin* + H_2O_2 group ($p < 0.05$).

Glutathione (GSH) analysis

When we examine the GSH levels given in Table 5 and Figure 1F, the lowest GSH level was in the H_2O_2 group and decreased significantly in *Curcumin* + H_2O_2 group ($p < 0.05$).

Total protein density (Bradford) measurements

When the total protein results given in Table 1, 2, 3, Figure 1B, Figure 1C and Figure 1D are examined, we can say that *Curcumin* promotes protein synthesis in *S. cerevisiae*. Especially when compared with H_2O_2

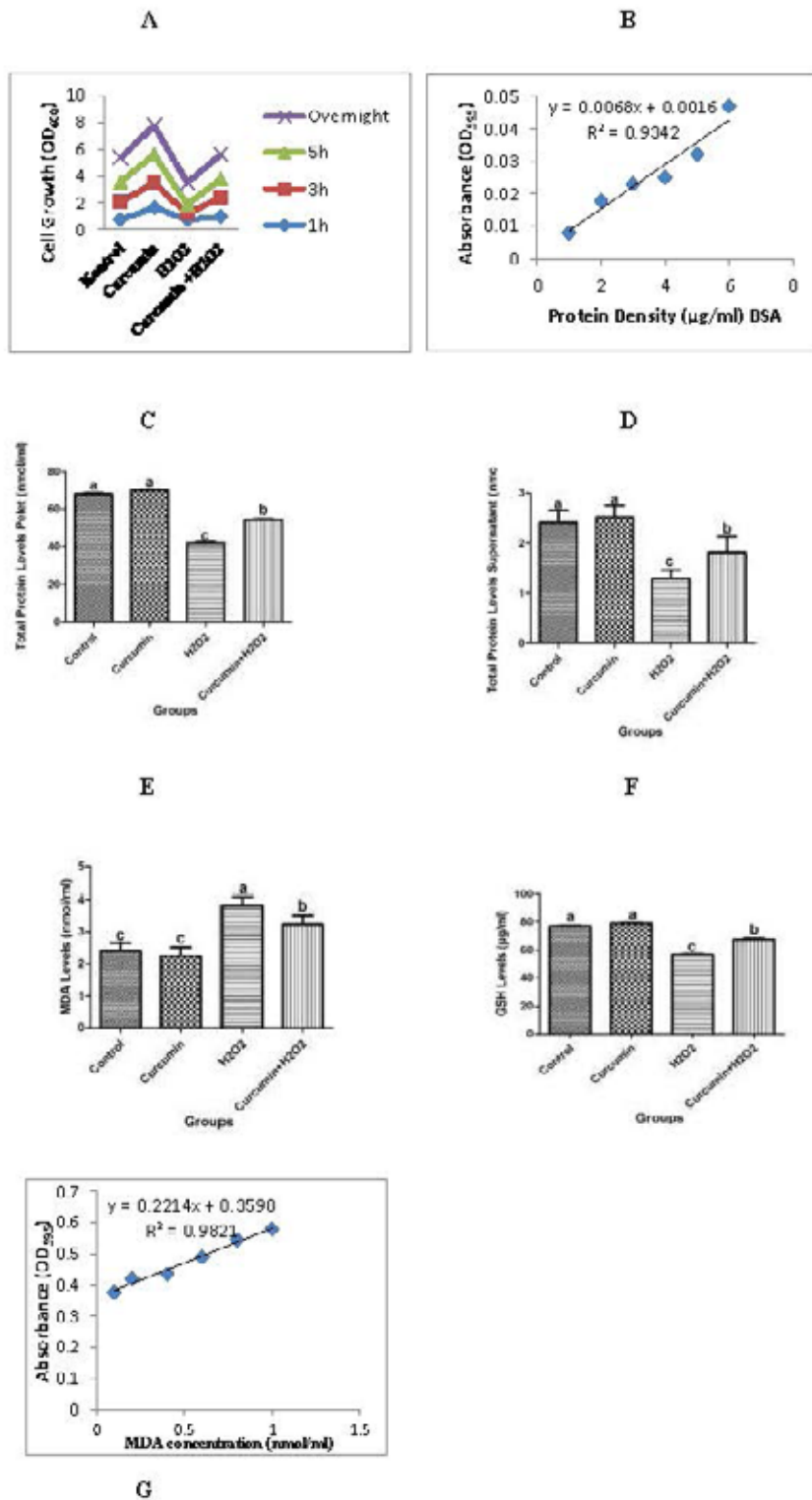


Figure 1. (A) Cell development of *S. cerevisiae* in Curcumin at different times, (B) Standard curve of bradford BSA (Bovine Serum Albumin), (C) Total protein density between groups (pellet), (D) Total protein density between groups (supernatant), (E) Intergroup MDA level, (F) Intergroup GSH level, (G) MDA standard curve

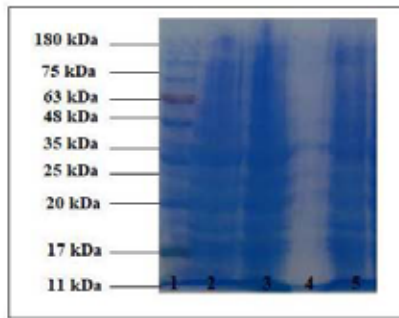


Figure 2. SDS-PAGE Pellet Protein Bands. Bands 1: Marker; 2: Control; 3: *Curcumin*; 4: H_2O_2 ; 5: *Curcumin* + H_2O_2

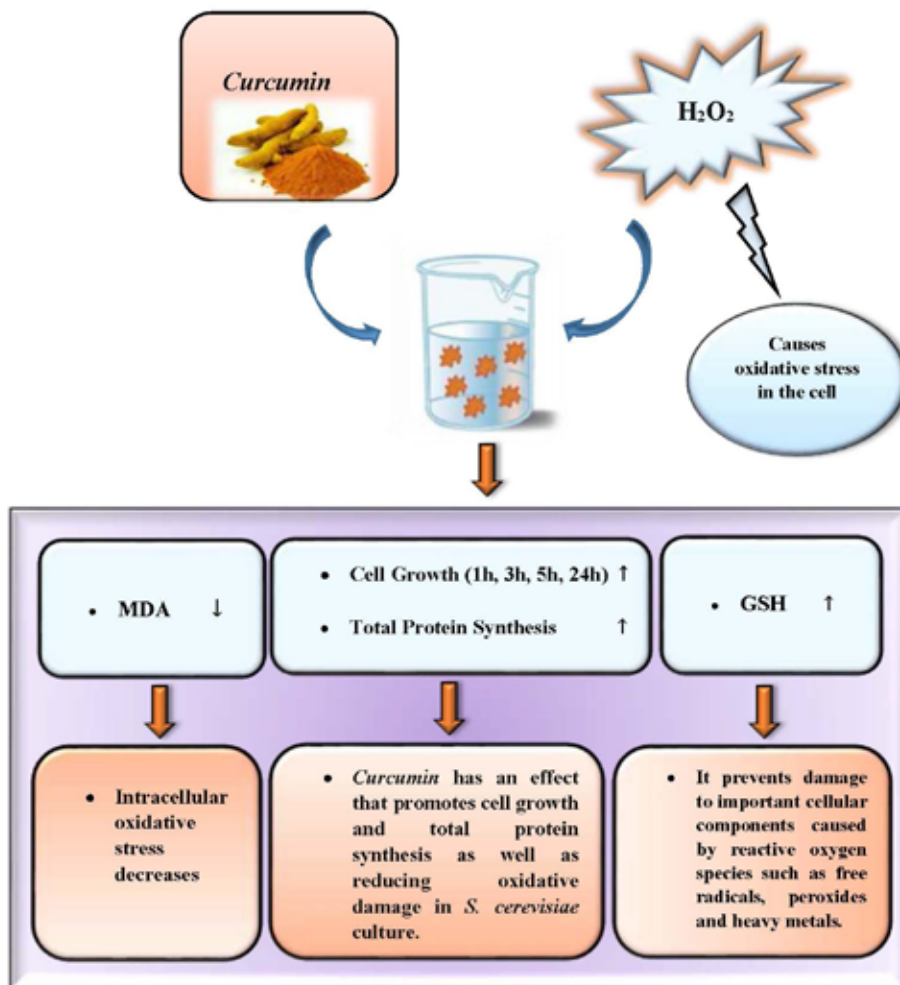
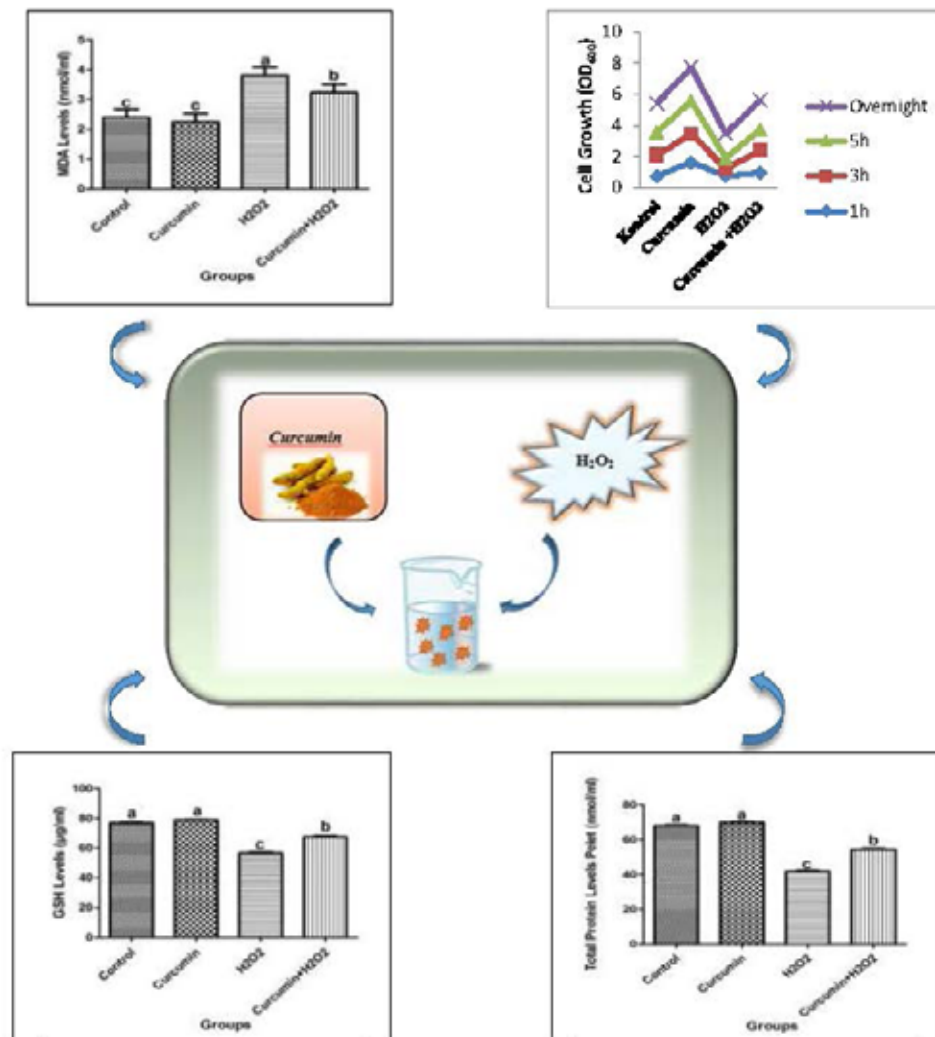


Figure 3. *Curcumin* increases cell growth, total protein synthesis and GSH level in *S. cerevisiae* culture and decreases the effect of oxidative damage caused by MDA.



- ✓ *Curcumin*, popularly known as *Curcumin* is a yellow powdered spice from the roots of *Curcuma longa*.
- ✓ Recently it has been widely used in the treatment of various diseases. It is also known to have protective effects such as antioxidant, anti-inflammatory, anti-microbial, anti-alzheimer, anti-tumor, anti-diabetic and anti-rheumatism.
- ✓ In addition, it has been determined that *Curcumin* provides protection against hypoglycemic, hepatoprotective, nephroprotective, cardioprotective and neuroprotective and myocardial infarction.
- ✓ Cell growth (1, 3, 5 and 24 hours), total protein synthesis and GSH levels (24 hours) increased in EGCG groups, while MDA level decreased (24 hours) when compared with H₂O₂ group.

Table 1. Cell Development of *S. cerevisiae* Curcumin at Different Times

Groups	1h	3h	5h	Overnight
Control	0,75 ± 0,02 ^c	1,32 ± 0,02 ^c	1,50 ± 0,02 ^c	1,82 ± 0,02 ^c
Curcumin	1,65 ± 0,02 ^a	1,81 ± 0,02 ^a	2,10 ± 0,02 ^a	2,15 ± 0,02 ^a
H ₂ O ₂	0,70 ± 0,02 ^d	0,47 ± 0,02 ^d	0,68 ± 0,02 ^d	1,58 ± 0,03 ^d
Curcumin + H ₂ O ₂	1,96 ± 0,02 ^b	1,41 ± 0,02 ^b	1,36 ± 0,02 ^b	1,86 ± 0,02 ^b

The difference between the groups with different letters ^{**a,b,c,d} is significant (p < 0.05). One-Way ANOVA *Post Hoc* LSD Test

Table 2. Total Protein Densities of Bradford Pellet

Groups (Supernatant)	Total Protein Densities (µg/ml)
Control	2,41 ± 0,02 ^a
Curcumin	2,50 ± 0,02 ^a
H ₂ O ₂	1,22 ± 0,02 ^c
Curcumin + H ₂ O ₂	1,82 ± 0,02 ^b

a-c: The difference between the groups bearing the different letters in the columns is significant (p < 0.05). One-Way ANOVA *Post Hoc* LSD Test

Table 3. Bradford Supernatant Total Protein Densities

Groups (Pelet)	Total Protein Densities (µg/ml)
Control	68,14 ± 0,02 ^a
Curcumin	69,70 ± 0,03 ^a
H ₂ O ₂	41,82 ± 0,02 ^c
Curcumin + H ₂ O ₂	54,16 ± 0,03 ^b

a-c: The difference between the groups bearing the different letters in the columns is significant (p < 0.05). One-Way ANOVA *Post Hoc* LSD Test

Table 4. MDA Level

Groups	MDA Level (nmol/ml)
Control	2,31 ± 0,02 ^c
Curcumin	2,12 ± 0,01 ^c
H ₂ O ₂	3,82 ± 0,03 ^a
Curcumin + H ₂ O ₂	3,10 ± 0,02 ^b

a-c: The difference between the groups bearing the different letters in the columns is significant (p < 0.05). One-Way ANOVA *Post Hoc* LSD Test

Table 5. GSH Level (Pelet)

Groups	GSH Level (Pelet) (µmol/ml)
Control	76,97 ± 0,03 ^a
Curcumin	78,90 ± 0,03 ^a
H ₂ O ₂	56,37 ± 0,02 ^c
Curcumin + H ₂ O ₂	67,69 ± 0,02 ^b

a-c: The difference between the groups bearing the different letters in the columns is significant (p < 0.05). One-Way ANOVA *Post Hoc* LSD Test

group, it is seen that the protein synthesis increased at a high rate in the *Curcumin* + H₂O₂ group.

Discussion

Recent studies have shown that many compounds found in plants with antioxidant properties can reduce the risk of developing cancer. *Curcumin* is one of these antioxidant plants. *Curcumin* in the structure of *Curcumin* has been found to have a protective biological effect in humans by reducing the risk of many diseases and cancer types. Ekinci [4] applied *Curcumin* treatment to diabetic rats and found that *Curcumin* treatment reduced lung damage caused by diabetes.

Mody et al. [14] showed that *Curcumin* application has a strong antibacterial activity against *Clostridium difficile*. Aslan [15] emphasized that different juices and combinations thereof have a protective role in reducing oxidative damage and increasing cell growth in *S. cerevisiae*. Aslan [16] stated that mulberry extract increased cell growth by providing significant protection against H₂O₂ damage in *S. cerevisiae*. Aslan

et al. [9] investigated the protective role of tomato against H_2O_2 -induced damage in *S. cerevisiae* and found that tomato has a protective feature due to its antioxidant properties. Aslan et al. [10] stated that grape seed reduces oxidative stress damage caused by H_2O_2 in *S. cerevisiae* and has a protective role on *S. cerevisiae* growth. They also found that MDA levels increased in H_2O_2 groups. Gumuscu [17] examined the possible protective effects of *Curcumin* and epigallocatechin gallate (EGCG) against paclitaxel-induced oxidative stress in rats. *Curcumin* and epigallocatechin gallate showed a strong effect on the protection of tissues and organs against oxidative damage caused by paclitaxel due to its strong antioxidant properties. In addition, GSH and CAT activity increased significantly in the *Curcumin* and epigallocatechin gallate treated groups, while MDA levels were increased in the paclitaxel treated groups.

Cotelı and Karatas [18] investigated the antioxidant effect of *Curcumin* in this study, and the curcuma's vitamins B_1 , B_3 and B_9 , β -carotene, A, E, C vitamin amounts, GSH, free radical scavenging effect with GSH, total phenolic and flavonoid concentrations due to the total antioxidant capacity is a very rich plant. Firat [1] investigated the effect of *Curcumin* on coronary artery disease and stated that *Curcumin* has a protective effect against cardiovascular diseases. Plavcova et al. [19] investigated the anti-inflammatory effect of *Curcumin* in *S. cerevisiae* and concluded that it is a natural phenolic compound that has a significant anti-inflammatory effect and is supportive in the treatment of many inflammatory diseases. Kerdsomboon et al. [20] investigated the protective effect of *Moringa oleifera* leaf extract (MOLE) against cadmium toxicity in *S. cerevisiae* and concluded that MOLE reduces intracellular cadmium deposition and oxidative stress. Jilani et al. [21] investigated the effect of olive leaf (*Olea europaea* L.) polyphenols on biological processability and antioxidant capacity in *S. cerevisiae* and concluded that olive leaf increased antioxidant activity. Kiruthika and Padma [22] investigated the protective role of *Zea mays* leaf extracts in *S. cerevisiae* against oxidative stress due to H_2O_2 and stated that *Zea mays* leaf extracts provide effective protection against oxidative stress.

Oprea et al. [23] investigated the chemoprotective effect of blueberry extracts against cadmium toxicity in

S. cerevisiae and concluded that blueberry extracts have a protective effect against cadmium-induced toxicity. Marques et al. [24] investigated the protective effect of *Ginkgo biloba* leaf extracts in DNA against oxidative damage caused by H_2O_2 in *S. cerevisiae* and stated that *Ginkgo biloba* leaf extracts stimulate the DNA protection mechanism by protecting DNA from oxidation. Selvam et al. [25] investigated the effect of *Curcumin* on the treatment and prevention of colon cancer and concluded that *Curcumin* has a therapeutic effect against colon cancer. Huang et al. [26] investigated the effects of OLE1 on cadmium-induced oxidative stress in *S. cerevisiae* and stated that OLE1 reduces the oxidative stress caused by cadmium by its antioxidant properties. Albuz [27] investigated the cytotoxic effects of ginger, *Curcumin* and clove which are used as food supplements in daily life. He stated that the cytotoxic effect of ginger and *Curcumin* is negligible especially for normal cells and that these two plants have a strong antioxidant effect.

Karaman and Koseler [28] investigated the relationship between *Curcumin* and chronic diseases and concluded that *Curcumin* showed positive results in the treatment of many diseases such as respiratory system diseases, neurological diseases, obesity, diabetes and cancer. Beyaz et al. [29] investigated the protective effects of ginger on oxidative stress caused by H_2O_2 in *S. cerevisiae* and concluded that ginger has a very strong therapeutic effect against oxidative stress. In addition, compared to H_2O_2 added groups in the ginger added MDA levels decreased and GSH levels were significantly increased. Dai et al. [8] researched that *Curcumin* protects against oxidative stress and inhibits the infection of the influenza A virus. Mounce et al. [30] they found that *Curcumin* inhibits *Zika* and *Chikungunya* virus replication in human cells. Aslan et al. [31] indicated that Black cumin has inhibitive effect and Aslan et al. [32] point out that Milk thistle has preservative effect against to oxidative damage in *S. cerevisiae*. In addition, Aslan et al. [33] revealed that *Nigella sativa* reduced lung damage. In another study, Aslan et al. [34] emphasized that Pomegranate juice has positive effect against *S. cerevisiae* cell growth. According to the other study the different sugar source supported the growing of *S. cerevisiae* cell growth [35].

Conclusion

In this study, it was concluded that the accumulation and toxic effect of H₂O₂ used to cause damage leads to oxidative stress. When the results of the study were evaluated, *Curcumin* promotes total protein synthesis in *S. cerevisiae* and increases cell growth. Furthermore, *Curcumin* is highly effective against oxidative damage caused by H₂O₂ in *S. cerevisiae* and protects the cell against oxidative damage (Figure 3). In addition, the lack of information about the parameters investigated in the literature review reveals the importance of the study and is thought to contribute to the literature.

Conflict of interest statement: We declare that we have no conflict of interest.

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