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Saccharomyces Cerevisiae Sulu Lizatı ile Sentezlenen Çinko Oksit Nanopartiküllerinin In-Vitro Yara İyileşmesi Modelinde Etkilerinin İncelenmesi

Investigation of The Effects of Zinc Oxide Nanoparticles Synthesized By *Saccharomyces Cerevisiae* Aqueous Lysate on In-Vitro Wound Healing Model

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ÖZ

Amaç: Bu çalışmanın amacı hamur mayası (*Saccharomyces cerevisiae*) sulu lizatı ile sentezlenen çinko oksit nanopartiküllerinin (ZnONPs) L929 fare fibroblast hücreleri üzerindeki toksik ve yara iyileştirici etkilerini incelemektir.
Yöntem: *Saccharomyces cerevisiae* sulu lizatı kullanılarak çinko oksit nanopartikülleri mikrodalga yöntemiyle sentezlenmiştir. ZnONPs karakterizasyonu Ultraviyole-Görünür bölge spektroskopisi (UV-Vis), SEM ve Zeta sizer ile gerçekleştirilmiştir. 1, 10, 100, 1000 µg/mL konsantrasyondaki ZnONPs'lerin toksik davranışları ve yara iyileşmesi üzerindeki etkileri *in-vitro* olarak L929 hücrelerinde incelenmiştir.
Bulgular: UV spektrumunda ZnONPs'ye spesifik 360-380 nm'de keskin pik görülmüştür. Zeta analizinde ZnO nanopartiküllerinin ortalama boyutu 512.8±16 nm ve zeta yükü ise -30.38±3.12 mV olarak ölçülmüştür. ZnONPs uygulanan L929 hücrelerinin doza bağımlı olarak toksik etki göstermediği bulunmuştur. 10, 100 ve 1000 µg/mL ZnONPs uygulanan L929 hücrelerinin yara kapanması miktarında kontrol grubu hücrelerine göre anlamlı oranda artış tespit edilmiştir.
Sonuç: *Saccharomyces cerevisiae* sulu lizatı ile sentezlenen çinko oksit (ZnO) nanopartiküllerinin in-vitro yara iyileştirici etkileri bu nanopartiküllerin ilaç ve kozmetik endüstrisinde kullanılabilme potansiyeli olduğunu göstermektedir.

Anahtar Kelimeler: Çinko oksit, Nanopartikül, Yara iyileşmesi, *Saccharomyces cerevisiae*, Hücre proliferasyonu.

ABSTRACT

Objective: The objective of this study was to examine the toxic and wound-healing behaviours of zinc oxide (ZnO) nanoparticles synthesized with an aqueous lysate of sourdough (*Saccharomyces cerevisiae*) on L929 mouse fibroblast cells.
Method: Zinc oxide nanoparticles were synthesized by microwave method using the aqueous lysate of *Saccharomyces cerevisiae*. Characterization of ZnO nanoparticles was accomplished with Ultraviolet-Visible region spectroscopy (UV-Vis), SEM and Zeta sizer. The toxic behavior of ZnONPs at concentrations of 1, 10, 100, 1000 µg/mL and their effects on wound healing were investigated *in-vitro* in L929 cells.
Results: A sharp peak was observed at 360-380 nm specific to ZnO in the UV spectrum. In the zeta analysis, the mean size of ZnO nanoparticles was 512.8±16 nm and the zeta charge was -30.38±3.12 mV. It was found that L929 cells treated with ZnONPs did not show dose-dependent manner. A significant increase was found in the wound closure amount of L929 cells applied 10, 100 and 1000 µg/mL ZnONPs compared to the control group cells.
Conclusion: In-vitro wound healing effects of zinc oxide (ZnO) nanoparticles synthesized with *Saccharomyces cerevisiae* aqueous lysate show that these nanoparticles have the potential to be used in the pharmaceutical and cosmetic industries.

Key words: Zinc Oxide, Nanoparticles, Wound Healing, *Saccharomyces cerevisiae*, Cell proliferation.

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1. INTRODUCTION

Size-controlled synthesis of metal particles has made important contributions to the development of nanotechnology. With the development of nanoscale synthesis methods, the production and industrial use of metals such as gold, silver, copper, iron and zinc has gained momentum. Zinc oxide nanoparticles (ZnONPs) are frequently used in biomedical applications due to their anti-bacterial, anti-fungal and anti-cancer behaviors. In addition, due to its UV light absorption feature, ZnONPs are included in the content of sunscreen creams in the cosmetic industry (1-3).

Three different strategies, chemical, physical, and biological have been developed for the synthesis of nanoparticles. Among them, the biological synthesis approach is the most promising synthesis approach due to its nominal cost, biocompatibility and environmentally friendly features. In the biological synthesis of ZnONPs, extracts of bacteria, yeast, as well as extracts from parts of plants such as roots, stems, leaves, fruits and bark are used. Biological synthesis of ZnONPs was carried out with extracts such as *Fusarium oxysporum* (4), *Aspergillus niger* (5), *Trichoderma harzianum* (6). *Saccharomyces cerevisiae* is a yeast species utilized in the production of bread, wine and beer. It has been reported that *Saccharomyces cerevisiae* contains molecules such as oleic acid, linoleic acid, maleic acid, thiamine, biotin (7,8). These biocomponents contained in *Saccharomyces cerevisiae* facilitate the nano-sized synthesis of metal particles.

In this study, ZnONPs were synthesized with the help of *Saccharomyces cerevisiae* aqueous lysate. After characterization studies, the toxic effects of ZnONPs on L929 mouse fibroblast cells were investigated. In addition, the effects of ZnONPs on wound healing of L929 mouse fibroblast cells were carried out.

2. METHOD

Materials

All the reagents, solvents, and materials were commercially purchased from Sigma-Aldrich and Merck. The compounds and solvents were appropriately purified, if necessary. All cell culture supplements and medium for cell culture studies were purchased from Gibco Company.

Preparation of *Saccharomyces cerevisiae* aqueous lysate

Saccharomyces cerevisiae (ATCC 9763) was obtained from the American type culture collection and was grown in YPD (1% Yeast extract, 2% peptone, 2% dextrose) medium at 30 ^{o}C and 180 rpm ambient conditions for 48 hours. The culture medium containing *Saccharomyces cerevisiae* was homogenized in an ultrasonic homogenizer (Bandelin UW2070). The obtaining homogenate was precipitated at 4000 rpm for 5 minutes. The supernatant part was taken and used in nanoparticle synthesis (9).

Synthesis of zinc oxide nanoparticles

20 mL of 10 mM Zinc sulfate and 20 mL of *Saccharomyces cerevisiae* aqueous lysate were mixed in a 100 mL beaker for 2 hours at room temperature. The solution was kept in a

750W microwave oven for 5 minutes. The obtained ZnONPs were precipitated at 4000 rpm for 5 minutes. The precipitated crystals were washed 5 times with cold distilled water. The obtained nanoparticles were dehydrated in an oven at 60 °C overnight (10).

Characterization of zinc oxide nanoparticles

The maximum energy peak absorbed by the prepared nanoparticles in the UV-Visible region (200-800nm) was analyzed in a spectrophotometer (Biotech Epoch). Particle size and surface charge measurements were made with Zeta sizer (Malvern Epotek).

Cell culture

L929 mouse fibroblast cells (ATCC) taken from liquid nitrogen were cultured in DMEM medium with 10% FBS, 1% penicillin/streptomycin containing L-glutamine and HEPES, in 25 cm^2 flasks, 5% CO_2 and 37 ^oC ambient conditions. When the cells filled the flasks 80-90%, they were passaged into new 25 cm^2 and 75 cm^2 flasks and the stocks were multiplied for use in the experiments (11).

Cytotoxicity Assay

L929 cells were added to 96-well plates with 100 µL of medium at $5x10^3$ cells per well. Cells were incubated for 24 hours in the incubator for adhesion. At the end of the incubation, ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate were applied to the cells at a concentration of 1, 10, 100, 1000 µg/mL. Cells were incubated for 24 hours. At the end of the incubation period, 10 µL of MTT (5 mg/mL) reagent was added to the cells in each well and, the cells were incubated for 4 hours. At the end of the incubation, the formed formazan dye was dissolved in DMSO. The absorbance of the resulting color was read in the spectrophotometer at a wavelength of 570 nm. Experiments were carried out in three independent repetitions (12).

In-vitro wound healing assay

L929 cells were added in 12-well plates at a density of $5x10^5$ cells per well. The cells were incubated for 24 hours in the incubator for adherence to the bottom of the plate. The middle of each well was drawn with a pipette tip to create a wound model. ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate were applied to the cells at a concentration of 1, 10, 100, 1000 µg/mL. Cell images were taken at 0 and 24 hours with inverted microscope (Zeiss Axio Vert.A1), and the amount of wound closure was calculated with the ImageJ program (13).

Statistical Analysis

Statistical analysis was accomplished utilizing GraphPad Prism version 7.0 software. Experiments were repeated 3 times and the results were given as mean ± SD. Dissimilarity between groups were analyzed by student's t test. Statistical matter is described as follows: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

3. RESULTS

Characterization of zinc oxide nanoparticles

Green synthesis is a low-cost method that does not require complicated equipment or toxic chemicals. In this study, zinc oxide nanoparticles were synthesized using *Saccharomyces cerevisiae* aqueous lysate (Figure 1A). The characterization of the synthesized nanoparticles was achieved by UV-Visible spectroscopy. When the UV spectrum is examined, the surface plasmon resonance peak at 365 nm supports zinc oxide synthesis (Figure 1B). The mean particle size of the prepared nanoparticles with Zeta sizer was calculated as 512.8±16 nm (Figure 1C). In addition, the zeta charge of ZnONPs was found to be -30.38±3.12 mV (Figure 1D).

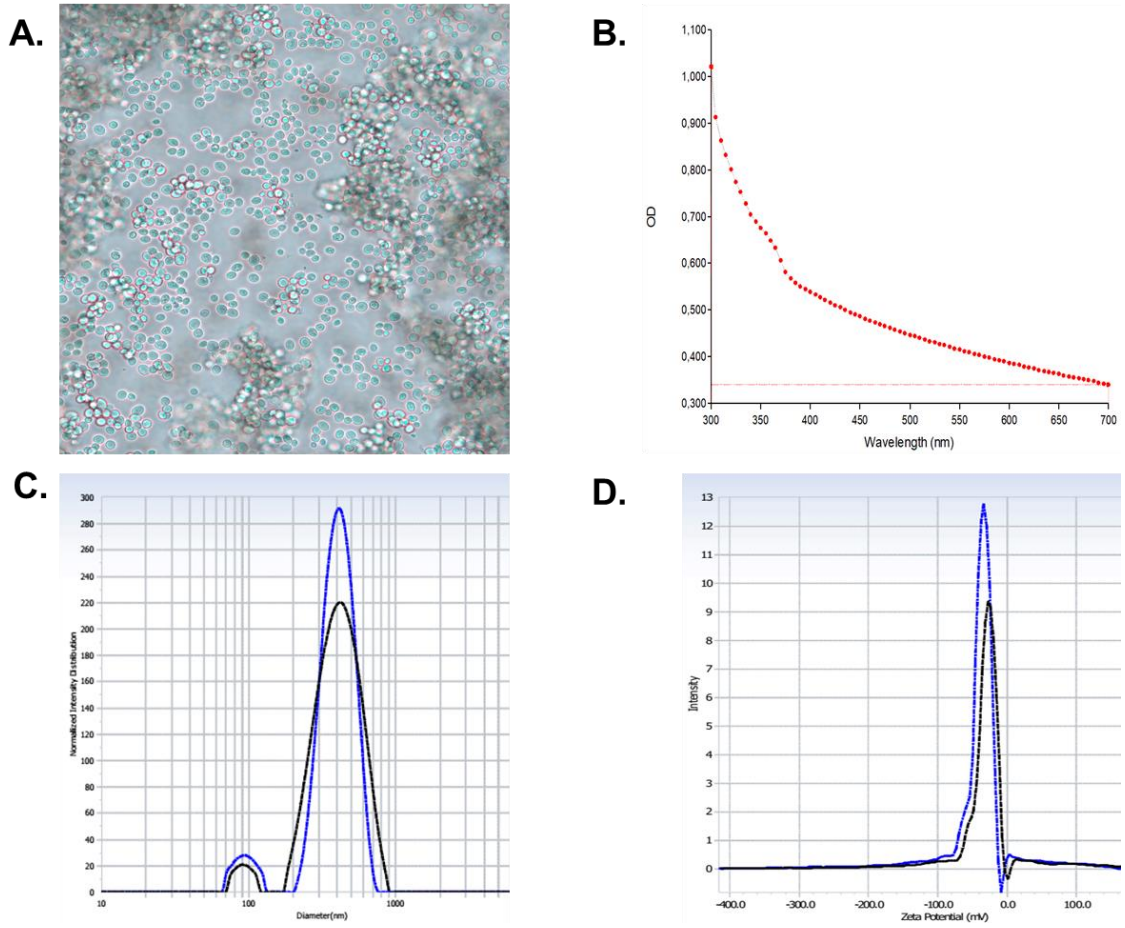

Figure 1. A) Inverted microscope images of *Saccharomyces cerevisiae* (Sourdough) at 40X magnification. **B)** UV spectrum of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate **C)** Size distribution graph of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate **D)** Zeta potential graph of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate

Toxic effects of zinc oxide nanoparticles on L929 cells

After the application of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate to L929 cells at a concentration of 1, 10, 100, 1000 µg/mL, the morphological changes were not observed in the these cells (Figure 2A). In addition, after the application of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate at a concentration of 1, 10, 100, 1000 µg/mL, any toxic effect was not detected L929 cells compared to the control group (Figure 2B).

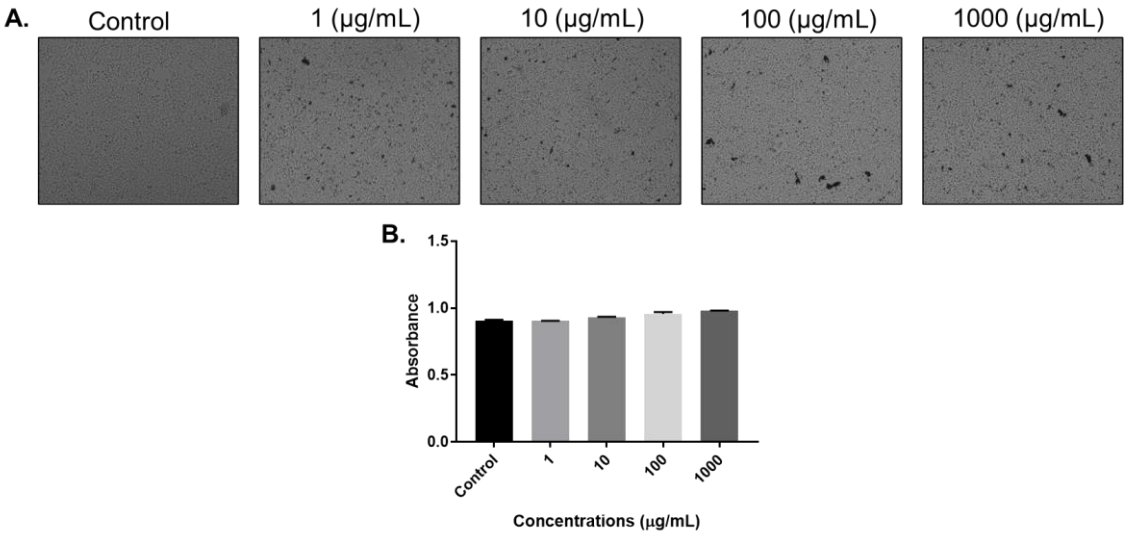

Figure 2. Toxic effects of ZnONPs synthesized by *Saccharomyces cerevisiae* aqueous lysate on L929 cells. **A)** Inverted microscope images of L929 cells at 5X magnification after ZnONPs application. **B)** Viability graph of L929 cells after ZnONPs administration.

Effects of zinc oxide nanoparticles on wound healing in L929 cells

After the application of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate to L929 cells at a concentration of 1, 10, 100, 1000 µg/mL, an increase in wound closure of these cells was observed (Figure 3A). In addition, after the application of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate at a concentration of 10, 100, 1000 µg/mL, there was a statistically important increase in wound closure of L929 cells as a dose-dependent (Figure 3B).

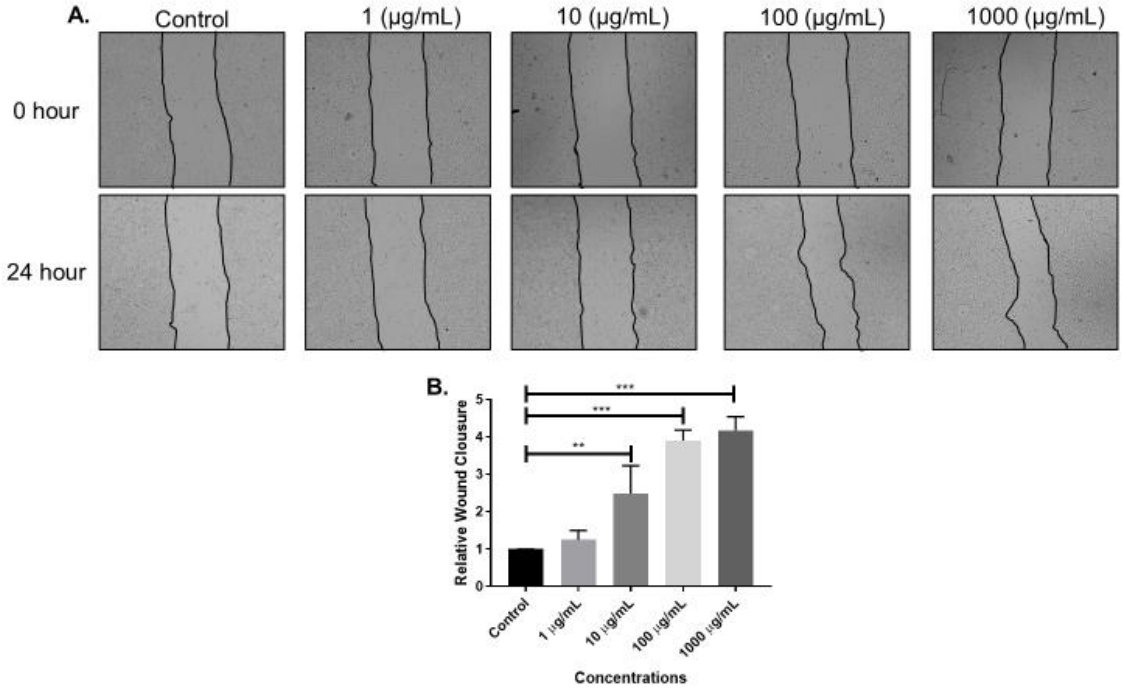

Figure 3. Effects of ZnONPs synthesized by *Saccharomyces cerevisiae* aqueous lysate on wound healing of L929 cells. **A)** Inverted microscope wound closure images of L929 cells at 5X magnification after ZnONPs application. **B)** Graph of relative wound closure of L929 cells after ZnONPs administration.

4. DISCUSSION

The use of metal oxide nanoparticles in biomedical applications has gained great momentum (14). Zinc oxide nanoparticles have a wide range of uses in the medical industry due to their potential properties such as drug delivery system, anti-cancer, anti-bacterial agent (15). In the biosynthesis of zinc oxide nanoparticles, microorganisms or extracts obtained from parts of plants such as roots, seeds, fruits and leaves are used. Since these extracts have a rich content of biogenic chemicals such as polyphenolic compounds, vitamins, amino acids, alkaloids and terpenoids, they are more preferred in the synthesis of nano-sized zinc oxide particles (16).

Synthesis of zinc oxide nanoparticles was achieved with extracts such as *Deverra Tortuosa, Azadirachta indica, Atalantia monophylla* rich in biogenic chemicals (17-19). It has been reported that *Saccharomyces cerevisiae* lysate is rich in ornithine, pantothenate, caprylic acid, choline, acetophenone, glycocholic acid, biotin and thiamine (20). Since B group vitamins are known to accelerate wound healing, ZnONPs were synthesized using *Saccharomyces cerevisiae* aqueous lysate rich in biotin and thiamine, and the effects of these nanoparticles on wound closure of L929 mouse fibroblast cells were investigated *in-vitro*.

UV-visible spectroscopy is one of the most used analytical techniques for the characterization of zinc oxide nanoparticles. The specific surface plasmon resonance peak of zinc oxide nanoparticles synthesized by green synthesis is in the range of 340-400 nm. Chen et al. reported the maximum absorbance peak of 360 nm in the UV spectrum of zinc oxide nanoparticles synthesized with *Scutellaria baicalensis* root extract (21). Hamk et al. reported that the surface plasmon resonance peak of zinc oxide nanoparticles they synthesized with *Bacillus subtilis* supernatant was at 341 nm (22). In this study, the maximum absorbance peak of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate was found to be 365 nm.

In-vitro wound healing assay is a technique in which the migration and proliferation potentials of cells are evaluated under various conditions. In this experiment, a line that mimics a wound is created with a micropipette tip in cells that proliferate as a monolayer. By applying therapeutic or toxic agents to the cells, the amount of closure of this wound created with a pipette tip is measured (23). When wound healing experiments performed with metal nanoparticles were examined, Erdoğan et al. reported that silver nanoparticles synthesized with *Citrus aurantium* reduced in-vitro wound closure in U87 glioblastoma cells (24). Majhi et al. reported that zinc oxide-coated silver nanoparticles enhanced wound healing in HaCaT human keratinocyte cells (25). Batool et al., on the other hand, reported that dressing containing 30 ppm ZnO synthesized with *Aloe barbadensis* leaf extract in a wound model created in albino mice in an in-vivo study completely closed the wound in 11 days (26). In this study, it was determined that in the experimental wound healing model formed in L929 mouse fibroblast cells, after 24 hours application of ZnONPs synthesized with *Saccharomyces cerevisiae* aqueous lysate to the cells, wound closure increased approximately 4 times more than in the control group.

5. CONCLUSION

With the synthesis of zinc oxide nanoparticles by green chemistry, costly equipment, use of toxic chemicals and waste of time are avoided. The wound healing effect of zinc oxide nanoparticles synthesized using *Saccharomyces cerevisiae* aqueous lysate, which is rich in biogenic molecules, in L929 mouse fibroblast cells was demonstrated in this study. The results of this study support the use of zinc oxide nanoparticles synthesized using *Saccharomyces cerevisiae* aqueous lysate in wound healing creams and dressings.

Conflict of interest statement

The authors declare that there is no conflict of interest for this research.

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