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Detoxification Efficiency of Micropropagated *Alternanthera reineckii* Briq. against Zinc Oxide Nanoparticles in Human Keratinocyte Cells

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ABSTRACT

Considering the rapid developments in nanotechnology, scientific research in the field of nanotoxicology is required in order to prevent the dangers of nanotechnology on human health. For this purpose, we tested the cytotoxic effect of ZnO nanoparticle (NP), which is included in many cosmetic products, on human keratinocyte cells (HaCaT). In addition, we evaluated to potentially inhibit this cytotoxic effect with an aquatic plant, *Alternanthera reineckii* Briq. produced by tissue culture method. The nodal explants of *A. reineckii* were cultured in Murashige & Skoog basal medium (MS) including the combinations of 0.25-1.25 mg/L Thidiazuron (TDZ) and 0.25 mg/L indole-3-butyric acid (IBA). Maximum number of shoots per explant (22.50 shoots/explant) was obtained in the culture medium with 0.75 mg/L TDZ+0.25 mg/L IBA. The highest shoot length (1.77 cm) was determined in MS medium with 0.25 mg/L TDZ+0.25 mg/L IBA. Acetone and water extracts were obtained from *A. reineckii* through Soxhlet extraction. The cytotoxic effect of ZnO alone on HaCaT was inhibited by acetone and water extracts. The cell viability, which decreased to 26.04% with the effect of ZnO, increased up to 67.83% with the application of acetone extract. Overall, our results revealed the protective potential of this plant against nanotoxicity induced by ZnO and shed light on future studies.

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Mikroçoğaltılan *Alternanthera reineckii* Briq.'nin İnsan Keratinosit Hücrelerinde Çinko Oksit Nanopartiküllerine Karşı Detoksifikasyon Etkinliği

ÖZET

Nanoteknolojideki hızlı gelişmeler göz önüne alındığında, nanoteknolojinin insan sağlığı üzerindeki tehlikelerinin önüne geçebilmek için nanotoksikoloji alanında bilimsel araştırmalara ihtiyaç duyulmaktadır. Bu amaçla birçok kozmetik üründe yer alan ZnO nanopartikülünün (NP) insan keratinosit hücreleri (HaCaT) üzerindeki sitotoksik etkisini test ettik. Ek olarak, doku kültür yöntemi ile üretilen bir su bitkisi olan *Alternanthera reineckii* Briq. ile bu sitotoksik etkiyi inhibe etme potansiyelini değerlendirdik. *A. reineckii*'nin nodal eksplantları, 0,25-1,25 mg/L Thidiazuron (TDZ) ve 0,25 mg/L indol-3-bütirik asit (IBA) kombinasyonlarını içeren Murashige & Skoog bazal ortamında (MS) kültüre edildi. Eksplant başına maksimum sürgün sayısı (22,50 sürgün/eksplant) 0,75 mg/L TDZ+0,25 mg/L IBA içeren kültür ortamında elde edilmiştir. En yüksek sürgün uzunluğu (1,77 cm) 0,25 mg/L TDZ+0,25 mg/L IBA içeren MS ortamında belirlenmiştir. *A. reineckii*'den Soxhlet ekstraksiyonu yoluyla aseton ve su özütleri elde edilmiştir. Tek başına ZnO'nun HaCaT üzerindeki sitotoksik etkisi, aseton ve su özütleri ile inhibe edilmiştir. ZnO'nun etkisiyle %26,04'e düşen hücre canlılığı, aseton özütü uygulamasıyla %67,83'e kadar yükselmiştir. Genel olarak, sonuçlarımız bu bitkinin ZnO tarafından indüklenen nanotoksisiteye karşı koruyucu potansiyelini ortaya koymuş ve gelecekteki çalışmalara ışık tutmuştur.

Botanik

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INTRODUCTION

In recent years, nanotechnology has appeared in many areas. Thanks to nanotechnology, analysis of nanometer-sized structures, determination of physical properties of nanometer-sized structures, and superior material production can be made (Das et al. 2019; Mohajerani et al. 2019; Jahan & Isildak, 2021). Nano-sized metal and metal oxide particles are indispensable raw materials of advanced technology and their application areas are spread over many different sectors. Metal and metal oxide nanoparticles (NPs) have high catalytic, magnetic, chemical and optical characteristics. These properties vary according to the surface properties, shapes and sizes of the NPs (Kavitha et al. 2022). Zinc oxide (ZnO) is also an important NP with the aforementioned properties (Sagadevan et al. 2018). ZnO NPs can be used in sensor, surface dyes, textile products, fabrics and materials such as plastics (Uma et al. 2019; Abdullah et al. 2020; Agustina et al. 2020). Moreover, as ZnO particles become transparent when they are reduced to nano-size, they are widely preferred in personal care products, especially sun creams (Gollavilli et al. 2020).

The use of NPs in many areas has also caused their side effects. Due to the very small size of nano materials and their large surface area, they show very high chemical and biological activity. If these particles enter the body and pass for a certain time, diseases such as inflammation, wheezing and coughing may occur (Monsé et al. 2019). Ways of exposure to NPs are by inhalation, digestion, injection into the skin or body (Braakhuis et al. 2015). Especially the use of nanocosmetics has increased in recent years. However, there are risks that may occur with their increased penetration through the skin. Risk of insoluble NPs in sunscreen preparations exists (Lee et al. 2020).

It is important to include herbal extracts in the product, especially in order to reduce the risk of nanomaterials found in cosmetic products. Thus, the possibility of reducing the toxic effect of the nanomaterial will increase. *Alternanthera reineckii* Briq. was used as plant material in this study. *A. reineckii* is an aquatic plant originating from South America. This plant is in a form adapted to living in and out of water thanks to the imbalance in the current at the banks of the Amazon river (Anderson et al. 2015). *A. reineckii* was propagated by tissue culture techniques. Plant tissue culture is the production of plants or plant products from the whole plant or various parts of the plant under sterile conditions in an artificial nutrient medium (El-Sherif 2019; Celik et al. 2020; Ozelci & Yigit, 2022). This method is mainly based on the totipotency property of plant cells. The ability to divide while forming the complete genome of the cell is called totipotency. Apart from the totipotency feature in plants, their growth and metabolism developments are also important (Rani & Kumar 2017). Recently, tissue culture technique has been widely used in many plant-based studies such as antioxidant activity (Dilkalal et al. 2021), stress physiology (Hosseini Tafreshi et al. 2021), and secondary metabolite production (Jirakiattikul et al. 2021). We have benefited from this technique because of its advantages such as preventing the collection of plants from nature and providing rapid and multiple plant production under *in vitro* conditions.

To the best of our knowledge, we found that the protective effect of *A. reineckii* has not been tested against ZnO-induced nanotoxicity. Therefore, in the present study, we propagated *A. reineckii in vitro* in the desired amount and examined its protective property against cytotoxic damage induced by ZnO NPs on the human keratinocyte cells.

MATERIALS and METHODS

ZnO NPs and Their Characterization

For ZnO NPs, nano powder sample, white powder in the nanoscale range <200 nm with a high purity of 99.9+% and CAS number 1314-13-2, purchased from US research Nanomaterials Inc, Houstan, TX, USA, is used in this study. Characterization of ZnO NP is performed with X-ray diffraction (XRD) and scanning electron microscope (SEM) analysis to identify the crystal structure, the average crystallite size and morphology of NP.

In Vitro Regeneration

A. reineckii plants were taken as sterile stock plants. Murashige and Skoog (MS) basal medium with vitamins were used as nutrient media in the culture studies. The nodal explants were placed in MS medium including 30 g/L sucrose, 7 g/L agar and the combinations of 0.25-1.25 mg/L Thidiazuron (TDZ) and 0.25 mg/L indole-3-butyric acid (IBA). It was sterilized by keeping it under 1.2 atmospheres pressure at 121°C for 20 minutes. Then, the nutrient media were sterilized by keeping them under 1.2 atmospheres pressure at 121°C for 20 minutes. The culture experiment was terminated at the end of eight weeks. In the activity studies, *A. reineckii* in the MS nutrient medium containing 0.75 mg/L TDZ + 0.25 mg L IBA,

where the best results were obtained, were used.

Extraction

Plants (10 g) left to dry at room conditions were subjected to extraction (Soxhlet extraction). The filtered extracts were then concentrated by means of a rotary evaporator. The stock extracts obtained were dissolved with 0.5% dimethyl sulfoxide (DMSO) before the experiments.

Culture of the Cell Lines

Dulbecco's Modified Eagle Medium (DMEM) was used for culturing the human keratinocyte cell line (HaCaT). DMEM in high glucose was supplemented with 1% penicillin-streptomycin, 1% L-glutamine and 10% heat-inactivated fetal bovine serum (FBS). The cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Antiproliferative Activities

Cells were seeded at 1×10^4 cells/well in 96-well flat-bottomed microtiter plates. After 24 h incubation, ZnO NPs (50 mg/L) and extracts with different concentrations of *A. reineckii* were added to the wells alone and in combination and kept in a CO_2 incubator at 37°C for 48 h. Final extract concentrations in the wells were numbered between 1-10 and showed in Table 1. Negative control (NC) cultures received 0.5% DMSO alone. MTT procedure was use for cytotoxic activity (Emsen et al. 2018).

Table 1. Different extract experiments obtained from *A. reineckii*
Çizelge 1. A. reineckii'den elde edilen farklı özüt deneyleri

Extracts at different concentrations *(Farklı konsantrasyonlarda özütler)*	Abbreviation of the extract treatments *(Özüt uygulamalarının kısaltması)*
Acetone/water extract at 1.95 mg/L concentration	AE1/WE1
Acetone/water extract at 3.91 mg/L concentration	AE2/WE2
Acetone/water extract at 7.81 mg/L concentration	AE3/WE3
Acetone/water extract at 15.63 mg/L concentration	AE4/WE4
Acetone/water extract at 31.25 mg/L concentration	AE5/WE5
Acetone/water extract at 62.5 mg/L concentration	AE6/WE6
Acetone/water extract at 125 mg/L concentration	AE7/WE7
Acetone/water extract at 250 mg/L concentration	AE8/WE8
Acetone/water extract at 500 mg/L concentration	AE9/WE9
Acetone/water extract at 1000 mg/L concentration	AE10/WE10

Statistical Analyses

Differences between effects of different TDZ-IBA doses on shoot regeneration of *A. reineckii* from nodal explant were tested on Duncan post hoc work, an ANOVA test ($p < 0.05$). Hierarchical clustering and heatmap analyses were used to measure the distances between viabilities in HaCaT cells treated with ZnO NPs alone or combined with acetone and water extracts of *A. reineckii*. SPSS 21.0 was preferred to perform the analyses.

RESULTS

Analysis of SEM Image and XRD Spectrum of ZnO NPs

XRD patterns of resulting material in the range of 2θ = 25–75^0 was obtained (Figure 1). The well-defined sharp Bragg peaks represented extremely crystalline nature of the material with the hexagonal crystal structure (known as zincite). The particle size was changing from 37.3 nm to few hundred nm. The phase identification was made using JCPDS database and the ZnO NPs were well organized and fitted the standard of ZnO (JCPDS no: 36-1451). SEM image of ZnO NP was given in Figure 2. ZnO NPs were distributed in random as in the SEM image.

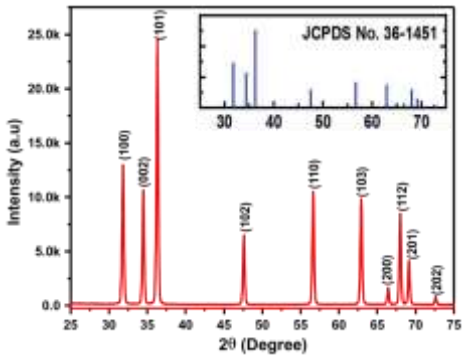

Figure 1. Characterization of ZnO NPs XRD pattern. Inset shows the X-ray diffraction pattern of ZnO nano powder. Standard pattern of ZnO (JCPDS 36-1451)

Şekil 1. ZnO NP'lerin XRD modelinin karakterizasyonu. Ekli küçük resim ZnO nano tozunun X-ışını kırınım modelini gösterir. ZnO'nun standart modeli (JCPDS 36-1451)

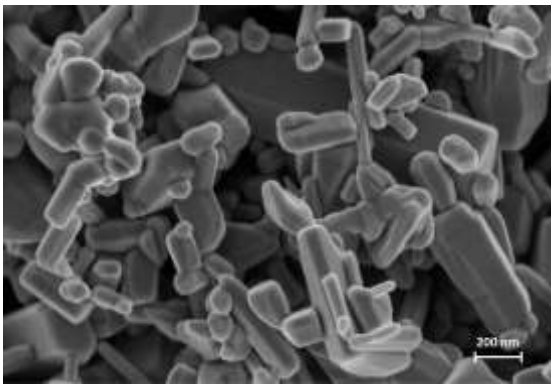

Figure 2. SEM image of ZnO NPs
Şekil 2. ZnO NP'lerin SEM görüntüsü

In Vitro Shoot Regeneration of *A. reineckii*

The nodal explants of *A. reineckii* were cultured for *in vitro* shoot regeneration in MS nutrient solution containing the combinations of 0.25-1.25 mg/L TDZ and 0.25 mg/L IBA. The first shoot formation was observed on the 12th day in the culture media fortified with 0.75 mg/L TDZ + 0.25 mg/L IBA. At the end of six weeks, the experiment was terminated and multiple shoot formation from the nodal explants was recorded (Table 2).

Shoot regeneration percentage was ranked between 66.66% and 100% (Table 2). The lowest shoot regeneration frequency (66.66%) was determined in the explants without growth regulators (control group). Mean shoot lengths ranked between 1.10-1.77 cm. The longest shoot (1.77 cm) was obtained in cultures with 0.25 mg/L TDZ + 0.25 mg/L IBA, while the shortest shoot (1.10 cm) was determined in cultures with 1.25 mg/L TDZ + 0.25 mg /L IBA.

Cytotoxicity Activities

Viability rates of HaCaT cells treated with ZnO NPs and *A. reineckii* extracts were tested. The application that decreased the cell viability (26.04±1.09%) the most was ZnO NPs alone. AE and WE experiments alone showed a certain amount of cytotoxic effect on cells at different concentrations. While AE10 reduced the cell viability rate to 74.61±1.42%, this rate was 86.40±2.06% for WE10 application. The experimental groups with the highest cell viability among the combined AE+ZnO NPs and WE+ZnO NPs applications were AE6+ZnO (67.83±1.84%) and WE2+ZnO (65.85±2.69%), respectively (Figure 4, 5).

Heatmap and HCA analyses ranked experiments according to cell viability data and included extract and ZnO NPs experiments into different clusters. Accordingly, the experiments tested for HaCaT cells were divided into 3 clusters. ZnO NPs experiment was located under cluster 3 alone and was separated from other clusters. Applications under cluster 1 were NC and AE, WE extract trials. The combined applications of extract+ZnO NPs were placed under cluster 2 (Figure 6a).

The colour gradient appearing on the heatmap also coincided with the cluster analysis. According to heatmap analysis, ZnO NPs trial differed from other extract applications with blue color. The other extract trials, except AE9 and AE10, had red color intensity. The viability rates of cells exposed to AE9 and AE10 treatments were 78.83±1.55% and 74.61±1.42%, respectively. Combined applications, except AE8, AE9, AE10 + ZnO NPs, were in black intensity with a medium shade (Figure 6b).

Figure 3. *In vitro* shoot regeneration of *A. reineckii.* Multiple shoot regeneration from nodal explants in MS medium including 0.75 mg/L TDZ + 0.25 mg L IBA after four weeks (a) and (b) eight weeks of culture
Şekil 3. *A. reineckii*'nin *in vitro* sürgün rejenerasyonu. Dört hafta (a) ve (b) sekiz haftalık kültürden sonra 0,75 mg/L TDZ + 0,25 mg L IBA içeren MS ortamında nodal eksplantlardan çoklu sürgün rejenerasyonu

DISCUSSION

While exploring potential application areas of nanotechnology, the risks and uncertainties that NPs may pose on living things and the environment should not be ignored. The size and density of NPs play an important role in the toxic effects of NPs (Braakhuis et al. 2014). Studies on the toxic effects of nanoparticles have been investigated in many different organisms and have produced similar results for different NPs. In a study with ZnO and titanium dioxide (TiO_2) NPs, their ecotoxic effects on the green algae *Pseudokirchneriella subcapitata* were evaluated. The researchers exhibited that algae growth was inhibited by the increase in NP concentration, and ZnO NP caused the cell membrane to become unstable (Lee & An 2013). It is also known

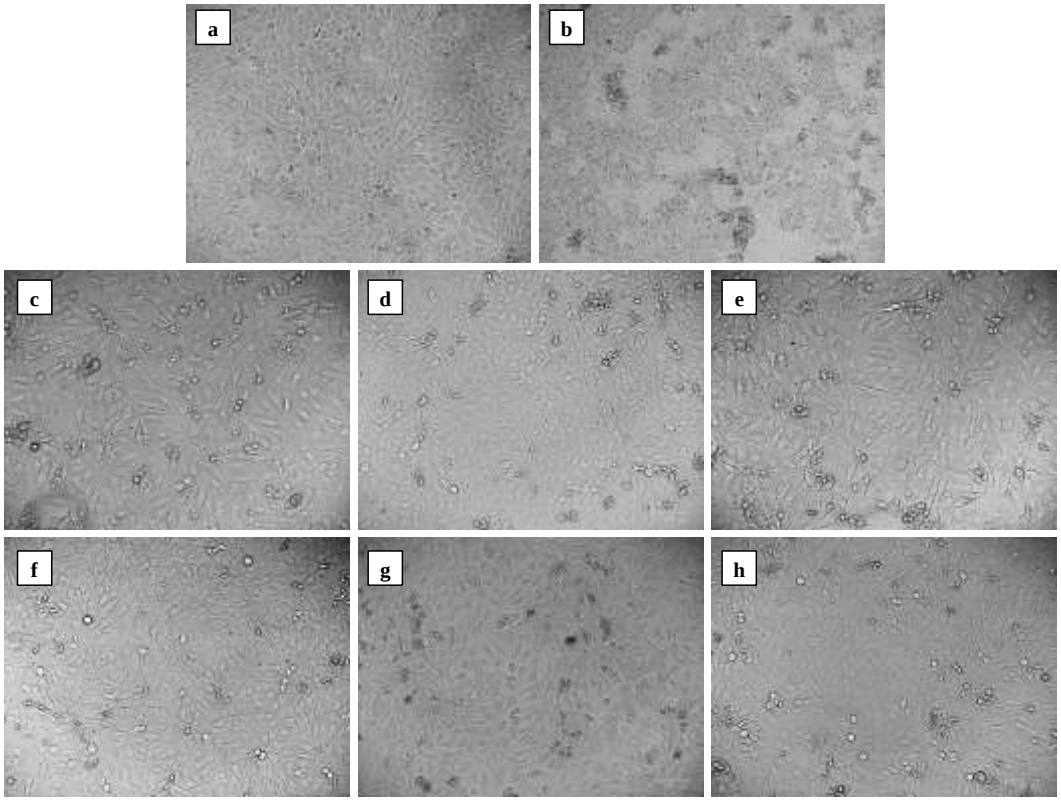

Figure 4. Effect of ZnO NPs alone (b), AE1+ZnO NPs (c), AE5+ZnO NPs (d), AE10+ZnO NPs (e), WE1+ZnO NPs (f), WE5+ZnO NPs (g), WE10+ZnO NPs (h) on cell viability in HaCaT cells observed under the fluorescent cell imager-bright field channel (magnification 175×). Control-treated cells were regarded as 100% viable (a).

Şekil 4. Tek başına ZnO NP'lerin (b), AE1+ZnO NP'lerin (c), AE5+ZnO NP'lerin (d), AE10+ZnO NP'lerin (e), WE1+ZnO NP'lerin (f), WE5+ZnO NP'lerin (g), WE10+ZnO NP'lerin (h) floresan hücre görüntüleyici-parlak alan kanalı (175× büyütme) altında gözlenen HaCaT hücrelerinde hücre canlılığı üzerindeki etkileri. Kontrol ile muamele edilen hücreler %100 canlı olarak kabul edilmiştir (a).

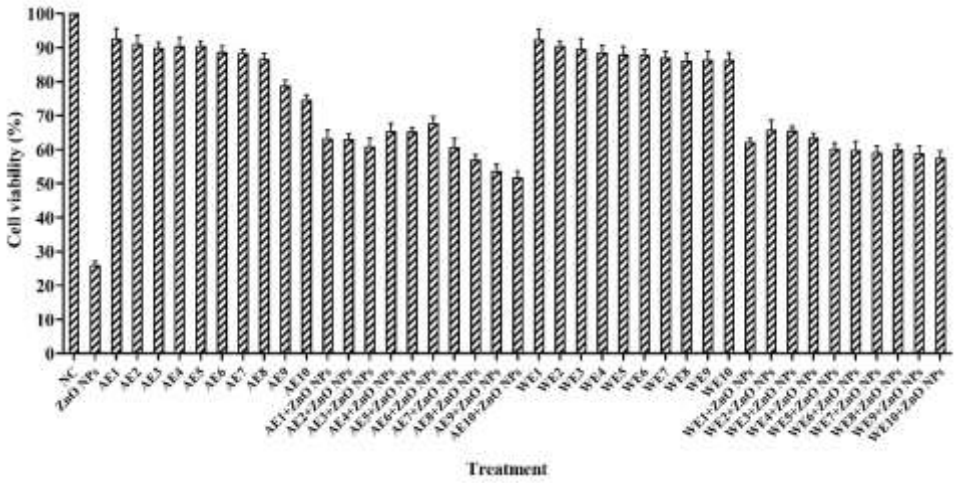

Figure 5. Viability rates obtained by MTT analysis in HaCaT cells treated with ZnO NPs alone or combined with acetone and water extracts of *A. reineckii*. Data represent mean ± standard deviation from 3 independent experiments.

Şekil 5. Tek başına ZnO NP'lerle veya A. reineckii'nin aseton ve su özütleri ile kombine edilmiş HaCaT hücrelerinde MTT analizi ile elde edilen canlılık oranları. Veriler, 3 bağımsız deneyden elde edilen ortalama ± standart sapmayı temsil etmektedir.

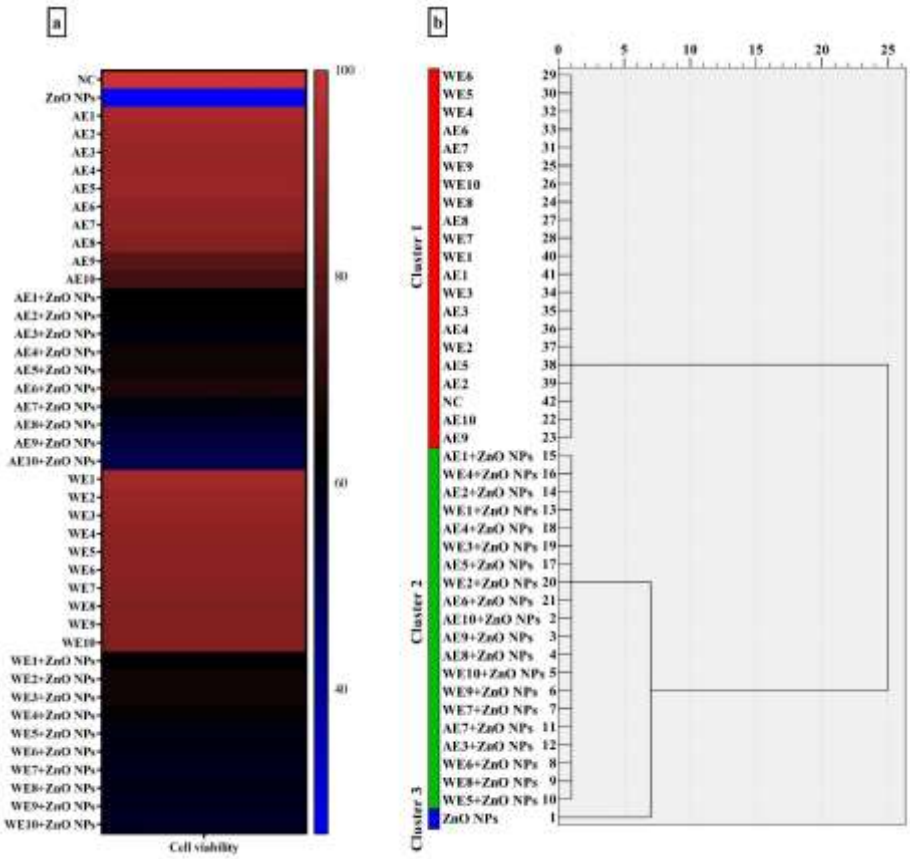

Figure 6. Heatmap of ZnO NPs alone or combined with acetone and water extracts of *A. reineckii* in HaCaT cells. High and low viabilities were represented by red and blue color, respectively. The scale of color intensity was positively correlated to cell viability (a). Dendrogram built from cell viability rates in HaCaT cells (b)

Şekil 6. HaCaT hücrelerinde A. reineckii'nin tek başına veya aseton ve su özütleri ile birlikte ZnO NP'lerinin ısı haritası. Yüksek ve düşük canlılık, sırasıyla kırmızı ve mavi renkle temsil edilmiştir. Renk yoğunluğu ölçeği, hücre canlılığı ile pozitif olarak ilişkiliydi (a). HaCaT hücrelerinde hücre canlılığı oranlarından oluşturulan dendrogram (b)

Table 2. Effects of different TDZ-IBA doses on shoot regeneration of *A. reineckii* from nodal explant
Çizelge 2. Farklı TDZ-IBA dozlarının nodal eksplanttan A. reineckii'nin sürgün rejenerasyonu üzerindeki etkileri

Growth regulators (mg/L) *(Büyüme düzenleyiciler)*				
TDZ	IBA	Shoot regeneration *(Sürgün rejenerasyonu)* (%)	Shoots per explants *(Eksplant başına sürgün)*	Shoot length *(Sürgün uzunluğu)*(cm)
0	0	66.66b	1.67c	1.15b
0.25	0.25	88.89ab	12.33b	1.77a
0.50	0.25	100.00a	17.67ab	1.59a
0.75	0.25	100.00a	22.50a	1.27b
1.00	0.25	88.89ab	21.67a	1.25b
1.25	0.25	83.33ab	16.00b	1.10b

Means followed by different small letters within same column were significantly different ($p < 0.05$)

that ZnO NPs causes genetic damage. Akbaba & Türkez (2018) tested the genetic damage level of ZnO NPs on lymphocytes by means of chromosome aberration and micronucleus analyses and reported that 500 ppm and higher concentrations showed genotoxic effects.

In the current study, the cytotoxic potential of ZnO NPs on HaCaT cells was evaluated and it was determined that the viability of the cells was greatly reduced. Similarly, there are different studies showing that ZnO NPs have different toxic mechanisms on HaCaT cells. In one study, the mechanism of skin toxicity caused by ZnO has been investigated. The researchers revealed that ZnO NPs could induce the inflammatory response in HaCaT cells. This induction was enhanced by reactive oxygen species (ROS)-extracellular signaling (Jeong et al. 2013). Another study showed that ZnO NPs could cause a potential

ROS generation. In this study, the data demonstrated a significant decrease of glutathione level in HaCaT cells exposed to ZnO NPs and it was suggested that there might be a link between the nanotoxicity mechanism and oxidative stress, active oxygen production, antioxidant defense mechanisms, and apoptosis (Lee et al. 2012). Studies have focused on the inhibition of nanotoxicity in cells. Resveratrol was preferred in some of the studies within this scope. Giordo et al. (2020) pronounced that resveratrol, an important antioxidant, inhibited mitochondrial dysfunction and oxidative stress caused by ZnO NPs in Zebrafish. In another similar study, genetic damage, oxidative stress and cytotoxicity in human pulmonary alveolar epithelial cells induced by ZnO NPs were tolerable by using resveratrol. The researchers' opinion is that the high antioxidant capacity of the compounds used inhibits the resulting nanotoxicity (Emsen & Turkez 2017).

The use of herbal products to inhibit nanotoxicity will reduce the level of side effects. Medicinal and aromatic plants used in many fields such as food, medicine and cosmetics are important in this regard (Giannenas et al. 2019). Tissue culture techniques, a current and biotechnological method, have made a significant contribution to the mass production of the medicinal and aromatic plants (Máthé et al. 2015). In order to obtain extract or active substance, the plants collected from nature can also disrupt the ecological balance. For this reason, the production of plants *in vitro* with tissue culture techniques makes a great contribution to such studies. In our study, we produced *A. reineckii* by tissue culture techniques and inhibited the nanotoxicity caused by ZnO through different extracts obtained from *A. reineckii*.

When shoot regeneration frequencies were examined, very high and very low hormone combinations had a negative effect on the shoot regeneration of the explants. Similarly, shoot tip explants of *Ceratophyllum demersum* L. were cultured and a decrease in shoot regeneration frequencies with high and low hormone application was reported (Emsen & Dogan 2018).

When shoot lengths were examined, the highest length value was obtained in the lowest concentrations of TDZ (0.25 mg/L). The increase in the ratio of TDZ in the culture medium caused the shoots to remain short. Similarly, negative effects of TDZ on shoot lengths were previously reported by Dewir et al. (2018) and Novikova & Zaytseva (2018). It has been reported that transferring *in vitro* cultures to nutrient media supplemented with low concentrations of TDZ (0.01 to 1.0 µM) would be a correct solution to avoid TDZ-induced adverse events such as short shoots (Novikova & Zaytseva 2018; Novikova et al. 2020). Another effective approach was to transfer the shoots in TDZ culture medium to hormone-free nutrient medium or to medium containing a plant growth regulator such as zeatin, 6-benzylaminopurine or GA_3 (Sujatha et al. 2008; Dhavala & Rathore 2010). In our current study, the shoot lengths were sufficient for us, as we conducted an activity study.

CONCLUSIONS

In general, the present study demonstrated that *in vitro* propagated *A. reineckii* showed protective role against ZnO-induced nanotoxicity. Especially the combined application of acetone extract obtained from this plant and ZnO highly inhibited the ZnO-induced cytotoxic effect. This result presented the idea that the extracts of *A. reineckii* can be added in certain proportions to products containing ZnO used in the cosmetic field.

Author's Contributions

BE designed the experiments and carried out extraction, cell culture, cytotoxicity experiments, IC carried out ZnO nanoparticle characterization, MD carried out *in vitro* regeneration process and all authors analysed the data and wrote the manuscript.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Abdullah, A.H.D., Putri, O.D., Fikriyyah, A.K., Nissa, R.C., Hidayat, S., Septiyanto, R.F., Karina, M., & Satoto, R. (2020). Harnessing the Excellent Mechanical, Barrier and Antimicrobial Properties of Zinc Oxide (ZnO) to Improve the Performance of Starch-Based Bioplastic. *Polymer-Plastics Technology and Materials 59*(12), 1259–1267.

Agustina, T.E., Melwita, E., Bahrin, D., Gayatri, R., & Purwaningtyas, I.F. (2020). Synthesis of Nano-Photocatalyst ZnO-Natural Zeolite to Degrade Procion Red. *International Journal of Technology 11*(3), 472–481.

Akbaba, G.B., & Türkez, H. (2018). Investigation of the Genotoxicity of Aluminum Oxide, ß-Tricalcium Phosphate, and Zinc Oxide Nanoparticles *In Vitro*. *International Journal of Toxicology 37*(3), 216–222.

Anderson, L.W.J., Fried, G., Gunasekera, L., Hussner, A., Newman, J., Starfinger, U., Stiers, I., van Valkenburg, J., & Tanner, R. (2015). Pest risk analysis for *Alternanthera philoxeroides*. EPPO, Paris.

Braakhuis, H.M., Kloet, S.K., Kezic, S., Kuper, F., Park, M.V.D.Z., Bellmann, S., van der Zande, M., Le Gac, S., Krystek, P., Peters, R.J.B., Rietjens, I.M.C.M., & Bouwmeester, H. (2015). Progress and Future of *In Vitro* Models to Study Translocation of Nanoparticles. *Archives of Toxicology 89*(9), 1469–1495.

Braakhuis, H.M., Park, M.V.D.Z., Gosens, I., De Jong, W.H., & Cassee, F.R. (2014). Physicochemical Characteristics of Nanomaterials That Affect Pulmonary Inflammation. *Particle and Fibre Toxicology 11*(1), 18.

Celik, M., Keskin, N., & Ozdemir, F.A. (2020). The Effects of UV Irradiation and Incubation Time on *In Vitro* Phenolic Compound Production in 'Karaerik' Grape Cultivar. *KSÜ Tarım ve Doğa Dergisi 23*(6), 1428–1434.

Das, A., Kamle, M., Bharti, A., & Kumar, P. (2019). Nanotechnology and It's Applications in Environmental Remediation: An Overview. *Vegetos 32*(3), 227–237.

Dewir, Y.H., Nurmansyah, N., Naidoo, Y., & Teixeira da Silva, J.A. (2018). Thidiazuron-Induced Abnormalities in Plant Tissue Cultures. *Plant Cell Reports 37*(11), 1451–1470.

Dhavala, A., & Rathore, T.S. (2010). Micropropagation of *Embelia Ribes* Burm f. through Proliferation of Adult Plant Axillary Shoots. *In Vitro Cellular and Developmental Biology - Plant 46*(2), 180–191.

Dilkalal, A., Annapurna, A.S., & Umesh, T.G. (2021). *In Vitro* Regeneration, Antioxidant Potential, and Genetic Fidelity Analysis of *Asystasia gangetica* (L.) T. Anderson. *In Vitro Cellular and Developmental Biology - Plant 57*(3), 447–459.

El-Sherif, N.A. (2019). Impact of Plant Tissue Culture on Agricultural Sustainability. *Handbook of Environmental Chemistry 77*, 93–107.

Emsen, B., Aslan, A., Turkez, H., Taghizadehghalehjoughi, A., & Kaya, A. (2018). The Anti-cancer Efficacies of Diffractaic, Lobaric, and Usnic Acid: *In Vitro* Inhibition of Glioma. *Journal of Cancer Research and Therapeutics 14*(5), 941–951.

Emsen, B., & Dogan, M. (2018). Evaluation of Antioxidant Activity of *In Vitro* Propagated Medicinal *Ceratophyllum demersum* L. Extracts. *Acta Scientiarum Polonorum-Hortorum Cultus 17*(1), 23–33.

Emsen, B., & Turkez, H. (2017). The Protective Role of Resveratrol against Zinc Oxide Induced Nanotoxicity. *Anatolian Journal of Botany 1*(2), 21–25.

Giannenas, I., Sidiropoulou, E., Bonos, E., Christaki, E., & Florou-Paneri, P. (2019). The History of Herbs, Medicinal and Aromatic Plants, and Their Extracts: Past, Current Situation and Future Perspectives. In: Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health. pp 1–18.

Giordo, R., Nasrallah, G.K., Al-Jamal, O., Paliogiannis, P., & Pintus, G. (2020). Resveratrol Inhibits Oxidative Stress and Prevents Mitochondrial Damage Induced by Zinc Oxide Nanoparticles in Zebrafish (*Danio Rerio*). *International Journal of Molecular Sciences 21*(11), 3838.

Gollavilli, H., Hegde, A.R., Managuli, R.S., Bhaskar, K.V., Dengale, S.J., Reddy, M.S., Kalthur, G., & Mutalik, S. (2020). Naringin Nano-Ethosomal Novel Sunscreen Creams: Development and Performance Evaluation. *Colloids and Surfaces B: Biointerfaces 193*, 111122.

Hosseini Tafreshi, S.A., Aghaie, P., Momayez, H.R., & Hejaziyan, S.A. (2021). Response of *In Vitro*-Regenerated *Myrtus communis* L. Shoots to PEG-Induced Water Stress. *Biocatalysis and Agricultural Biotechnology 34*, 102033.

Jahan, I., & Isildak, I. (2021). Lemon Peel Extract for Synthesizing Non-Toxic Silver Nanoparticles through One-Step Microwave-Accelerated Scheme. *KSÜ Tarım ve Doğa Dergisi 24*(1), 1–10.

Jeong, S.H., Kim, H.J., Ryu, H.J., Ryu, W.I., Park, Y.H., Bae, H.C., Jang, Y.S., & Son, S.W. (2013). ZnO Nanoparticles Induce TNF-α Expression via ROS-ERK-Egr-1 Pathway in Human Keratinocytes. *Journal of Dermatological Science 72*(3), 263–273.

Jirakiattikul, Y., Rithichai, P., Songsoem, K., & Itharat, A. (2021). Elicitation of Salicylic Acid on Secondary Metabolite Production and Antioxidant Activity of *In Vitro Musa acuminata* L. Cv. 'Gros Michel' Shoots. *Current Applied Science and Technology 21*(3), 569–578.

Kavitha, P., Shanthi, C., & Kannan, R. (2022). A Facile Green Synthesis of Nanostructured Gold–Silver@Carbon (Au–Ag@C) Nano Catalyst and Its Applications. *Kuwait Journal of Science 49*(2), 1–12.

Lee, C.-C., Lin, Y.-H., Hou, W.-C., Li, M.-H., & Chang, J.-W. (2020). Exposure to ZnO/TiO2 Nanoparticles Affects Health Outcomes in Cosmetics Salesclerks. *International Journal of Environmental Research and Public Health 17*(17), 1–12.

Lee, S.H., Pie, J.E., Kim, Y.R., Lee, H.R., Son, S.W., & Kim, M.K. (2012). Effects of Zinc Oxide Nanoparticles on Gene Expression Profile in Human Keratinocytes. *Molecular and Cellular Toxicology 8*(2), 113–118.

Lee, W.-M., & An, Y.-J. (2013). Effects of Zinc Oxide and Titanium Dioxide Nanoparticles on Green Algae under Visible, UVA, and UVB Irradiations: No Evidence of Enhanced Algal Toxicity under UV Pre-Irradiation. *Chemosphere 91*(4), 536–544.

Máthé, Á., Hassan, F., & Abdul Kader, A. (2015). *In Vitro* Micropropagation of Medicinal and Aromatic Plants. In: Medicinal and Aromatic Plants of the World. Springer, Dordrecht, pp 305–336.

Mohajerani, A., Burnett, L., Smith, J.V., Kurmus, H., Milas, J., Arulrajah, A., Horpibulsuk, S., & Kadir, A.A. (2019). Nanoparticles in Construction Materials and Other Applications, and Implications of Nanoparticle Use. *Materials 12*(19), 3052.

Monsé, C., Raulf, M., Hagemeyer, O., Van Kampen, V., Kendzia, B., Gering, V., Marek, E.-M., Jettkant, B.,

Bünger, J., Merget, R., Merget, R., & Brüning, T. (2019). Airway Inflammation after Inhalation of Nano-Sized Zinc Oxide Particles in Human Volunteers. *BMC Pulmonary Medicine 19*(1), 266.

Novikova, T.I., Asbaganov, S.V., Ambros, E.V., & Zaytseva, Y.G. (2020). TDZ-Induced Axillary Shoot Proliferation of *Rhododendron mucronulatum* Turcz and Assessment of Clonal Fidelity Using DNA-Based Markers and Flow Cytometry. *In Vitro Cellular and Developmental Biology - Plant 56*(3), 307–317.

Novikova, T.I., & Zaytseva, Y.G. (2018). TDZ-Induced Morphogenesis Pathways in Woody Plant Culture. In: Ahmad N, Faisal M (eds) Thidiazuron: From Urea Derivative to Plant Growth Regulator. pp 61–94

Ozelci, D., & Yigit, E. (2022). Micropropagation of the *Morus nigra* L. (Black Mulberry) cv. 'Eksi Kara'. *KSÜ Tarım ve Doğa Dergisi 25*(1), 49–56.

Rani, A., & Kumar, S. (2017). Tissue Culture as a Plant Production Technique for Medicinal Plants: A Review. *International Conference on Innovative Research in Science, Technology and Management 6*(1), 784–795.

Sagadevan, S., Raj, K.P., Aziz, F.A., Chowdhury, Z.Z., Johan, M.R.B., & Podder, J. (2018). Structure, Properties, Photocatalytic and Antibacterial Activity and Applications of Zinc Oxide Nanoparticles-an Overview. *Journal of Bionanoscience 12*(4), 457–468.

Sujatha, K., Panda, B.M., & Hazra, S. (2008). De Novo Organogenesis and Plant Regeneration in *Pongamia pinnata*, Oil Producing Tree Legume. *Trees - Structure and Function 22*(5), 711–716.

Uma, H.B., Ananda, S., & Nandaprakash, M.B. (2019). High Efficient Photocatalytic Treatment of Textile Dye and Antibacterial Activity via Electrochemically Synthesized Ni-Doped ZnO Nano Photocatalysts. *Chemical Data Collections 24*, 100301.