INVESTIGATION OF BIOCHEMICAL EFFECTS OF NANO ZINC-OXCIDE (ZnO) IN *SACCHAROMYCES CEREVISIAE*

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Abstract. Zinc-oxide nanoparticle (ZnO NP) is one of the metal oxide materials widely used in many fields due to its superior properties. Numerous studies have been conducted on the toxic effects of these NPs in biological systems; however, there is a limited number of studies investigating their effects on biochemical parameters. The aim of this study is to determine the effects of ZnO NP on some biochemical parameters and defense system in yeast (*Saccharomyces cerevisiae*) culture medium. The analysis (Gas chromatographic analysis) of fatty acids and sterols (ergosterol, campestrol, stigmasteol, betasitosterol) was performed. The results of the present study revealed that zinc oxide nanoparticles had various effects on biochemical parameters in *S. cerevisiae* yeast culture to which they were applied at different doses. A significant statistical difference was found between the control group and the other groups for biochemical parameter content. It is thought that the results of the antioxidant defense system will be a reference for similar studies on other living models and will contribute to the literature by supporting future studies using in vivo systems.
Keywords: *nano-zinc oxide, fatty acid, GSH, vitamin, Saccharomyces cerevisiae*

Introduction

The materials with a particle size of less than 100 nm are categorized as nanoparticles (NP), or nanomaterials. Nanomaterials are referred to as particles, no matter their source, and range in size from 1 nanometer to several microns. Any size smaller than 1 micron can be classified as NPs, whereas all sizes larger than one micron are classified as microparticles. Although NPs have persisted in nature for millions of years, humans have been producing and using them for various purposes for decades since their superior properties were discovered (Nowack and Bucheli, 2007).

Nanotechnology is a very promising discipline based on the manipulation of nanoscale materials synthesized by combining the concepts and theories of basic science disciplines (Rai and Biswas, 2018). The increasing demand and application of NP technology have increased its use in various metal industries, biomedical science and health technology. Rapid developments in the field of nanotechnology lead to nano-sized materials such as nano-particles, nano-crystals, and nano-tubes, to be introduced further into our daily lives. Nowadays, more than a thousand nanotechnology products are used and integrated into the composition of commercial products.

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Zinc (Zn) is a trace element found in the structure of most prokaryotic and eukaryotic living organisms (*Fig. 1*) It is necessary for the proper functioning of numerous metabolic processes, including homeostasis, immunological response, oxidative stress, cell differentiation and ageing (Mocchegiani et al., 2000). In recent years, Zn-derived compounds that have been produced experimentally have drawn more attention due to their significant role in vital functions as well as their unique properties for agricultural, environmental, industrial and health applications. In particular, zinc oxide (ZnO) NPs have a wide range of biomedical applications, including anti-diabetic, anti-cancer, anti-fungal, anti-bacterial and similar properties to drug release (Sangani et al., 2015). Although they have been employed in targeted drug transport technology, the cytotoxicity of ZnO NPs is not yet fully known. The results of the study have showed that ZnO NPs have a high antibacterial effect in gram-negative and gram-positive bacteria concentrations. Furthermore, it has been observed that biosynthetically produced ZnO NPs outperform chemically produced ZnO NPs in terms of antibacterial activity. These considerations have allowed ZnO NPs to be rapidly diversified as well as produced in very large quantities (Hazra et al., 2013).

Although the application of nano-structures is quite novel, it has raised issues related to the potential toxicity of nano-materials, interconnected with all aspects of human life. The investigation of the physicochemical properties and performance of nanomaterials is necessary to assess their potential hazards and toxic effects on biological ecosystems and the human body. The toxicity of nanomaterials and their effects on the human body and surrounding biosystems can vary depending on the nature of NPs, particle size, shape and their use. In recent years, the fact that these substances have harmful effects on living organisms and the environment has increased concerns in scientific and social circles. The contact of the human body with ZnO NPs with the expanding application area has been rising rapidly, as well as their dispersion in the environment. It has been found that these metal oxide NPs have both beneficial and detrimental effects in their interaction with the biological environment (Beyersmann, 2002). In an experimental study, the inhalation of NPs was found to support pulmonary inflammation in animals. Another study on intratracheal ZnO inoculation reported cytotoxic and neutrophilic inflammatory patterns in *Sprague-Dawley* rat after 24 h (Greenwood and Earnshaw, 1997). It was also observed to cause genotoxic and cytotoxic effects by harming the brain, neurons and bronchial epithelial cells in humans.

It is critical to establish a fundamental understanding of cell genetics and physiological structure and organization. The effects of many molecules that play important roles in living systems have been discovered through the study of cultures such as yeast.

Although there have been studies on the toxic effects of ZnO NPs in biological systems, few studies have been undertaken to examine their effects on biochemical parameters and defense systems. The levels of some biochemical parameters under the yeast conditions in order to determine the effects of ZnO NPs on living organisms were examined in this study.

Material and methods

Preparation of in vitro yeast cell culture media

First, YEPD broth were prepared for the growth and proliferation of *S. cerevisiae* yeast cells, which we will use in our experiment. 2 g yeast extract, 4 g glucose and 4 g

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bactopeptone were used per 200 mL of broth. After the preparation of the broth, the following groups were formed:

- Control group (C): For *S. cerevisiae* cells, a culture medium containing 2 g yeast extract, 4 g bactopeptone and 4 g glucose in 200 mL of pure water was prepared.
- Hydrogen peroxide group (H_2O_2): For *S. cerevisiae* cells, a culture medium containing 2 g yeast extract, 4 g bactopeptone and 4 g glucose in 200 mL of pure water was prepared. Then, 200 µl hydrogen peroxide (H_2O_2) was added to this medium (Demir, 2019). Each dose was assigned as a separate group and experimental studies were conducted.
- Zinc-oxide group (Z-1): For *S. cerevisiae* cells, a culture medium containing 2 g yeast extract, 4 g bactopeptone and 4 g glucose in 200 mL of pure water was prepared. Then, 100 mg ZnO was added to this medium (Demir, 2019). Each dose was assigned as a separate group and experimental studies were conducted.
- Zinc-oxide group (Z-2): For *S. cerevisiae* cells, a culture medium containing 2 g yeast extract, 4 g bactopeptone and 4 g glucose in 200 mL of pure water was prepared. Then, 250 mg ZnO was to this medium (Demir, 2019). Each dose was assigned as a separate group and experimental studies were conducted.

After all the groups were incubated at 30°C for 72 h, the density of the cells was measured in a spectrophotometer at 600 nm. The cells in each culture medium were taken into sterile tubes and centrifuged at 6000 rpm and +4°C and then the cell pellets were collected. These pellets were washed with 0.9% NaCl solution and cleaned from liquid wastes. Process steps were carried out as follows.

Extraction of cell pellets for protein, GSH, GSSG and MDA measurement

After the cell pellets were washed with 0.9% NaCl, their wet weights were determined and homogenized in Tris-HCl, Tris base and EDTA (pH: 7.4) buffer in a cold environment. The samples were then centrifuged (Klejdus et al., 2004). After centrifugation, the supernatant was used in total protein, reduced glutathione (GSH), oxidized glutathione (GSSG) and malondialdehyde (MDA) analysis.

Measurement of GSH and GSSG amount in high performance liquid chromatography (HPLC) device

1.0 mL of the supernatant obtained for the analysis was deproteinized by adding 1.0 mL of 10% perchloric acid, and after centrifugation, 1.0 mL of the sample was taken and analyzed in Shimadzu HPLC (Yang et al., 2009). The measurements were made at a wavelength of 214 nm using LC – 10 AD VP as pump in the device, SPD- 10A VP as UV–visible detector, PDA detector, CTO-10AS VP as column furnace, SIL-10AD VP as autosampler, DGU-14A as degasser unit, and Class VP 6.26 operating program (Shimadzu, Kyoto, Japan). The mobile phase consisted of a mixture of 0.1 mL trichloroacetic acid (TCA) and methanol (94%/6%, v/v). The samples were separated using the ODS-3 HPLC column. They were calculated using the Class VP 6.26 software according to the calibration curve prepared from standard mixtures (Shimadzu, Kyoto, Japan).

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Measurement of lipid peroxidation (MDA) concentration

The proteins were precipitated upon treatment with 10% perchloric acid by taking 1.0 mL of the supernatant obtained for the analysis, and after the mixture was centrifuged at 5000 rpm for 5 min, 1.0 mL sample was taken into autosampler vials and analyzed in Shimadzu HPLC (Karataş et al., 2002). A mixture of 30 mmol KH_2PO_4 and methyl alcohol (Sayes et al., 2007). (82.5–17.5%, pH = 4.0 with H_3PO_4) was used as the mobile phase and the ODS-3 HPLC column (150 mm × 4.6–5 μm) was employed (Karatepe, 2004).

Extraction of lipids

Lipids were extracted from the cell pellet according to the method described by Hara and Radin (1978). The cell pellet was homogenized in a mixture of 3/2, (v/v) hexane/isopropyl alcohol, and then this mixture was centrifuged to obtain the supernatant.

Preparation of fatty acid methyl esters

Afterwards, 2% methanolic sulfuric acid was added to the lipid extract in the supernatant phase obtained for the preparation of fatty acid methyl esters and mixed well. This mixture was left to methylation at 55°C for 15 h (Hara and Radin, 1978). The tubes were then cooled to room temperature and 5% sodium chloride (NaCl) was added and mixed. The fatty acid methyl esters in the tubes were extracted with n-hexane and the hexane phase was pipetted on top and treated with 2% potassium bicarbonate ($KHCO_3$). The caps of the tubes were closed and turned upside down and kept for 3 h after adding $KHC0_3$ solution. At the end of the period, the supernatant phase was placed in smaller test tubes, the pellet was poured and the samples were evaporated in a 40°C oven, then 1 ml was added to the test tubes with sample remains and vortexed. 2 ml vials were filled with a 1 ml sample. Gas chromatography device was used to perform the analysis (Christie, 1989).

Fatty acid analyses

Analyses were performed using the Shimadzu GC 17 gas chromatography. For the analysis, a capillary column with SP-2560, 25 m × 0.25 mm i.d., 0.20 μm (Supelco, Sigma, USA) features was used. The injection temperature was set to 240°C and the detector temperature to 280°C. The column temperature program would increase in the range of 120°C–220°C, and it was set as 5°C per minute up to 200°C, and 4°C per minute from 200°C to 220°C. The temperature was held at 220°C for 8 min and for a total of 35 min. Nitrogen gas was used as carrier gas (Tvrzicka et al., 2002).

Analysis of alpha-tocopherol and ergosterol by HPLC method

Potassium hydroxide (5% KOH) solution dissolved in methanol was added to the samples separated for analysis, and were kept at 85°C for 15 min. Distilled water was added to the samples cooled to room temperature and mixed. Lipophilic molecules were extracted with 2 × 5 mL hexane and the solvent was evaporated under nitrogen gas. It was then dissolved in 1.0 mL (50% + 50%/v/v) acetonitrile/methanol mixture and analyzed into autosampler vials. Acetonitrile/methanol (60% + 40%/v/v) mixture was used as mobile phase at flow rate of 1.0 mL/min. While PDA-UV detector was used for

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analysis, Supelcosil LC 18 (15 × 4.6 cm, 5 µm; Sigma, USA) was used for column. Analyses were made at a wavelength of 202 nm (Katsanidis and Addis, 1999; Lopez-Cervantes et al., 2006).

Statistical analysis

One-way analysis of variance (ANOVA) and Post Hoc Tukey-HSD test were used to determine differences between the groups. The data were presented as mean ± S.E.M. Data were considered as statistically significant at the level of $p < 0.05$. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis of the data.

Results and discussion

When the effect of ZnO NP on MDA level was analyzed, a reduction was observed in the experimental groups compared to the control group. In particular, the reduction was remarkable in the group treated with a low dose of ZnO NP ($p < 0.0001$). The MDA level elevated in the group treated with H_2O_2 ($p < 0.05$) (*Fig. 2*). When zinc groups applied at different doses were compared among themselves, it was observed that there was an increase in MDA level with increasing concentration.

It was observed that the SOD level did not change much against the exposure to ZnO NP ($p > 0.05$) but elevated in the group treated with H_2O_2 ($p < 0.05$) (*Fig. 3*).

It was determined that the CAT level of ZnO NP in the yeast cell elevated in all groups compared to the control group. The elevation in the experimental groups took place depending on the dose ($p < 0.001$; $p < 0.0001$) (*Fig. 4*).

When the GST level was compared with the control group, it was determined that GST level dropped as the concentration in all experimental groups elevated. There was an elevation in the H_2O_2 group ($p < 0.05$) (*Fig. 5*).

When the effect of ZnO NP on GSH level was analyzed, it was found that it elevated in the group treated at low concentration ($p < 0.05$) and slightly dropped at high concentration and in the group treated with H_2O_2 ($p < 0.01$; $p < 0.001$) (*Fig. 6*).

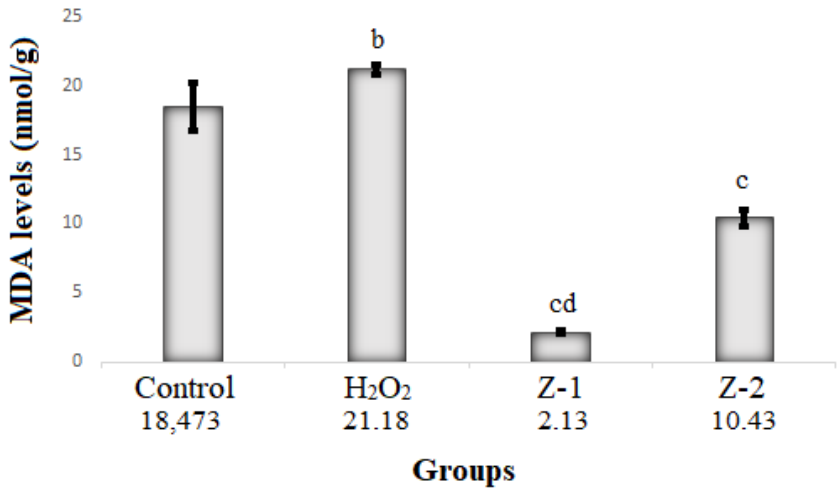

Figure 2. *The effect of Zinc Oxide on MDA level in S. cerevisiae cells (b: p < 0.05, c: p < 0.01, cd: p < 0.0001)*

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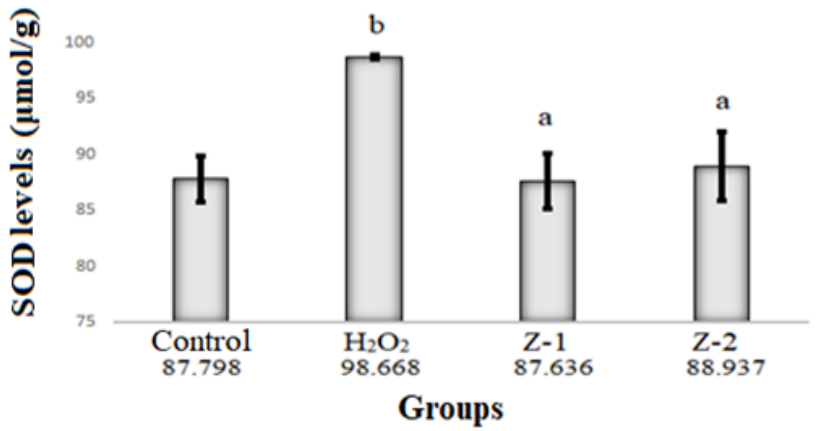

Figure 3. *The effect of Zinc Oxide on SOD level in S. cerevisiae cell (a: p > 0.05, b: p < 0.05)*

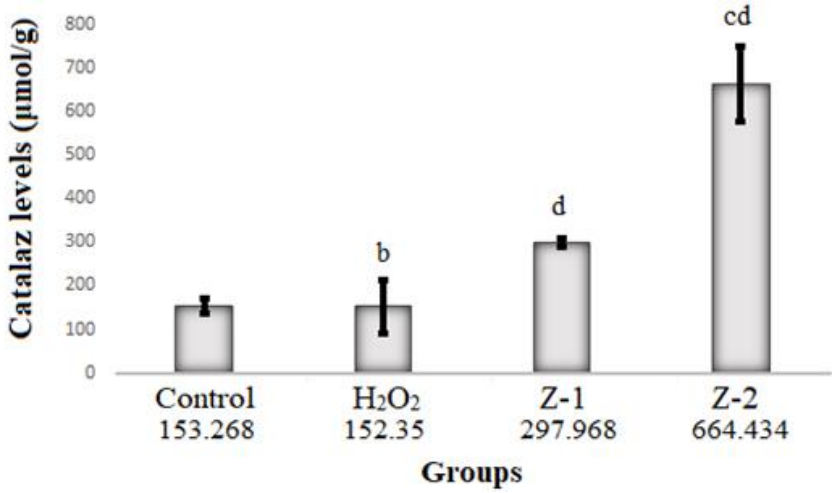

Figure 4. *The effect of Zinc Oxide on CAT level in S. cerevisiae cell (b: p < 0.05, d: p < 0,001, cd: p < 0.0001)*

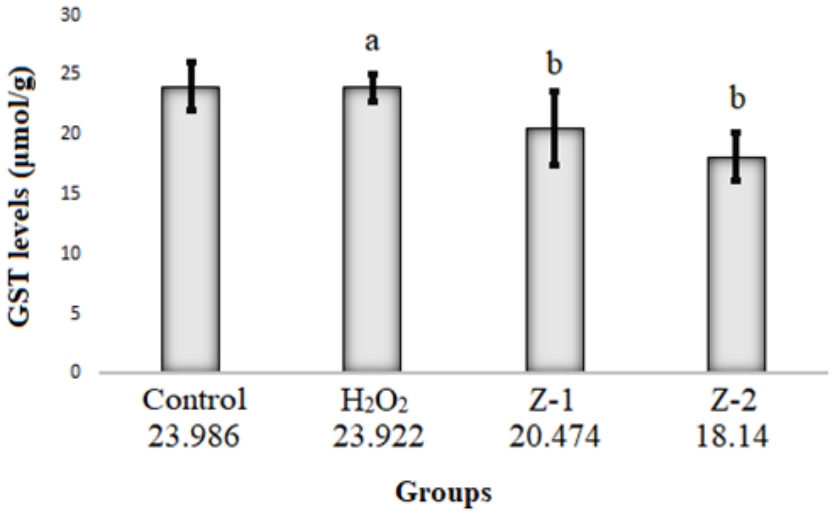

Figure 5. *The effect of Zinc Oxide on GST level in S. cerevisiae cell (a: p > 0.05, b: p < 0.05)*

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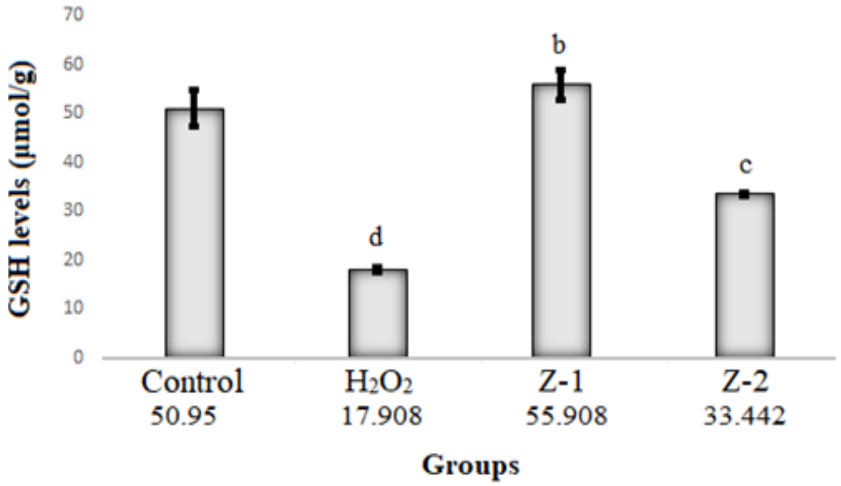

Figure 6. *The effect of Zinc Oxide on GSH level in S. cerevisiae cell (b: $p < 0.05$, c: $p < 0.01$, d: $p < 0.001$)*

Oxidized glutathione (GSSG) levels elevated at low concentrations ($p < 0.001$) and partially dropped at high concentrations ($p < 0.05$) compared to the control group. There was a significant elevation in the H_2O_2 group ($p < 0.0001$) (*Fig. 7*).

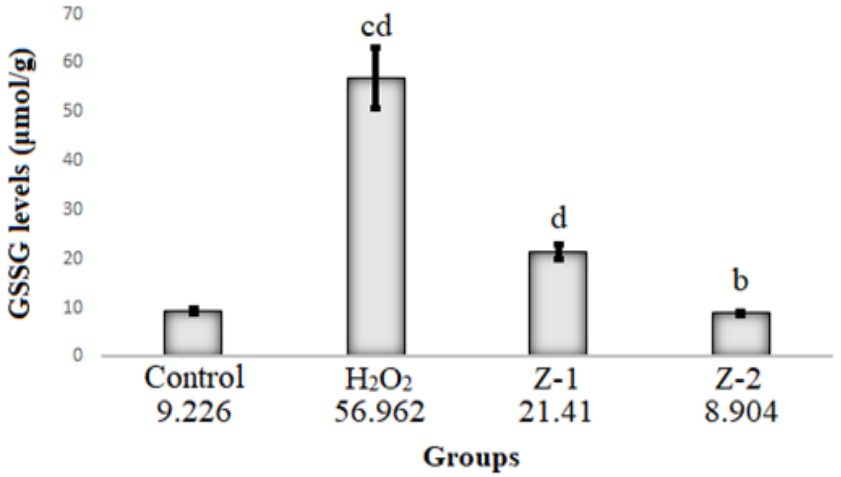

Figure 7. *The effect of Zinc Oxide on GSSG level in S. cerevisiae cell (b: $p < 0.05$, d: $p < 0.001$, cd: $p < 0.0001$)*

When the effect of ZnO NP on total protein level was examined, it was observed that there was an elevation in the group treated at low concentration ($p < 0.001$) and a drop at high concentration and in the group treated with H_2O_2 ($p < 0.05$; $p < 0.0001$) (*Fig. 8*).

Table 1 shows the effect of ZnO NP on the amount of fatty acids in *S. cerevisiae* yeast cells. The results showed that the level of myristic acid (C14:0) changed inversely proportional to the concentration. Myristoleic acid (C14:1), palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0) and eicosenoic acid (C20:1) levels elevated in all experimental groups compared to the

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control group. Pentadecanoic acid (C15:0), heptadecenoic (C17:1), and elaidic acid (C18:1 n-9) levels elevated at low concentration and dropped at high concentration and in the group treated with H_2O_2 compared to the control group; pentadecanoic acid (C15: 1), palmitoleic acid (C16:1), heptadecanoic acid (C17:0) and oleic acid (C18:1) levels dropped in all groups compared to the control group.

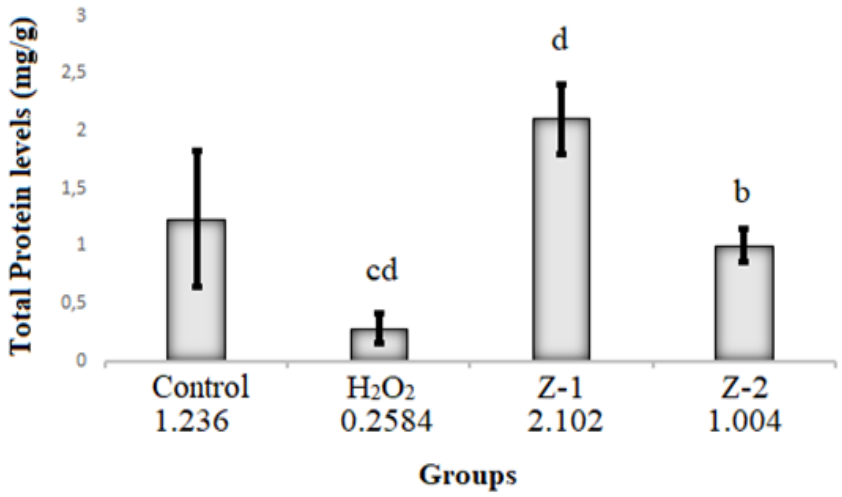

Figure 8. *The effect of Zinc Oxide on total protein level in S. cerevisiae cell (b: p < 0.05, d: p < 0.001, cd: p < 0.0001)*

Table 2 shows the effect of ZnO NP on some vitamin and sterol amounts of *S. cerevisiae* yeast cells. Based on the results obtained, it was observed that vitamin D2, α-tocopherol and sitosterol levels elevated depending on the dose; vitamin D3, vitamin K1, ergosterol and β-sitosterol levels elevated at high concentrations and dropped at low concentrations.

Table 1. *The effect of Zinc Oxide on the amount of fatty acids in S. cerevisiae cell*

Fatty acids	Control	H_2O_2	Z-1	Z-2
C14:0	1.338 ± 0.45	0.483 ± 0.02^{d}	1.542 ± 0.42^{b}	0.944 ± 0.19^{c}
C14:1	0.042 ± 0	0.052 ± 0.005^{b}	0.147 ± 0.04^{cd}	0.114 ± 0.04^{d}
C15:0	0.175 ± 0.008	0.123 ± 0.004^{c}	0.336 ± 0.03^{d}	0.169 ± 0.02^{b}
C15:1	1.461 ± 0.27	0.709 ± 0.15^{d}	0.920 ± 0.14^{c}	1.139 ± 0.15^{b}
C16:0	24.128 ± 0.75	46.874 ± 3.12^{d}	30.152 ± 3.57^{b}	30.986 ± 4.83^{b}
C16:1	33.900 ± 0.80	30.411 ± 0.96^{b}	22.961 ± 4.36^{c}	17.032 ± 3.76^{d}
C17:0	0.146 ± 0.01	0.090 ± 0.01^{c}	0.132 ± 0.004^{b}	0.137 ± 0.02^{b}
C17:1	0.115 ± 0.003	0.065 ± 0.02^{d}	0.066 ± 0.005^{d}	0.126 ± 0.006^{b}
C18:0	14.754 ± 1.04	13.680 ± 0.52^{b}	20.492 ± 1.64^{c}	22.101 ± 2.21^{c}
C18:1 n-9	12.849 ± 0	10.28 ± 0	11.614 ± 1.09^{b}	16.264 ± 2.21^{c}
C18:2	4.242 ± 0.36	5.197 ± 0.57^{b}	6.817 ± 1.26^{b}	6.473 ± 0.47^{b}
C18:3	0.241 ± 0.03	0.130 ± 0.008^{c}	0.345 ± 0.02^{c}	0.876 ± 0.26^{cd}
C20:0	0.072 ± 0.01	0.092 ± 0.01^{c}	0.111 ± 0.02^{c}	0.182 ± 0.04^{d}
C20:1	0.159 ± 0.04	0.060 ± 0.008^{d}	0.507 ± 0.15^{cd}	0.264 ± 0.14^{c}

a: p > 0.05, b: p < 0.05, c: p < 0.01, d: p < 0.001, cd: p < 0.0001

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Table 2. *The effect of zinc oxide on amount of vitamins and sterols in S. cerevisiae cell*

Vitamins and sterols	Control	H_2O_2	Z-1	"Z-2
Vitamin D2	0.550 ± 0.18	0.858 ± 0.18^{c}	0.704 ± 0.09^{c}	1.338 ± 0.49^{d}
Vitamin D3	1.852 ± 0.27	3.840 ± 0.60^{d}	1.640 ± 0.24^{b}	2.604 ± 0.71^{c}
α-tocopherol	7.190 ± 0.79	7.802 ± 1.73^{b}	17.276 ± 1.37^{cd}	19.562 ± 6.37^{cd}
Vitamin K1	5.264 ± 0.34	7.196 ± 1.46^{c}	3.206 ± 0.84^{c}	6.440 ± 0.24^{c}
Ergosterol	24.128 ± 0.75	46.874 ± 3.12^{d}	23.634 ± 1.98^{a}	30.986 ± 4.83^{c}
Sitosterol	1.342 ± 0.64	0.298 ± 0.21^{cd}	1.450 ± 0.23^{b}	4.890 ± 1.12^{cd}
β-sitosterol	2.000 ± 0.61	3.790 ± 1.48^{d}	1.940 ± 0.73^{b}	3.934 ± 1.10^{d}

a: $p > 0.05$, b: $p < 0.05$, c: $p < 0.01$, d: $p < 0.001$, cd: $p < 0.0001$

The results of the present study revealed that zinc oxide nanoparticles had various effects on biochemical parameters in *S. cerevisiae* yeast culture to which they were applied at different doses. A significant statistical difference was found between the control group and the other groups for biochemical parameter content.

A balance prevails between oxidants and antioxidants in organisms under normal conditions. If this balance is disturbed against antioxidants, this would cause oxidative stress. According to Syama et al. (2013) NPs cause oxidative stress in animals and have a detrimental effect on the antioxidant system.

Endocytosis and cohesion are two of the most common mechanisms for NPs to penetrate the cell. It is still not exactly known the mechanism of the damage caused by NPs, which begin to cause toxicity as soon as they penetrate the cell. However, their main known effects are oxidizing and disintegrating the cell membrane, reducing energy production in the cell, increasing reactive oxygen complexes and releasing toxic chemicals. The oxidation of membrane proteins and lipids represents the most toxic effect on the cell membrane structure and lipid peroxidation is a major benchmark for scientific research. The MDA level in a cell is utilized as a scale to assess the damage caused by a dangerous agent.

Zinc and the SOD enzyme catalyze the breakdown of the superoxide radical into less harmful O_2 and H_2O_2 and is then detoxified by CAT and glutathione peroxidase (GPx). The NADPH oxidases are inhibited and ROS production is reduced. Zinc also helps to antagonize redox-active transition metals such as iron and copper, which stimulate the formation of free radicals through fenton reactions. These transition metals form complexes with cellular components such as carbohydrates, enzymes, nucleotides, DNA and citrates. Once metal complexes develop, the metal entraps and forms a reactive hydroxyl radical (OH) by interacting with H_2O_2. This results in lipid peroxidation and extensive damage to proteins, DNA and tissues. Zinc causes a reduction in localized oxidative damage by substituting copper and iron in the body. In a study, zinc supplements reduced MDA, one of the by-products associated with oxidative stress, in the plasma of healthy human beings (Gammmoh, 2017).

In their study that aimed to assess the therapeutic effect of ZnO NPs on oxidative damage and impairment in the antioxidant defense system caused by cyclophosphamide (CP) in male albino rats, Shkal et al. (2020) found that treatment of rats with ZnO NPs in combination with CP alleviated the oxidative damage and impairment in the antioxidant defense system caused by CP. They also determined that ZnO NPs reduced free radicals and lowered MDA levels by protecting cell membranes against oxidative

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damage. They reported that administration of ZnO NP in combination with CP caused a significant drop in TBARS reactive substance levels. Administration of ZnO NPs in combination with CP restored enzyme levels by reducing the formation of lipid peroxidation. ZnO NPs dropped free radical levels by improving the activities of antioxidases. Researchers have attributed such improvements in the antioxidant defense system to the antioxidant properties and/or free radical scavenging capacity of ZnO NPs (Kasperczyk et al., 2012). Decrease in MDA concentration of *S. cerevisiae* supports the findings of the present study. The decrease in the concentration of MDA in groups ZnO (100 and 205 mg) suggests stimulated lipid peroxidation causing to cell damage and antioxidant defense mechanisms can prevent formation of excessive free radicals.

SOD and CAT are the two major enzymes that protect the biological system as the first line of defense against oxidative damage in cells. SOD protects cells against superoxide anion, one of the radicals that cause serious damage to cell components, and CAT protects cells from the harmful effects of these reactive oxygen species by acting on hydrogen peroxide-a non-radical but highly dangerous for cells, which can turn into hydroxyl radical. NPs affect the SOD and CAT enzyme activities of living organisms. In this study, it was observed that SOD level did not significantly differ against the exposure to ZnO NP, whereas CAT levels elevated depending on the dose in all groups compared to the control group. SOD is an anti-superoxide enzyme that catalyzes the dismutation of superoxide to H_2O_2 to be used by CAT (Canli et al., 2019). H_2O_2 releasing as a result of SOD activity was thought to elevate the CAT level depending on the dose.

Both enzymatic and non-enzymatic biomarkers are affected by NPs. Various glutathione products (rGSH, tGSH, GSSG)–effective antioxidants in metabolism–are among the most frequently used biomarkers. Glutathione is a reductive tripeptide that eliminates free radicals. Consequently, many studies have shown that either elevated or dropped glutathione levels can be a marker of oxidative stress in the body CAT (Canli et al., 2019). This indicates that NPs can alter metabolism by either intervening in enzymatic processes or binding to enzyme active sites. GSH is the most critical non-protein thiol in all living cells and plays a vital role in intracellular protection against toxins such as Cu and Zn CAT (Canli et al., 2019). Maintaining intracellular GSH levels is not only critical for the health of living organisms but also protects cells from the harmful effects of oxidative stress. GST is an enzyme that acts in intracellular GSH metabolism and maintenance of GSH levels and protects cells from oxidative damage by using GSH as a cofactor or substrate. In this study, ZnO nanoparticles applied to *S. cerevisiae* culture medium dropped the GST level depending on the dose, but elevated GSH and GSSG levels with inverse correlation at a low dose and lowered at high dose. The elevated amount of GSH at low doses suggests that the yeast cell may have developed a defense mechanism against ZnO NP and the lowered amount of GSH at high doses suggests that the cell adapts to oxidative damage.

Fatty acids of C14:0, C14:1, C15:0, C15:1, C16:0, C16:1n-7, C17:0, C18:0, C18:1n-9, C18:2n-6, C18:3, C20:0 and C20:1 were determined in the yeast cells in the present study. Unsaturated fatty acids, C16:1n-7, C18:1n-9, C18:2n-6 and C18:3 are very important in human nutrition and health. In case of stress, the liquid mosaic structure is preserved by regulating the synthesis of unsaturated fatty acids in the membrane structure. Different doses of ZnO increased the amounts of palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0) and eicosenoic acid (20:1). According to this result, it can be asserted that different

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doses of ZnO induce both fatty acid synthase activity and Δ12desaturase enzyme activities. Because palmitic acid (C16:0) is the end product of the fatty acid synthase enzyme and linoleic acid (C18:2n-6), the Δ12desaturase enzyme. There was an increase in these fatty acids.

The basic structure of eukaryotic cell membranes is mostly composed of sterols. Ergosterol–the main sterol in yeast–is important both for membrane fluidity and permeability and the activity of membrane-bound enzymes. Also, it is a major pharmacological mediator and is known to be a precursor of vitamin D2 (Saddick et al., 2017). Ergosterol is commercially produced by yeast culture for use in production of vitamin D2 and cortisone. *S. cerevisiae* under oxidative stress has an adaptive response to ROSs similar to human cells. It is also known that ergosterol, the main sterol of the yeast cell, accounts for the permeability and fluidity properties of the membrane structure and the activities of membrane-bound enzymes. Ergosterol produced by yeast is also widely used in the production of vitamin D and cortisone (Saddick et al., 2017). In recent years, most studies have been focused on increasing production of yeast ergosterol. On the other hand, α-tocopherol is a vitamin E precursor known to exhibit the highest antioxidant activity (Arnezeder and Hampel, 1990). In this study, levels of vitamin D2, vitamin D3, α-tocopherol, ergosterol, β-sitosterol and sitosterol increased in yeast cells under the influence of ZnO NP. It was determined that yeast cells reacted differently with respect to dose and substance content of the used materials.

Conclusion

The development of nanotechnology has resulted in the emergence of nanoscience as a discipline and is now used in many products for different purposes. Due to their high stability and anti-corrosion properties, ZnO NP has been widely used in various products such as biomedical applications, biosensors, LCDs, solar cells, optics, electricity and ceramics. Besides, they also appear in products that are constantly used in daily life such as rash/sunscreens, paper, paint, food, plastics and cosmetics. Soon, NPs are anticipated to dominate the fields of biotechnology and materials science. In addition to their potential advantages, further information and studies are needed on the toxicity of NPs, which rapidly introducing into our lives.

Since ZnO NPs occupy a large surface area and have small sizes, they are known to pass through cell membranes, lead to toxic effects, interact with cell macromolecules, and create differences in their structures. Based on the findings of our experimental study, antioxidant enzyme (SOD, KAT) activities of *S. cerevisiae* cells exposed to ZnO NP increased, indicating that the cell was subjected to oxidative stress. However, intracellular defense prevented the elevation of MDA levels. The drop in total protein levels– from the biochemical parameters used to assess the overall health of organisms exposed to toxic substances–at high ZnO NP dose indicated that the type, dose and properties of NPs are important factors affecting toxicity. ZnO NP elevated some of the fatty acid, vitamin, and sterol levels in yeast cells but also lowered some others.

In conclusion, the amounts of ZnO NP used in the experiment were observed to trigger biochemical alterations in yeast cells. Furthermore, the stress in the organism was found to develop due to extremely high doses of ZnO NPs. Previous studies have found that all NPs are not toxic in nature and exert a positive effect on the physiological or morphological properties of living organisms. The fact that NPs are becoming increasingly widespread and have become an important part of human life has made it

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even more essential to examine their effects on the ecosystem without overlooking them. Based on findings of previous studies and the present study, although NPs may be beneficial at low doses, they may have toxic effects at high doses and adversely affect the organism and the ecosystem. For a more comprehensive understanding of the effects of NPs on other living organisms and their biochemical, physiological and molecular consequences, further studies are needed.

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Declaration of conflicting interests and ethics. The authors declare no conflict of interest. This study complies with research and publishing ethics and rules.

Author contributions. The authors declared that they contributed equally to the article.

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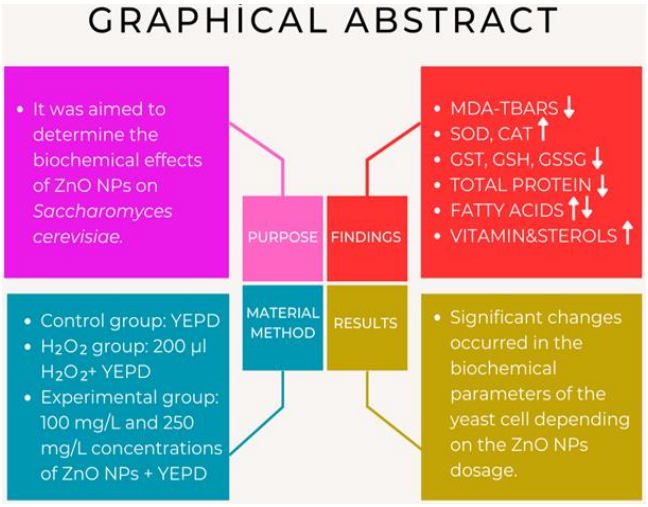
APPENDIX



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