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Research Article

Investigation of Toxicity Parameters of La-TiO_2 Nanoparticles in *Allium cepa* L. Root Tip Cells

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Abstract

This study examined the dose-dependent physiological, anatomical, and cytogenetic effects of lanthanum-doped titanium dioxide (La-TiO_2) nanoparticles (NPs) in Allium cepa L. root tip cells. These NPs are widely used in electronics, optics, energy storage, and photocatalysis.. La-TiO_2 NPs were synthesized via the microwave method, characterized by SEM and XRD, and then in vivo parameters were examined. Physiological parameters including root length, weight increase and germination percentage; cytogenetic indicators including chromosomal abnormality (CA), micronucleus (MN) frequency, and mitotic index (MI); and anatomical effects on meristematic cell damage were examined through sectioning, and the obtained data were statistically evaluated. The onion bulbs used as test materials were divided into five groups: Control and groups 10 ppm, 25 ppm, 50 ppm and 100 ppm exposed to different doses of La-TiO_2 nanoparticles. After 72 hours, physiological parameters decreased with increasing La-TiO_2 NP dose. Chromosomal abnormalities (CA) and micronucleus (MN) frequency increased. Anatomical damage included epidermal cell damage, thickened cortex walls, flattened nuclei, and unclear vascular tissues in La-TiO_2-treated groups. In conclusion, while literature studies have generally focused on the toxic parameters of commercially available NPs, genotoxic and cytotoxic studies conducted after the synthesis and characterization of NPs, as applied in our study, are limited. In previous studies, NP size was generally kept larger. However, in our study, the size of La-TiO_2 NPs was determined to be approximately 8 nm, and it was found to have a significant genotoxic effect on toxicity.

Keywords: *A.cepa L.*, La-TiO_2 nanoparticle, micronucleus, cytogenetics, meristematic damage

La-TiO_2 Nanopartiküllerinin *Allium cepa* L. Kök Ucu Hücrelerindeki Toksisite Parametrelerinin İncelenmesi

Öz

Bu çalışmada yaygın kullanılan nanopartiküllerden (NP) biri olan ve elektronik ve optik uygulamalar, enerji depolama ve fotokatalizör olarak da kullanılan lantan katkılı titantum dioksit (La-TiO_2) nanopartiküllerinin *Allium cepa* L. kök ucu hücrelerinde doz bağımlı, anatomik, fizyolojik ve sitogenetik etkileri incelenmiştir. Mikrodalga yöntemiyle sentezlenen La-TiO_2 NP'lerin SEM, XRD gibi karakterizasyon analizleri yapılıp istenen özellikte bir nanopartikül sentezinden sonra in vivo parametrelere geçilmiştir. Fizyolojik parametreler açısından: kök uzunluğu, ağırlık artışı ve çimlenme yüzdesi; sitogenetik indikatörler açısından kromozom anormalliği (KA), mikronükleus (MN) sıklığı, mitotik indeks (MI); anatomik olarak meristematik hücre hasarları kesitler alınarak incelenmiş ve elde edilen veriler istatistiksel açıdan değerlendirilmiştir. Test materyali olarak kullanılan soğanlar kontrol ve La-TiO_2 naopartikülü ile farklı dozlarda uygulanan 10 ppm, 25 ppm, 50 ppm ve 100 ppm olmak üzere beş gruba ayrılmıştır. 72 saat yapılan uygulama sonucunda La-TiO_2 nanopartiküle maruz bırakılan gruplarda uygulanan doz miktarındaki artışa bağlı olarak fizyolojik parametrelerde azalma meydana geldiği belirlenmiş olup, KA ve MN sıklığı açısından bir artış olduğu tespit edilmiştir. Anatomik hasarlar bakımından epidermal hücre hasarı, kalınlaşmış korteks hücre duvarı, yassılaşmış hücre çekirdekleri ve belirgin olmayan iletim dokusu La-TiO_2 uygulanan gruplarda gözlemlenmiştir. Sonuç olarak literatür çalışmalarında genellikle ticari olarak temin edilen nanopartiküllerin (NP'ler) toksik parametreleri çalışılmışken, bizim çalışmamızda uyguladığımız NP sentezi ve karakterizasyonu sonrası gerçekleştirilen genotoksik ve sitotoksik çalışmalar sınırlıdır. Yapılan çalışmalarda, NP boyutu genellikle daha büyük tutulmuştur. Çalışmamızda ise La-TiO_2 NP'lerinin boyutu yaklaşık 8 nm olarak belirlenmiş ve toksisite üzerinde genotoksik açıdan önemli bir etkiye sahip olduğu belirlenmiştir.

Anahtar Kelimeler: *A.cepa L.,* La-TiO_2 nanopartikül, mikronükleus, sitogenetik, meristematik hasar

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1. Introduction

As a technical term, 'nano' refers to 10^{-9} or one in a billion, and nanotechnology operates within the size range of 1–100 nm [1]. Nanoparticles are known for their specific shapes, adjustable pore sizes, enhanced surface area, and high reactivity [2].

The synthesis methods used for titanium dioxide nanoparticles (TiO_2 NPs) can alter their properties, affecting their biological interactions. Among these properties, the size, shape, crystal structure, and surface coating of TiO_2 NPs are among the most critical factors influencing their interactions with other systems. These features influence surface area, aggregation behavior, (reactive oxygen species) ROS generation, and interactions with cellular structures.

As the size of nanoparticles decreases, their surface area increases significantly compared to larger particles. More importantly, the proportion of molecules or atoms on the particle surface increases, thereby enhancing their chemical reactivity. These properties, which make nanoparticles highly desirable in various applications, also play a crucial role in their interactions with biological systems. The size, shape, chemical composition, physicochemical stability, crystal structure, surface area, and surface energy of nanoparticles and nanomaterials significantly influence their toxicity levels [3].

Due to their small size, NPs can easily penetrate cells and move between cells, tissues, and organs [4]. As NP size decreases, the ratio of total atoms or molecules on the surface increases exponentially, making NPs more biologically active per unit mass compared to larger particles [5]. Studies have shown that NPs can disrupt gene expression mechanisms in plants. These disruptions have been linked to the induction of genotoxicity [6].

Advancements in nanotechnology have led to the increasing production and widespread use of nanoparticles. Due to their production, utilization, and waste, exposure to these particles has also increased for both humans and other living organisms. Consequently, concerns regarding the toxic effects of nanoparticles have grown in recent years. Despite the rising use of NPs, there are still significant gaps in toxicity studies at the cellular and molecular levels [7].

The Allium test, which commonly utilizes *A. cepa L.* (onion), is an inexpensive, sensitive, easy-to-apply, and rapid method that provides DNA- and chromosome-level information. Due to its many advantages, the *A. cepa* and *V. faba* root tip chromosome abnormality tests have been validated by the World Health Organization (WHO) International Programme on Chemical Safety, the United States Environmental Protection Agency (USEPA), and the International Labour Organization for screening chemicals and assessing their in situ genotoxic effects [8].

Bioassay methods are essential in toxicity evaluations for identifying the harmful effects of environmental pollutants. These assessments do not only consider the growth and biochemical indicators of test organisms but also the effects on the genetic material of cells. Such impacts can lead to disruptions in cellular division processes, which may be transmitted to subsequent generations or result in the development of pathologies during the organism's ontogenesis. This

test method takes into account growth indicators along with disruptions occurring in the apical meristem root cells during cell division [9].

Titanium dioxide (TiO_2) nanoparticles are widely used in the production of various materials, including cosmetics, sunscreens, toothpaste, and pharmaceutical products. They can also enter groundwater from sources such as paints, sunscreen lotions, food additives, and nanoparticle-coated plastics, glass, and metal waste [10]. The National Institute for Occupational Safety and Health (NIOSH) has established an acceptable exposure limit (PEL) of 0.0015 mg/mL and a recommended exposure level (REL) of 0.0001 mg/mL for TiO_2 nanoparticle-based materials [11]. This highlights the necessity of evaluating the potential risks and toxic effects of TiO_2 nanoparticles in surface waters.

Detailed genotoxicity studies on the effects of TiO_2 nanoparticles at concentrations below 20 mg/mL in the *Allium cepa* model and their intracellular effects remain limited. Klancnik et al. [12] suggested that TiO_2 nanoparticles may exhibit higher toxicity in *Allium cepa* compared to growth medium conditions, with the effect being time- and concentration-dependent. Ghosh et al. [13] reported that exposure to 320 mg/mL TiO_2 nanoparticles led to micronucleus formation and chromosomal abnormalities, correlating with a reduction in root length. Another study confirmed dose-dependent cytogenetic and genotoxic impacts of TiO_2 NPs on *A. cepa* based on observed chromosomal abnormalities and comet assay results [14].

Lanthanum (La) is a rare earth metal that is not found in a free state in the Earth's crust but rather in minerals alongside other rare earth elements. The average lanthanum concentration in the Earth's crust is approximately 30 mg/kg. Lanthanum is used in the robotics, electronics and energy storage industries, leading to human-made contamination of soil and water and generating environmental concerns [15]. Unregulated mining activities and inadequate waste management practices further contribute to lanthanum pollution. Additionally, the common use of micronutrient fertilizers has been recognized as a major source of excessive lanthanum accumulation in agricultural soils, exacerbating environmental concerns.

Lanthanide elements have attracted significant research interest due to their exceptional optical, magnetic, and redox properties, which make them valuable for various industrial applications. Specifically, lanthanum (La), when used as dopants in TiO_2, prevent the phase transition from anatase to rutile, induce lattice distortions in the surface layer, create defects that decrease crystallite size, enhance specific surface area, improve thermal stability, increase oxygen vacancy concentration, and readily interact with organic compounds to facilitate their mineralization [16]. Lanthanide have been widely used as TiO_2 dopants due to their ability to significantly alter its physicochemical properties. Doping of TiO_2 with lanthanides occurs through two mechanisms: i. La^{3+} ions can integrate into the TiO_2 lattice by direct bonding or substituting Ti^{4+} ions, forming a Ti–O–La–O–Ti structure, which causes lattice distortions due to the difference in ionic radii. ii. When there is insufficient energy for lattice substitution, lanthanide ions remain dispersed on the TiO_2 surface, forming Ti–O–La bonds.[17-18]

The impact of lanthanum on plants is influenced by factors like soil conditions, plant species, La concentration, exposure time, and growth environment. High concentrations of La can

interfere with cell division, DNA integrity, nutrient absorption, and photosynthesis, resulting in toxic effects. Plants employ detoxification strategies such as vacuolar storage, osmolyte production, and antioxidant defense systems. However, at high La concentrations, these defense mechanisms can be overwhelmed, resulting in negative effects on plant growth and development. Plants can absorb and accumulate lanthanum from the soil, allowing it to enter the food chain, which may eventually lead to bioaccumulation in animals and humans over time [19].

Kotelnikova et al. [20] conducted a study on *A. cepa L*. using 0-200 mg/L La^{3+} ion concentrations and observed a significant decrease in the MI. The presence of La induced common chromosomal abnormalities, including metaphase delay, disturbed metaphase, c-mitosis, stickiness, and anaphase abnormalities, resulting in increased cell adhesion and a higher frequency of disturbed metaphases in onion root cells. Similarly, D'Aquino et al. [21] examined the effects of La on the MI in *Triticum durum* and found that exposure to 10 µM La led to a reduction in root length and a decrease in the MI within root tissues. This study is the first to report the toxicity of La-TiO_2 nanoparticles at high exposure levels (100 mg/mL) in *Allium cepa* and demonstrates a dose-dependent effect, which follows a linear trend up to 100 mg/mL. The primary objective of this study is to explain the genotoxicity mechanism of nanoparticles formed by the combination of La and TiO_2. Based on quantitative data, a positive correlation has been established between nanoparticle uptake by cells and the resulting physiological, cytotoxic, and anatomical damage. Using optical microscopy, various chromosomal abnormalities such as chromosome stickiness, chromosome breaks, lagging chromosomes, and chromosomal bridges were observed at different stages of the cell cycle. The necessity of examining the effects of La-TiO_2 nanoparticles on cellular division processes is supported by previous studies on the toxic effects of La and Ti elements separately [21],[22]. However, previous studies have investigated nano powders individually, rather than in combination. In this study, the aim is to synthesize La-TiO_2 nanoparticles in the desired nanoscale using the microwave synthesis method and to determine the toxicity level resulting from the combination of Ti and La. This will be achieved through the *Allium cepa* test system, utilizing various toxicity biomarkers (e.g., germination rate, root development, morphological changes, and chromosomal damage). Therefore, The aim of this study is to evaluate the toxic effects of La-TiO_2 nanoparticles synthesized via the microwave method and to assess their impact under bioassay conditions using *A. cepa L.*

2. MATERIALS AND METHODS

2.1 Synthesis of La-TiO_2 NPs via Microwave Method

Titanium isopropoxide (Alfa Aesar, 97%), ethanol (Sigma-Aldrich, 99.9%), lanthanum chloride heptahydrate (Sigma-Aldrich, $LaCl_3 \bullet 7H_2O$), and hydrochloric acid (Sigma-Aldrich, 37%) were purchased for the study.

The synthesis of La-TiO_2 nanoparticles was carried out using a microwave device (Milestone, Start D, USA), ensuring a La/Ti (n/n) molar ratio of 0.05 in the synthesis medium. Solution A was prepared by mixing titanium isopropoxide ($Ti(OPri)_4$) and ethanol (C_2H_5OH), while

solution B consisted of lanthanum chloride heptahydrate ($LaCl_3 \bullet 7H_2O$), ethanol (C_2H_5OH), deionized water (H_2O), and hydrochloric acid (HCl). Both solutions were stirred at 350 rpm for 20 minutes. Then, solution A was added dropwise to solution B using a Pasteur pipette. After the addition was complete, the solution was subjected to microwave heating at 120°C for 30 minutes at 600 W to complete the synthesis process.

Following the synthesis, the nanoparticle solution was centrifuged at 7000 rpm (Eppendorf, 5430R) and washed twice with ultra-pure water. In the final step, the synthesized nanoparticles were dried in an oven (Memmert, UN55) at 70°C for 24 hours. The crystal structure and phase analysis of the synthesized La-TiO_2 nanoparticles were examined using X-ray diffraction (XRD), while FE-ESEM-EDS analyses were performed to determine the microcrystalline structure and surface characteristics.

For in vivo studies, a stock solution containing La-TiO_2 nanoparticles (250 ppm, 500 mL) was prepared by subjecting it to ultrasonic treatment (Lab Companion UC-10) for 30 minutes. The stock solution was then diluted with ultra-pure water to prepare additional La-TiO_2 nanoparticle solutions at concentrations of 10, 25, 50, and 100 ppm.

2.2 Preparation of Root Tips and Nanoparticle Concentrations

The *A. cepa L.* bulbs used in this study were obtained from a local vendor in Yozgat, and those of approximately the same size and healthy condition were selected. The doses of La-TiO_2 nanoparticles (10, 25, 50, and 100 ppm) used in this research were based on previous Ti and La studies in the literature [12], [14], [20], [23]. The groups were formed as follows: Group I: control group (distilled water), Group II: 10 ppm La-TiO_2 nanoparticle distilled water solution, Group III: 25 ppm La-TiO_2 nanoparticle distilled water solution, Group IV: 50 ppm La-TiO_2 nanoparticle distilled water solution, and finally, Group V: 100 ppm La-TiO_2 nanoparticle distilled water solution.

2.3 Physiological Changes in Root Tips and Application Method

The onion bulbs selected for the study were sterilized by soaking in a 2% sodium hypochlorite solution for 10 minutes as a pre-treatment. In the final step, the root parts were thoroughly washed with distilled water. For the germination process, twenty onions were placed in plastic containers for each of the five groups to promote sprouting. The germination experiment was carried out in an incubator for 72 hours, at a temperature of ± 2 (20 ˚C) and in the dark. Onions with roots reaching approximately 10 mm in length were considered to have germinated. At the end of the period, the weight difference (g) before and after the application was measured using a precision scale. Germination percentages were calculated, and root lengths were measured in millimeters. The germination percentages were determined using the equation below (1). All experiments were performed in triplicate.

$$\text{Germination Percentage} = \left(\frac{\text{Number of Germinated Bulbs}}{\text{Total Number of Bulbs}} \right) x\ 100 \qquad (1)$$

2.4 Determination of Cytogenetic Changes in Root Tips

The germinated root tips were cut to approximately 1 cm in length. The cut root tips were fixed for cytogenetic analysis in a solution prepared in a 3:1 ratio of 3: glacial acetic acid 1: distilled water (Clarke fixative) for two hours, washed in 96% ethanol for 15 minutes, and then stored in 70% ethanol at +4 °C until use. The root tips were hydrolyzed in 1N HCl at 60°C for 17 minutes. Afterward, they were treated with 45% acetic acid for 30 minutes, incubated in acetocarmine for 24 hours, and crushed in 45% acetic acid. The mitotic abnormalities were photographed using a binocular research microscope (Olympus, BX43) with a digital camera (Olympus, SC50) at 400x magnification [24], [25]. Micronucleus was detected based on the criteria established by Fenench et al. [26]. The mitotic index was calculated as a percentage using the formula given in equation (2).

$$\text{Mitotic Index (\%)} = \left(\frac{\text{Number of Dividing Cells}}{\text{Total Number of Cells Analyzed}} \right) x\ 100 \qquad (2)$$

2.5. Determination of Anatomical Changes in Root Tips

To determine anatomical damage in the meristematic cells of the root tip, *A. cepa* L. root tips were rinsed with distilled water to eliminate La-TiO_2 residues, then cross-sections were taken and stained with 2% methylene blue. The stained samples were analyzed under 400X magnification. [27].

2.6. Statistical Evaluation

The data were analyzed statistically using SPSS V 23.0 (IBM Corp, USA, 2015) software. All results are presented as mean ± SD (standard deviation), and differences between means were evaluated using One-way ANOVA followed by Duncan's Multiple Range Test. A p-value below 0.05 ($p<0.05$) was considered statistically significant.

3. Results and Discussion

3.1 Characterization of La-TiO_2 NPs (XRD and SEM-EDX Analyses)

Among traditional preparation methods, sol-gel, co-precipitation, hydrothermal, and solvothermal synthesis are included. Sol-gel and co-precipitation methods require multiple processing steps and calcination, which can lead to low yields and agglomeration formation. Hydrothermal or solvothermal methods, on the other hand, typically require continuous processing for several hours. Therefore, producing crystalline TiO_2 doped with rare earth elements through a fast, moderate, and scalable synthesis method has been a significant challenge for a long time [28].

Microwave-assisted methods provide a promising alternative to overcome the disadvantages of traditional methods. Studies on nanoparticle synthesis using this method are also available in the literature [29], [30], [31]. Compared to traditional methods, the biggest advantage of microwave synthesis is its speed, high efficiency, and repeatability [32], [33]. While solvothermal processes take hours, the microwave method can be completed in just minutes [28].

XRD spectra were obtained for the synthesized La-TiO_2 crystal structure and phase analysis using a PANalytical EMPYREAN model powder diffractometer with Cu Kα radiation (λ= 1.5418 Å). XRD diffractograms were obtained with a scan degree of 10-80° (2θ) and a scanning range of 3 min^{-1}.

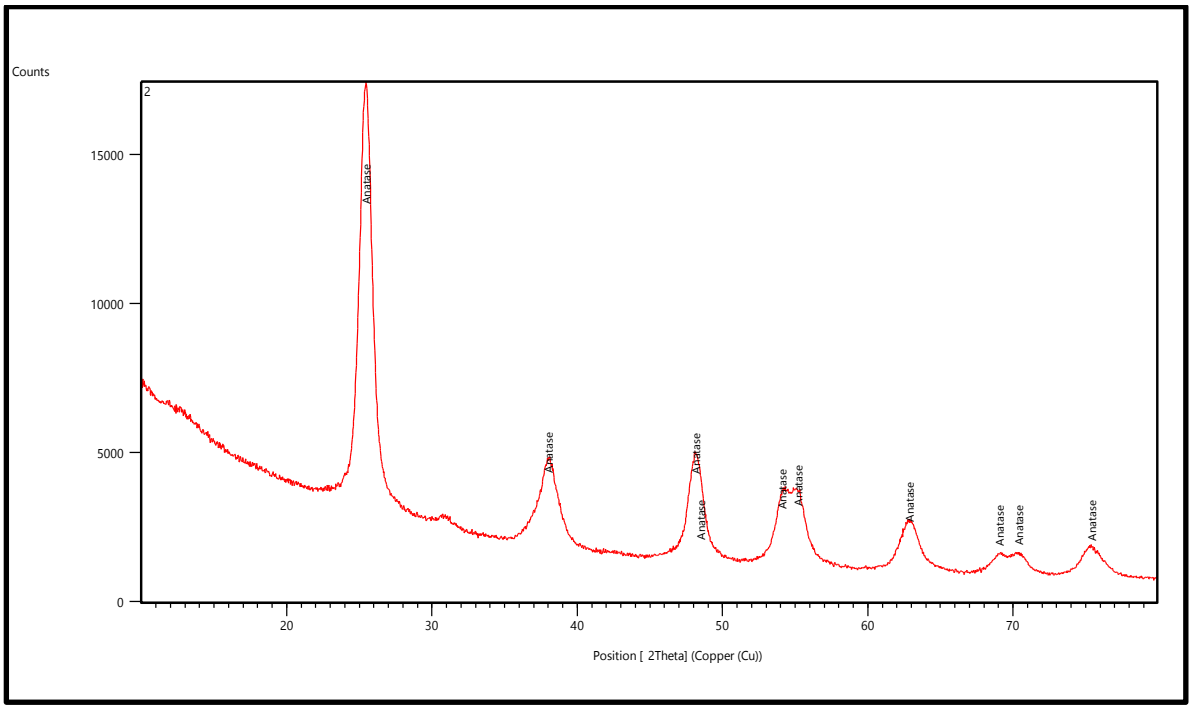

Figure 1. XRD pattern of the synthesized La-TiO_2 nanoparticle

The XRD patterns of the synthesized La-TiO_2 are shown in Figure 1. As seen from the obtained diffractograms, the most intense peak was observed at approximately 2θ = 25.44°. Additionally, other prominent peaks (~37.98°, 48.24°, and 55.17°) correspond to the anatase structure. The crystal sizes of the synthesized materials were determined to be approximately 8 nm using the Scherrer equation. The crystal structure of TiO_2 nanoparticles influences their toxicity. There is a consensus that the anatase phase produces more reactive oxygen species compared to rutile and brookite phases. However, amorphous TiO_2 material has been found to be more toxic than anatase.

The effects of anatase and rutile nanoparticles on plants may differ; for example, anatase nanoparticles are reported to damage the nucleus and cell membrane of algal cells, while rutile nanoparticles damage chloroplasts and internal organelles. However, some studies have shown that the toxicity of anatase and rutile nanoparticles on plants is similar [34]. FE-ESEM-EDS

(FEI, QUANTA, FEG 450) analyses were performed to determine the microcrystalline structure and surface characteristics of the synthesized nanoparticle.

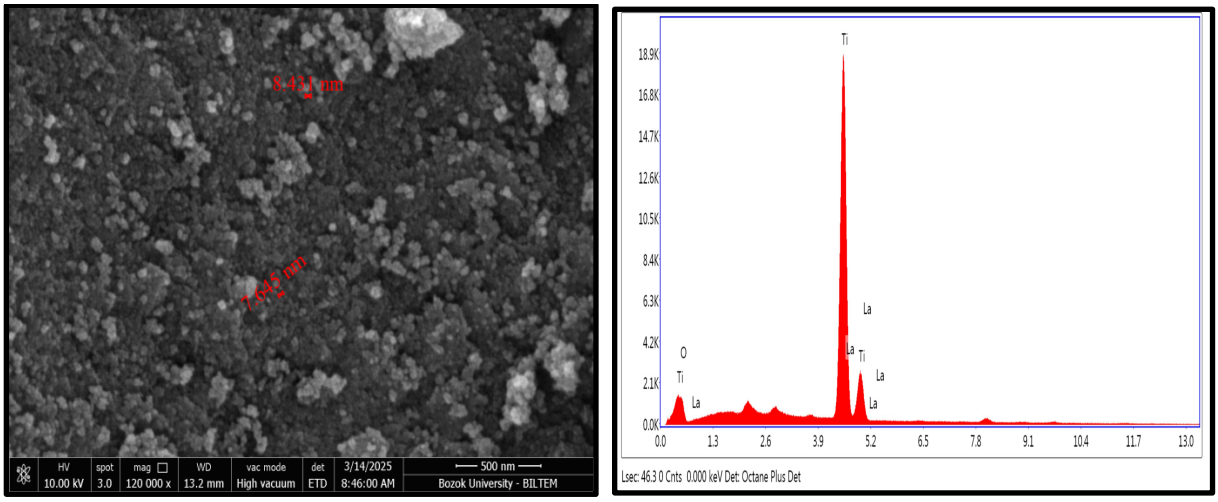

Figure 2. Surface images and SEM-EDX spectra of La-TiO_2 nanoparticles

When examining the SEM images of La-TiO_2 nanoparticles (Figure 2), it can be seen that all the particles are irregular spherical nanoparticles. Agglomeration, accumulation, and clumping can be observed in some areas of the nanoparticles. According to the EDX results, the weight percentage of lanthanum in the environment is 4.67%, while the titanium (Ti) content is 73.51%. Although the La/Ti (n/n) ratio added to the environment is 0.05, the EDX results show that this ratio is approximately 0.022, indicating it has incorporated into the TiO_2 structure. In a study conducted with Hordeum vulgare L. using a hydrothermal method, XRD patterns showed that anatase powder has tetragonal symmetry, and the diameters of TiO_2 aggregates ranged from a few nanometers to several micrometers, with an average size between 5-10 nm [35].

3.2. Effect of La-TiO_2 Nanoparticles on Physiological Parameters

The effects of La-TiO_2 nanoparticles on weight gain, root length, germination percentage in A. cepa, and chromosomal abnormalities in *A. cepa* root tip cells are presented in Figures 3-4 and Tables 1 and 2.

Table 1. Effect of La-TiO_2 nanoparticles on live weight gain (g) after application. * The data are presented as mean ± SD. Means with different letters (a, b, c, d, e) in the same column represent statistically significant differences ($P<0.05$), ($n = 10$).

Groups	Initial	Final	Weight Gain (g)

Group I	11.25±2.42[b*]	17.89±2.85[a*]	+6.64
Group II	11.02±1.65[b*]	14.97±1.25[b*]	+3.95
Group III	9.58±1.09[c*]	11.67±1.13[c*]	+2.09
Group IV	9.43±0,8[d*]	10.67±1.8[d*]	+1.24
Group V	10.21±1.3[d*]	10.93±1.42[e*]	+0.72

In the study, it was determined that the application of La-TiO_2 nanoparticles had an inhibitory effect on weight gain. At the end of the 72 hour application period, the average weight gains were measured as 6.64g for Group I, 3.95g for Group II, 2.09g for Group III, 1.24g for Group IV, and 0.72g for Group V. It was found that there was an inverse relationship between the applied La-TiO_2 nanoparticle dose and the weight gain. Although there is no comprehensive study investigating the effects of La-TiO_2 nanoparticles on weight gain, there are separate studies conducted on Ti and La. The accumulation of these metals in wastewater, aquatic ecosystems, and soil, as well as their presence in agricultural products and human hair samples, has been linked to the results of industrial activities and agricultural applications [36].

Table 2. Effect of La-TiO_2 nanoparticles on root length (cm) after application. Group I: Control, Group II: 10 ppm La-TiO_2, Group III: 25 ppm, Group IV: 50 ppm, Group V: 100 ppm

Groups	Minimum	Maximum	Average
Group I	6.42	8.79	7.80±1.31[a*]
Group II	4.21	5.89	4.87±0.92[b*]
Group III	3.11	3.92	2.73±0.47[c*]
Group IV	1.08	2.09	1.33±0.43[d*]
Group V	0.1	0.6	0.28±0.59[e*]

[*]Data is presented as mean ± SD. Statistical differences between means were determined using the "Duncan" test and one-way analysis of variance. Means with different letters (a, b, c, d, e) in the same column represent statistical significance ($P<0.05$), ($n = 10$).

La-TiO_2 nanoparticles were also shown to have an inhibitory effect on another physiological parameter, root length (Figure 3 and Table 2). It was determined that with the increase in the La-TiO_2 nanoparticle dose, the root length decreased. The average root lengths for the groups were measured as 7.80±1.31 cm for Group I, 4.87±0.92 cm for Group II, 2.73±0.47 cm for Group III, 1.33±0.43 cm for Group IV, and 0.28±0.59 cm for Group V. The maximum root length was observed in the control group, while the minimum root length was observed in Group V treated with 100 ppm La-TiO_2 nanoparticle dose. On average, there was a 96.4% reduction in root length in the highest dose of 100 ppm compared to the control group.

Table 3. Effect of La-TiO_2 Nanoparticles on Germination Percentage. Group I: Control, Group II: 10 ppm, Group III: 25 ppm, Group IV: 50 ppm, Group V: 100 ppm

Groups	Total bulb number	Germinated	Non-germinated	Germination percentage

Group I	50	50	0	%100
Group II	50	43	7	%86
Group III	50	31	19	%62
Group IV	50	24	26	%48
Group V	50	18	32	%36

Treatment of *A. cepa* test material with different doses of La-TiO_2 nanoparticles showed an inhibitory effect on germination. The germination percentages were determined as 100% for Group I, 86% for Group II, 62% for Group III, 48% for Group IV, and 36% for Group V. It was observed that with the increase in La-TiO_2 nanoparticle dose, there was a significant decrease in the germination percentage of *A. cepa*. In a study conducted with lanthanum alone, the measured root lengths of germinated seedlings in the solutions showed a significant decrease compared to the control. In the La^{3+} solution test, a statistically significant decrease in root length was observed at a concentration of 50 mg/l and higher concentrations [20]. It has been shown that La can enter root meristematic cells and interact with important components of the nucleus [41]. As a result of this interaction, the cessation of root growth is attributed to the inhibition of cell division, rather than inhibition of cell elongation. It has been shown that La can penetrate plant cells and cause changes in the organization of microtubules [42]. This suggests that at high concentrations, it may lead to the cessation of root growth.

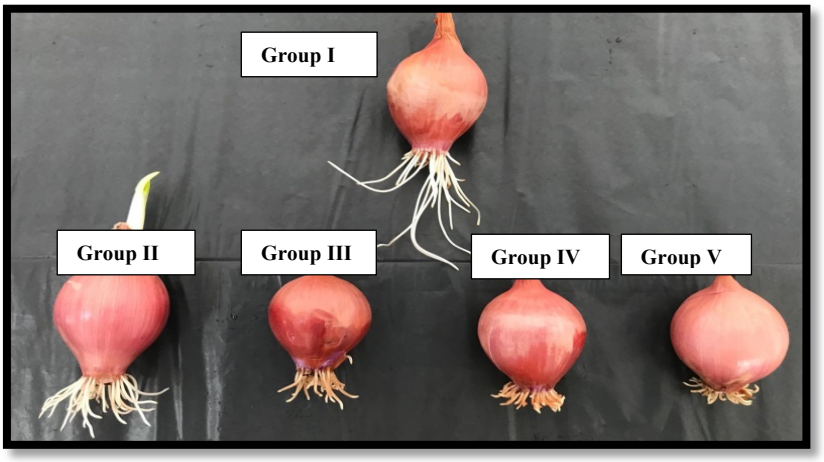

Figure 3. Germination Status After Application of La-TiO_2 Nanoparticles (Group I: Control (distilled water), Group II: 10 ppm, Group III: 25 ppm, Group IV: 50 ppm, Group V: 100 ppm)

3.3. Effect of La-TiO_2 Nanoparticles on Cytogenetic Parameters

In our study, the cytogenetic effects of La-TiO_2 nanoparticles were investigated by determining MN formation, MI ratio, and chromosomal damage. The effects of the studied nanoparticle on MN formation (Table 4) and (Figure 4) are shown. It was determined that La-TiO_2 nanoparticles, depending on the application dose, promoted MN formation in A. cepa root tip cells. As a result of microscopic observations, an average of 0.60±0.72 MN formation was observed in the control group. While only a few MN formations were detected in Group I (control group), it was found that as the nanoparticle dose increased, MN formation also increased. The average MN formations were measured as 10.90±1.43 in Group II, 19.70±3.32 in Group III, 41.80±5.18 in Group IV, and 69.20±7.36 in Group V. The appearance of cytotoxic effects in mitotic cells is associated with changes in cells during the early stages of the cell cycle, particularly in cells prior to mitosis. For example, this could be related to the inhibition of DNA synthesis in the S phase or blockage in the G2 phase [43]. Abnormalities that occur during these phases lead to many abnormalities in root cells, such as MN. Additionally, an increase in the frequency of chromosomal abnormalities (CA) was observed in *A. cepa* meristematic cells exposed to TiO_2 nanoparticles, depending on the concentration. For the determination of MI, 1,000 cells from each root tip in each group, with a total of 10,000 cells, was examined. For the determination of micronucleus formation, 100 cells from each root tip in each group and a total of 1,000 cells were analyzed.

Table 4. Effect of La-TiO_2 Nanoparticles on Micronucleus (MN) Formation and Mitotic Index (MI) at Different Doses. Group I: Control, Group II: 10 ppm, Group III: 25 ppm, Group IV: 50 ppm, Group V: 100 ppm

Groups	MN Frequency Mean ± SD	Mitotic Index (MI) Average	Percent MI %
Group I	$0.60\pm0.72^{e*}$	$887\pm31.19^{a*}$	8.87
Group II	$10.90\pm1.43^{d*}$	$746\pm29.46^{b*}$	7.46
Group III	$19.70\pm3.32^{c*}$	$631\pm27.83^{c*}$	6.31
Group IV	$41.80\pm5.18^{b*}$	$492\pm23.51^{d*}$	4.92
Group V	$69.20\pm7.36^{a*}$	$339\pm17.15^{e*}$	3.39

* Data is presented as mean ± standard deviation (SD). "Duncan" test was used for statistical difference in means, and "one-way" analysis of variance was used to determine the results. Means with different letters (a, b, c, d, e) within the same column represent statistically significant differences ($P<0.05$), ($n = 10$).

Table 5. Frequency of Chromosomal Abnormalities at Different Doses of La-TiO_2 NPs

Groups	Number of root tips	Number of Mitotic Cells	CB	SC	UDC	FRG	CM	BN
Group I	10	100	0.00±0.00e*	0.40±0.68e*	0.10±0.32e*	0.00±0.00e*	0.00±0.00e*	0.00±0.00e*
Group II	10	100	11.28±2,07d*	8.42±1.17d*	7.46±2,42d*	3.74±2,31d*	2.96±0.12d*	1.10±0.11d*
Group III	10	100	19.46±3,14c*	18.27±1.46c*	13.73±2,04c*	8.16±2,94c*	7.25±2,14c*	2.85±1,17c*
Group IV	10	100	35.73±4.32b*	32.65±1.29d*	25.18±1.73b*	17.63±1.92b*	14.55±2.47b*	5.05±2.03b*
Group V	10	100	72.79±5,23a*	69.43±2.37a*	58.16±2,34a*	37.78±2,13a*	32.17±1,84a*	11.19±1,94a*

*Chromosomal damage counts; 100 cells from each root tip in each group were counted, totaling 1,000 cells. CB: chromosome bridge, SC: sticky chromosome, UDC: unequal distribution of chromatin, FRG: fragment, CM: c-mitosis, BN: binuclear cell. Data are presented as mean ± SD. Means with different letters (a, b, c, d) in the same row indicate statistical significance ($P<0.05$), (n = 10).

The effect of La-TiO_2 application on the MI percentage is shown in Table 4. In the control group, an average MI of 8.87% was observed, while after La-TiO_2 exposure, a rapid decrease in MI percentage was observed. Compared to the control group, MI percentage decreased by 1.41% in Group II, 2.56% in Group III, 3.95% in Group IV, and 5.48% in Group V, with this decrease being statistically significant ($P<0.05$). The MI is an important parameter for determining root growth rate, as it is related to cell proliferation, which contributes to better understanding plant growth. This parameter is directly related to the frequency of cell division [21] and is thus important for explaining the effects of La on plant growth and development. In the study by Kotelnikova et al. [20], La^{3+} solution showed a significant decrease in mitotic index (MI) values at all tested concentrations. In our experiment, tests with La-TiO_2 solutions also showed a significant correlation between MI and root length. As the MI decreased at higher doses, a decrease in root length was also observed. The frequency of chromosomal abnormalities for high concentrations of La-TiO_2 may be low due to the significant decrease in the number of dividing cells (low MI values). In another study on *Allium cepa L.*, it was observed that mitotic index significantly decreased at all tested La^{+3} concentrations (0–200 mg/L). The presence of lanthanum caused abnormalities such as residual chromosomes in metaphase, stickiness, C-mitosis, and disrupted metaphase and anaphase [20]. Our findings are consistent with the values reported by Kotelnikova et al. [20]. In another study, observed chromosomal abnormalities and comet assay results validated the dose-dependent cytogenetic and genotoxic effects of TiO_2 nanoparticles on *A. cepa* [14].

Microscopic examinations revealed that the frequency of chromosomal damage induced by La-TiO_2 nanoparticles was as follows: chromosome bridge > sticky chromosome > unequal distribution of chromatin > fragment > c-mitosis > binuclear cells. The highest effect of the nanoparticles on chromosomes was identified as chromosome bridge. In the control group (with the exception of a few sticky chromosomes and unequal chromatin distribution), no statistically significant chromosomal damage was observed, while in all four treated groups, chromosomal damage increased with the application dose, and these increases were statistically significant ($P < 0.05$). Similar studies supporting our findings observed numerous c-metaphases in soy plants exposed with La concentrations ranging from 20 to 160 mM. This was likely considered the cause of the reduction in root growth, and binuclear cells were found as a result of La exposure [22]. Another study observed that the most common abnormalities in *Allium cepa L.* root tip cells exposed to La were residual chromosomes in metaphase, stickiness, c-mitosis, and disrupted metaphase and anaphase [20]. In an experiment with *Vicia faba L.* seedlings, it was shown that La caused damage to the DNA structure [40].

Pakrashi et al. [14] worked with commercially purchased Titanium (IV) oxide nanoparticles (<25 nm) and revealed TiO_2 nanoparticle exposure. Various abnormalities such as chromosome stickiness, chromosome breaks, residual chromosomes (laggards), and clustered chromosomes were observed in cells exposed to different TiO_2 NP concentrations varying between 12.5 mg/mL and 100 mg/mL. In comparison with our study, dose-dependent increases were observed. The highest chromosomal damage identified as chromosome bridge was 61.8±3.4 in their study, whereas in our study, this damage was found to be 72.79±5.23.

SEM and XRD analyses confirmed that the synthesized La-TiO_2 nanoparticles have an approximate size of 8 nm. Studies have shown that a reduction in nanoparticle size increases toxicity in plants. Specifically, as nanoparticle size decreases, their surface area increases, leading to higher chemical reactivity. Furthermore, smaller nanoparticles can more easily penetrate cell membranes and infiltrate plant tissues more deeply, thereby increasing the risks of cytotoxicity and genotoxicity. Several studies support this phenomenon. Demir et al. [41]. (2014) reported that titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles of different sizes induced primary DNA damage, as determined by the comet assay. Based on these findings, researchers have suggested that ZnO nanoparticles may act as clastogenic, genotoxic, and cytotoxic agents in Allium cepa root tip cells. These findings indicate that reducing nanoparticle size may enhance plant toxicity. Additionally, the incorporation of La into TiO_2 nanoparticles is also expected to increase toxicity.

Filho et al. [42]. (2019) demonstrated that exposing A. cepa roots to TiO_2 nanoparticles at a concentration of 1000 mg/L resulted in a significant increase in chromosomal abnormalities and micronucleus (MN) formation. Similarly, Castiglione et al. [43] (2016) observed an increase in chromosomal abnormalities in *Vicia faba* upon exposure to TiO_2 nanoparticles. Another study by Bellani et al. [44] (2020) reported cytotoxic effects, including chromosomal abnormalities, in *Lens culinaris* exposed to TiO_2 nanoparticles. Furthermore, in our study, specific chromosomal abnormalities such as bridge formation and fragmentations were observed to be more pronounced at 100 mg/L compared to separate studies on La and TiO_2

nanoparticles. This suggests that the incorporation of La into TiO_2 enhances toxicity. These findings are consistent with previous research reporting increased chromosomal abnormalities upon exposure to TiO_2 nanoparticles [14, 20, 42].

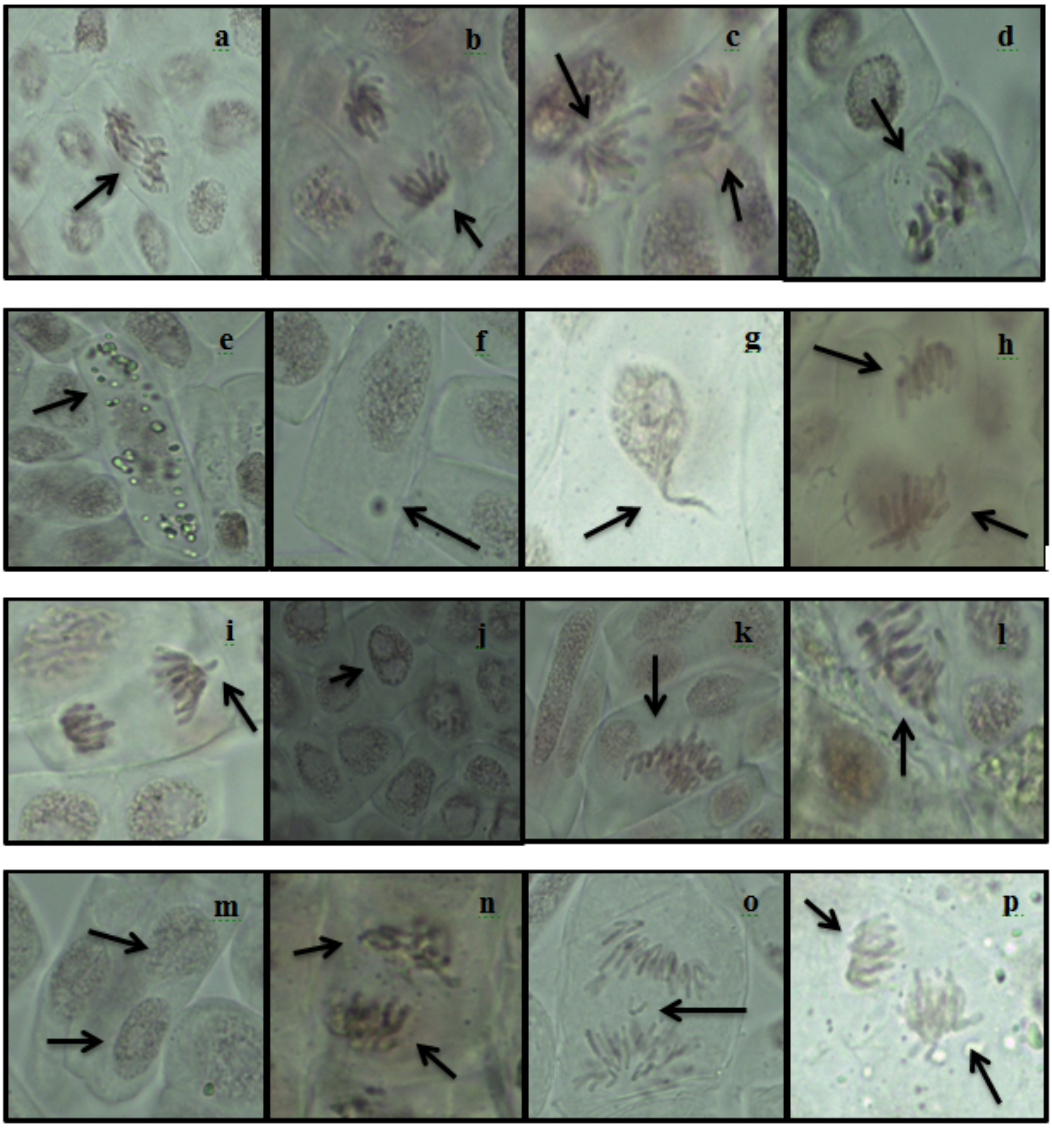

Figure 4. Chromosomal abnormalities observed in *A. cepa* L. root cells exposed to La-TiO_2 nanoparticles. (a: chromosome bridge, b: Pole flattening in anaphase, c: Pole shift in anaphase, d: Irregular prophase, e: Intracellular substance accumulation, f:Mn, g: nuclear budded nucleus, h,p: unequal distribution of chromatin, i: vagrant chromosome, j: vacuolated nucleus, k: sticky chromosome l: c-mitosis, m: binuclear cell, n: Irregular distribution in anaphase o: fragment).

3.4. Effect of La-TiO_2 NPs on Anatomical Parameters

Anatomical damages observed in *Allium cepa L.* root tip cells after the application of La-TiO_2 nanoparticles are shown in Figure 5. No anatomical changes or damages were caused in the control group *Allium cepa L.* root tip cells and tissues, while an increase in the rate of anatomical changes and damages was observed with the increasing doses of La-TiO_2 nanoparticles in the

treatment groups. Microscopic examination revealed that La-TiO_2 nanoparticles caused several anatomical damages in *A. cepa* root tip cells. The frequency of these damages increased with the concentration of La-TiO_2.

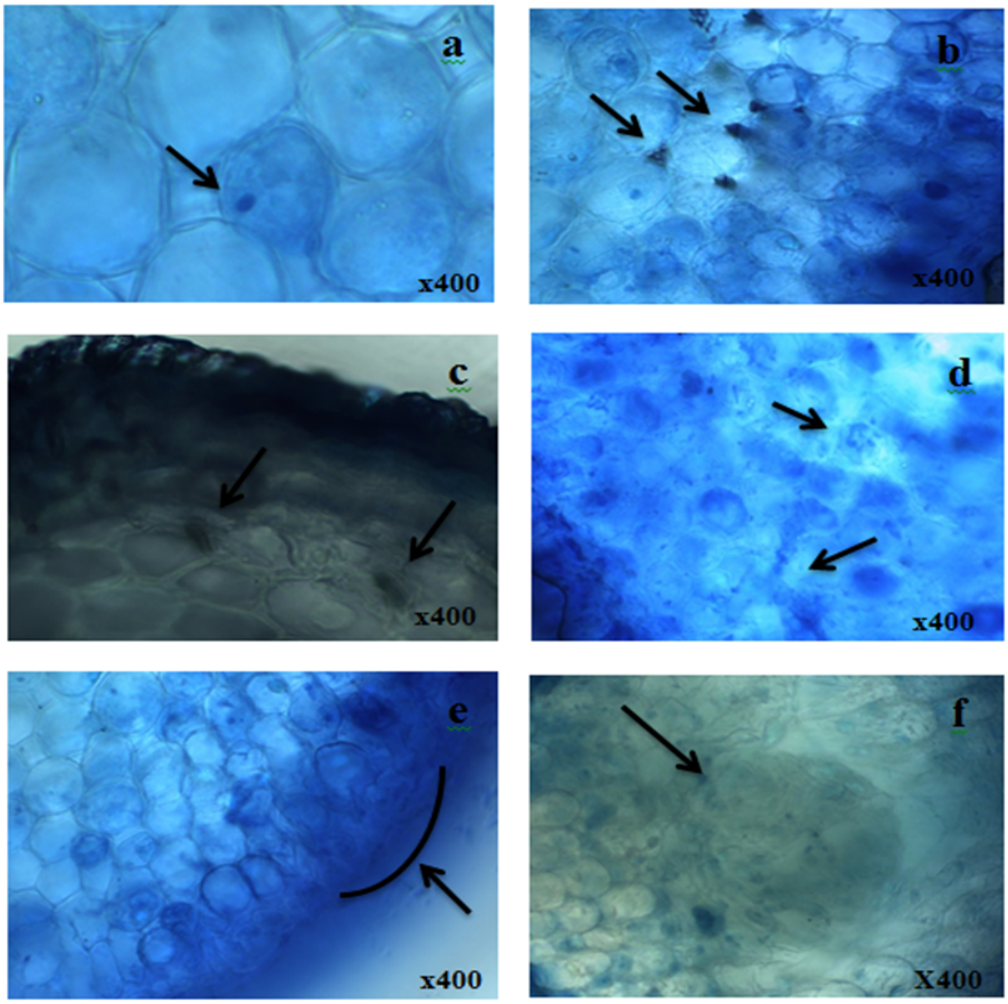

Figure 5. Anatomical damages in meristem cells caused by La-TiO_2; a: flattened cell nucleus, b: cortex cell wall thickening, c: necrosis, d: accumulation of substances in cortex cells, e: epithelial cell deformation, f: unclear transmission tissue.

In this research, several anatomical damages, for example flattened cell nuclei, epidermal cell deformation, cortex cell wall thickening, necrosis and unclear transmission tissue, were identified in the groups after nanoparticle application. These results demonstrate that root tip cells show different anatomical responses when exposed to La-TiO_2 nanoparticles. Anatomical changes such as epidermal deformation and cortex cell wall thickening were observed in low-dose (10 ppm) nanoparticle applications. Necrosis, seen as brown-black spots, was observed in the high-dose groups (50 and 100 ppm), indicating an anatomical damage developed in response to toxicity. In the literature, it is stated that smaller nanoparticles can more easily pass through porous structures in the epidermis and other membranes, allowing them to enter the organism in deeper concentrations, interacting more strongly with vital parts of the plant [45]. For example, TiO_2 nanoparticles of 14 nm size were observed to accumulate easily in root

parenchyma, while particles of 25 nm size mainly accumulated in the vascular cylinder [46]. Generally, as the size of nanoparticles decreases, higher toxicity is observed at the same concentration. Since there is no comprehensive study on the anatomical damage caused by La-TiO_2 nanoparticles synthesized using the microwave method in root tip cells, the obtained results have been discussed by comparing them with data from studies conducted on similar nanoparticles.

Some studies have reported that slight lanthanide accumulations taken from soil were observed in the cell walls, intercellular spaces, plasma membrane, vesicles, and vacuoles of the root epidermal cells of ferns that hyperaccumulate lanthanides [47]. Filho et al. [42] also observed damage in the plasma membrane and cell wall of roots exposed to TiO_2 nanoparticles, indicating that these barriers were not effective in preventing TiO_2 NP uptake by meristematic cells.

It has been observed that high concentrations of La inhibit growth, disrupt cell division, and cause root adhesion in plants. These effects suggest that La could negatively impact the overall health and resistance of plants [20], [47].

Conclusion

Many studies have investigated the genotoxic, cytotoxic, and physiological effects of various nanoparticles (NPs) such as Ag, TiO_2, ZnO, Fe, and Cd on plants, reporting different results. It has been shown that smaller-sized nanoparticles cause toxicity in plants even at concentration levels as low as 10 ppm. In this study, La-doped TiO_2 NPs were synthesized using the microwave synthesis method and characterized in terms of shape, size, and experimental techniques using XRD and SEM analyses. The toxic effects of different concentrations of La-TiO_2 NPs (10, 25, 50, and 100 mg/L) on *A. cepa L.* plants were investigated. In previous studies toxic effects of TiO_2 NPs and La on plant cells were explored individually. In our work, La was incorporated into TiO_2 NPs through the microwave synthesis method to study their synergestic effect on cytotoxicity, genotoxicity and anatomical parameters of *A. cepa L.* plant. Moreover, while most literature studies involve commercially available NPs, genotoxic and cytotoxic studies conducted after the synthesis and characterization of NPs are limited. In the studies conducted, the NP size was generally kept larger in the range of 20-100 nm. In our study, the size of La-TiO_2 NPs was determined to be approximately 8 nm, and it was found to have a significant impact on cytotoxicity, genotoxicity, germination rate, root elongation, and plant anatomical levels. Additionally, with the decrease in nanoparticle size and the incorporation of La into the structure, the toxic effect on chromosomal damage was found to be higher than most of the other TiO_2 and La studies.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contribution

Uzun Akgeyik, A. concept; Uzun Akgeyik A. and Akgeyik, E. design; Uzun Akgeyik A. and Akgeyik, E. resources; Uzun Akgeyik A. and Akgeyik, E. materials; Akgeyik, E. data collection and processing; Uzun Akgeyik, A. data validation; Uzun Akgeyik, A. analysis and interpretation; Akgeyik, E. literature search; Uzun Akgeyik, A. writing; Uzun Akgeyik A. and Akgeyik, E. All authors have read and agreed to the published version of the manuscript.

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