



Cytotoxic and Genotoxic Assessment of Silicon Dioxide Nanoparticles by Allium and Comet Tests

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Abstract

Silicon nanoparticles gained a great interest due to its use in biomedical research. It is considered as safe and has been used in nanomedicine. But literature still states its toxicity depending upon the size and dose of silicon nanoparticles. So, current study was aimed to evaluate the cytotoxicity and genotoxicity of silicon dioxide nanoparticles (SiO₂NPs) by Allium anaphase–telophase and Comet tests. Characterization of SiO₂NPs showed the particle size as 16.12 ± 3.07 nm. The mean diameter of SiO₂NPs was having range of 404.66 ± 93.39 nm in solution. Highest total anomalies (18.80 ± 0.45) were observed at 100 µg/mL, whereas least (11.2 ± 0.84) were observed by the 12.5 µg/mL concentration. There was concentration–response association in increased CAs and DNA damage. The highest concentration (100 µg/mL) of SiO₂NPs induced the significant DNA damage (149.67 ± 1.15), whereas the least was observed by the negative control (2.67 ± 0.58). The current study revealed the cytotoxic and genotoxic effects of SiO₂NPs on the root meristem cells of *A. cepa*.

Keywords Silicon dioxide nanoparticles · Mitotic index · DNA damage · *Allium cepa*

Nowadays nanoparticles (NPs) are widely used due to their distinctive physical properties like enhanced surface and chemical reactivity, more surface area to mass ratio, high cell permeability (Hochella et al. 2008; Oberdörster et al. 2005). SiO₂NPs are used for a wide range of applications like in rubber, plastic, adhesives, ceramics industries (Barbe et al. 2004; Rothen-Rutishauser et al. 2006). Moreover, it is also used extensively for biomedical applications including drug delivery, biomedical imaging and gene therapy, because of its ideal properties of resistant to biodegradation

and biocompatibility in cellular environments (Hirsch et al. 2003; Moghimi et al. 2005; Slowing et al. 2008). Thus, threat of accidental release of these and other NPs to environment and ultimately to food chain is inevitable (Oberdörster et al. 2005). Documented studies on different organisms and cells suggested that SiO₂NPs can have lethal effects on their development and growth (Arnold et al. 2013; Rodea-Palomares et al. 2010). So, wide use of SiO₂NPs needs a comprehensive understanding of their potential genotoxic effects on animal and human health.

Allium test is widely used to analyze genotoxicity and cytotoxicity of environmental samples and as well as for chemicals (Gupta et al. 2018; Liman et al. 2013). This test is extensively used due to its many properties like high sensitivity, economic and easy to proceed, less number of chromosomes ($2n = 16$), and reproducibility of results (Rahman et al. 2017; Gupta et al. 2018, Caritá and Marin-Morales 2008; Chaparro et al. 2010; Liman et al. 2015). It also has been successfully employed as biomarker for nano and micro materials (Ghosh et al. 2011; Kaygisiz and Cığerci 2017; Liman 2013; Rajeshwari et al. 2016). Comet assay is used to evaluate DNA damage and has been used to find the genotoxicity of nanoparticles due to its reliability, simplicity, low cost, sensitivity and versatility (Ghosh et al. 2015; Cvjetko et al. 2017; Cığerci et al. 2015; De et al. 2016; Demir et al.

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2014; Mangalampalli et al. 2018). Although, studies have been reported on the genotoxicity and cytotoxicity of silicon nanoparticles in human and other mammalian cells, but limited studies have been observed on cytotoxic and genotoxic behavior of SiO₂NPs in plant cells. So, current study was designed to explore the cytotoxicity and genotoxicity of SiO₂NPs by Comet and Allium ana-telophase tests. Characterization of SiO₂NPs was also performed.

Materials and Methods

Silicon dioxide (10–20 nm particle size, CAS Number: 7631-86-9) were purchased from sigma Aldirich. Scanning and Transmission electron microscopic images were taken from Phenom ProX (Phenom-World BV, Eindhoven, Netherlands) and JEM-2100 (JEOL, Tokyo, Japan) with voltage of about 15 KV (SEM) and 200 KV (TEM). Energy Dispersive Xray Spectroscopy (EDX) and Zetasizer (Malvern Nano ZS90) were used to determine the size distribution, elemental analysis and zeta potential of particles.

A. Cepa organic bulbs were obtained from local market. Bulbs of about 25–30 mm were used. Nominal stock concentration of NPs was prepared by 300 mL double distilled water and making it of 500 µg/mL. Inductively coupled plasma-mass spectrometry (Thermo Scientific ICAP RQ ICP-MS, USA) was used to determine SiO₂NPs concentration in the stock suspension, which was 115 ± 10 µg/mL in the suspension. Different concentrations (12.5, 25, 50 and 100 µg/mL) of SiO₂NPs were prepared and suspended in distilled water. This was followed by sonication for about 30 min on ultrasonic water bath (Bandelin Sonorex Digitec DT100, Germany, 320 W, 35 kHz). The roots on reaching 2–3 cm in length were exposed to arbitrarily selected concentrations of SiO₂NPs, distilled water (negative control) and MMS (10 µg/mL, positive control) at room temperature (21 ± 4 °C) for 4 h in the dark. After the exposure time period, ethanol:glacial acetic acid (3:1, v/v) solution was used to fix the root tips (0.7–1 cm) at 4 °C for 24 h. This was followed by washing with distilled water and then stored in 70% alcohol at 4 °C. The root tips were washed with distilled water and hydrolyzed at 60 °C by using 1 N HCl for about 8–10 min. Following this, 20–25 min of staining was done at room temperature by using Feulgen dye. The frequency of CAs (stickiness, distributed anaphase–telophase, chromosome laggards and anaphase bridge) and MI were measured as demonstrated by (Saxena et al. 2005) with minor modifications. It was done by using Nikon Eclipse Ci-L light microscope (Japan) that was equipped with a CMOS camera (Argenit, Kameram, Turkey). As per treatment, five slides were randomly taken and counted for scores. By using the given equation, CAs and MI were calculated .

$$MI = (\text{total number of dividing cells} / \text{total cell numbers}) \times 100.$$

$$CA = (\text{total number of abnormal cells} / 100 \text{ anaphase-telophase cells}) \times 100.$$

Comet assay was performed as demonstrated by (Tice et al. 2000) with slight modifications. Same concentrations (12.5, 25, 50 and 100 µg/mL) of NPs were used to expose the mersistem cells of *A. cepa* root. Ice cold Tris–MgCl₂ nuclei isolation buffer (4 mM MgCl₂·6H₂O; 0.5% w/v Triton X-100, 0.2 M Tris, pH 7.5) was used to isolate nuclei from ten seedlings. Following this, 50 µL of cells were taken and mixed with 50 µL of LMPA (1.5% prepared) and poured over the slides that were pre coated with NMPA (1% prepared). After that, lysis was done in alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for about 20 min at 4 °C. After lysis, slides were placed in electrophoresis tank and gel was run at 300 mA and 25V for 20 min at 4 °C. Neutralization was carried out by the 0.4 M Tris (pH 7.5) and stained with 70 µL EtBr solution (20 µg/mL). Fifty comets per slide were scored as from 0-undamaged to 4-complete damage, using fluorescence microscope (BAB, TAM-F, Turkey) equipped with a CCD camera (BAB, TC-5, Turkey). The analysis of arbitrary unit (AU) values of each treatment were calculated according to (Ciğerci et al. 2015).

Results and Discussion

Scanning electron microscope (SEM) was used to analyze the adsorption of SiO₂NPs by the root surface. Figure 1a shows the roots from the samples that were lacking nanoparticle organization while Fig. 1b shows the roots that has adsorbed SiO₂NPs at the surface. Figure 1d reveals the results of electron dispersive X-ray (EDX) studies which made it clear that detected particles were having silicon. By using TEM, physiochemical properties of SiO₂NPs were recorded and presented in a Table 1. About 100 particles were randomly measured to calculate the mean size of SiO₂NPs particles using TEM. Figures 1 and 2 shows size measurements of SiO₂NPs that was obtained as 16.12 ± 3.07 nm. The average diameter of SiO₂NPs was obtained in the range of 404.66 ± 93.39 nm by using water suspension with ultrahigh purity. On the other hand, pdI value of SiO₂NPs was also recorded that was 0.513. Table 1 also shows the electrophoretic mobility of SiO₂NPs particles and zeta potential (ζ) in ultrahigh purity water. Henry's equation was used to calculate electrophoretic mobility (Baalousha et al. 2012).

The effects of SiO₂NPs on MI and mitotic phases in the root tips of *A. cepa* after 4 h are shown in Table 2. Concentration dependent increase in MI was observed by the all concentrations of SiO₂NPs but these were lower than the negative control group. Negative control showed the

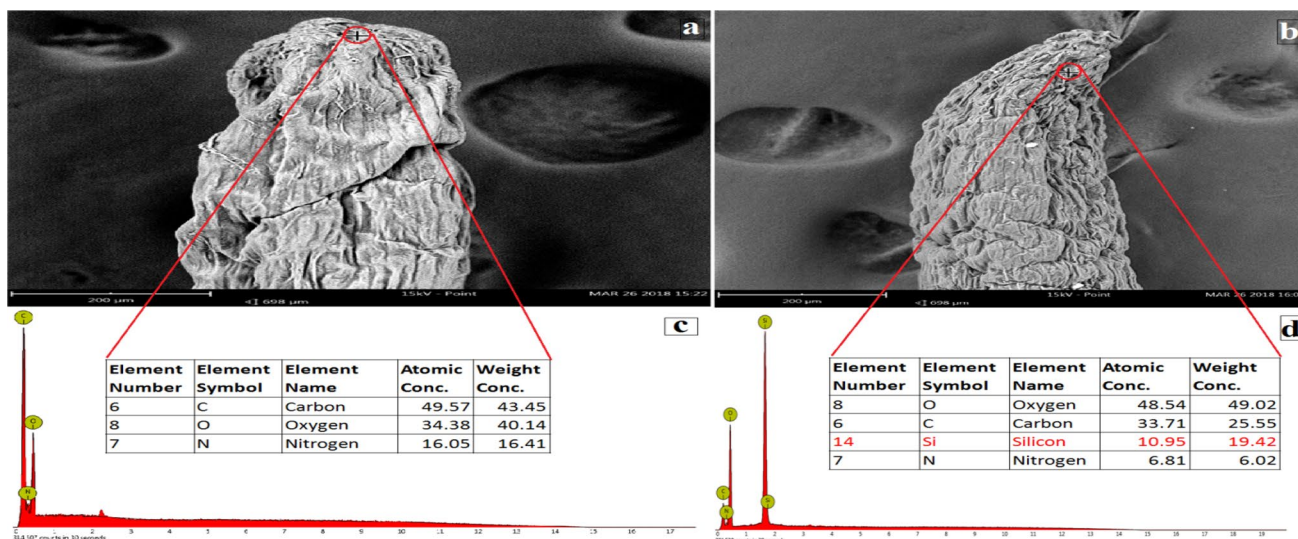


Fig. 1 SEM images of surface adhesion of SiO₂NP son Allium root; **a** and **c** control roots showing the absence of particle adhesion to the surface and EDX analysis of SiO₂NPs, **b** and **d** showing SiO₂NPs adhered to the root surface and EDX analysis of SiO₂NPs

Table 1 Characterization of silicon dioxide nanoparticles (SiO₂NPs)

Particles	Size using TEM (nm)	Average diameter (nm)	Polydispersion index (PdI)	Zeta potential ζ (mV)	Electrophoretic mobility (μm ² /s V)
SiO ₂ NPs	16.12 ± 3.07	404.66 ± 93.39	0.513	- 19.0	- 1.48

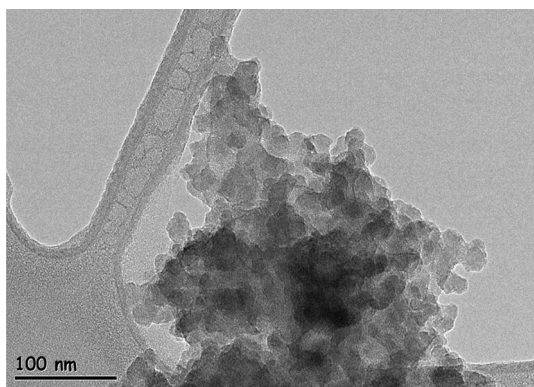


Fig. 2 Transmission electron microscopy (TEM) images of silicon dioxide nanoparticles (SiO₂NPs)

highest MI value (71.96 ± 1.16) while least (43.25 ± 0.52) was observed by the positive control group. There was an increase in the mitotic phases after the exposure of SiO₂NPs, compared to the negative control group, except for the prophase at all concentrations and telophase at 25 μg/mL. Significant decrease of MI at lower concentrations may be due to inhibition of DNA synthesis (Sudhakar et al. 2001) or

blocking of G2 phase, preventing the cell from entering mitosis (EI-Ghamery et al. 2000) or mitotic inhibition of chemical(s) (Sharma and Vig 2012) or blockage of specific cell cycle proteins which further stop DNA polymerase and other enzymes leading to antimitotic effect (Hidalgo et al. 1989; Türkoğlu 2015). Dose dependent increase in MI by exposure of SiO₂NPs demonstrated that higher concentrations of SiO₂NPs are less phytotoxic. It could be due that SiO₂NPs appears to block mitosis at lower doses by kinetically stabilizing spindle microtubules and not by changing the mass of polymerized microtubules (Jordan et al. 1993). SiO₂NPs also have been used to check the cytotoxic effects of these on different cancerous cell lines (Gong et al. 2012; Lin et al. 2006; Wagner et al. 2009). (Park et al. 2009) also demonstrated the toxic effects of silica NPs in the embryonic stem cell even below the cytotoxic doses. Previously, it has been reported that silica NPs can easily agglomerate and influences the uptake of NPs into the cell (Lin and Haynes 2010; Napierska et al. 2010). Demir et al. (2015) also demonstrated that at highest concentration (100 μg/mL) of SiO₂NPs agglomerated in *Drosophila melanogaster* cells which further decreases the penetration of NPs in to the cells. So, less amount of NPs might be eliminated by the own defence mechanism of plant cells (Demir et al. 2015). In our current study, lowest phytotoxicity of SiO₂NPs at highest concentration could be suggestive of this mechanism. However, further studies on the cellular defense mechanism at molecular level after SiO₂NPs exposure should be carried out.

CAs (chromosome laggards, disturbed anaphase–telophase, stickness and anaphase bridge) induced by SiO₂NPs in *A. cepa* root meristematic cells are shown in Table 3 and

Fig. 3. It was observed that SiO₂NPs induced significant CAs in *A. cepa* root anaphase–telophase cells compared to the control group. There was a concentration dependent increase in the total CAs by the SiO₂NPs ($r = 0.967$, $p = 0.01$). Highest total anomalies (18.80 ± 0.45) were observed at 100 $\mu\text{g}/\text{mL}$, whereas least (11.2 ± 0.84) were observed at the 12.5 $\mu\text{g}/\text{mL}$ dose. No significant difference was found between the total anomalies, at the highest concentration of studied NPs and the positive control

group ($p > 0.05$). The highest frequency of disturbed anaphase–telophase was observed at all concentrations of SiO₂NPs (except at 12.5 $\mu\text{g}/\text{mL}$) and stickiness at 50 and 100 $\mu\text{g}/\text{mL}$ compared to the positive control group. The *A. cepa* cytotoxic test has been used to monitor the genotoxicity of NPs (Kaygisiz and Cigerci 2017; Kumari et al. 2011; Liman 2013). Previously, a cell proliferation assay showed nontoxic effects at low dosages, but cell viability decreases at high dosages by the composite silica NPs on

Table 2 The effects of SiO₂NPs on mitotic index and mitotic phases in the root tips of *A. cepa* after 4 h

Treatments	Concentrations ($\mu\text{g}/\text{mL}$)	CCN	MI \pm SD	Mitotic phases (%) \pm standard deviation (SD) ^a			
				Prophase	Metaphase	Anaphase	Telophase
Control	–	5108	71.96 \pm 1.16a	91.38 \pm 0.57a	1.47 \pm 0.25a	1.36 \pm 0.19a	5.79 \pm 0.25a
MMS	10	5140	43.25 \pm 0.52b	82.55 \pm 0.49b	3.37 \pm 0.36b	2.66 \pm 0.54b	11.42 \pm 0.35b
SiO ₂ NPs	12.5	5200	53.50 \pm 0.99c	88.46 \pm 0.48ce	2.55 \pm 0.31c	2.44 \pm 0.31b	6.54 \pm 0.44c
	25	5142	57.09 \pm 0.60d	89.71 \pm 0.24d	2.18 \pm 0.07d	2.42 \pm 0.14b	5.69 \pm 0.39a
	50	5095	59.41 \pm 0.42e	87.91 \pm 0.54c	2.74 \pm 0.32c	2.25 \pm 0.29b	7.10 \pm 0.42d
	100	5201	61.66 \pm 0.91f	88.87 \pm 0.33e	1.93 \pm 0.12d	2.37 \pm 0.19b	6.83 \pm 0.36 cd

CCN counting cell numbers, MI Mitotic Index, Con. concentrations

^aMeans with the same letter do not differ statistically at the level of 0.05

Table 3 Chromosomal anomalies induced by SiO₂NPs in *A. cepa* root meristematic cells

Treatment groups	Concentrations ($\mu\text{g}/\text{mL}$) ^a	Anaphase–telophase anomalies %					
		CCN	DAT	CL	S	AB	TA \pm SD ^a
Control	–	500	5.2	–	2.6	–	7.8 \pm 0.84 ^a
MMS	10	500	5	5.2	6.2	3.4	19.8 \pm 0.45 ^b
SiO ₂ NPs	12.5	500	5	1.6	3.4	1.2	11.2 \pm 0.84 ^c
	25	500	7.4	1.8	3.2	1.6	14 \pm 1 ^d
	50	500	7	1.4	6.4	1.6	16.4 \pm 0.89 ^e
	100	500	7.4	2.4	7.2	1.8	18.80 \pm 0.45 ^b

SD standard deviation, CCN counting cell numbers, DAT disturbed anaphase–telophase, CL chromosome laggards, S stickiness, AB anaphase bridge, TA total anomalies, Con. concentrations

^aMeans with the same letter do not differ statistically at the level of 0.05

Fig. 3 Anaphase–telophase anomalies in root tips of *A. cepa* induced by (SiO₂NPs). **a** Disturbed anaphase–telophase, **b** chromosome laggards, **c** stickiness, **d** anaphase bridge

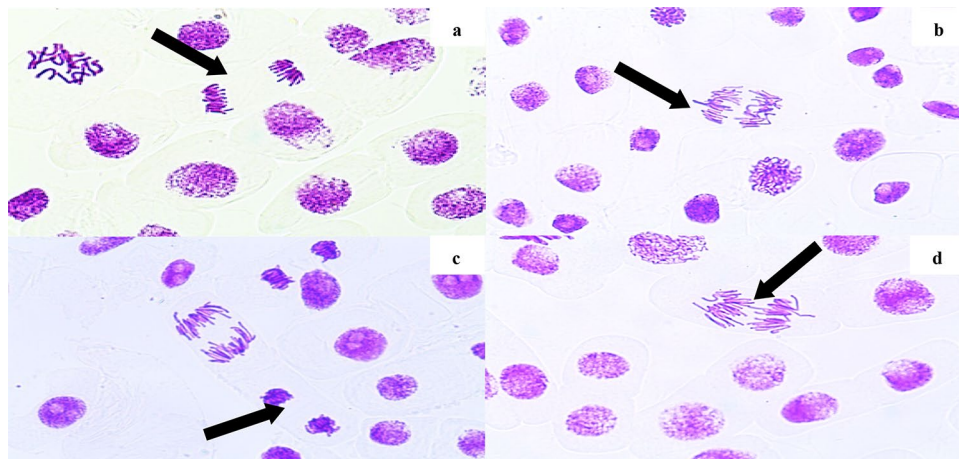


Table 4 Detection of DNA damage in nuclei of *A. cepa* root meristems exposure to SiO₂NPs using the Comet assay.

Treatments	Concentrations (µg/mL)	DNA damage (arbitrary unit ± SD) ^a
Negative control	–	2.67 ± 0.58 ^b
MMS	10	153.33 ± 1.53 ^c
SiO ₂ NPs	12.5	122 ± 1.73 ^d
	25	135.67 ± 2.08 ^e
	50	144 ± 1 ^f
	100	149.67 ± 1.15 ^g

SD standard deviation

^aMeans with the same letter do not differ statistically at the level of 0.05

normal fibroblast and tumor cells (Chang et al. 2007). No toxic effects have been observed by the SiO₂NPs to human mesothelioma cells and mouse embryonic fibroblast cells (Brunner et al. 2006). Amorphous fumed nano-silica (14 nm) showed the cytotoxicity in the human colon epithelial cell-line, when exposed for up to 24 h (Gerloff et al. 2009). Various in vitro studies of Silica NPs had showed the cytotoxic effects and oxidative stress in various model systems (Lin et al. 2006; Mu et al. 2012; Napierska et al. 2010; Sayes et al. 2007). It was demonstrated in human peripheral blood lymphocytes and HEK293 cells that different sizes of silica NPs have potential to interact with DNA and that can lead to primary DNA damage in these cells (Demir et al. 2013; Kaewamatawong et al. 2006; Kim et al. 2010). Whereas, in another study, no significant chromosome breakage and chromosome loss were observed in A549 human lung carcinoma cells (Gonzalez et al. 2010).

In the current study, different chromosomal anomalies were observed like disturbed anaphase–telophase (Fig. 3a) and chromosome laggards (Fig. 3b). These could be due to the disturbed microtubules or failure of the chromosome to move toward the poles or deformation of spindle structure (Rajeshwari et al. 2016; Singh and Roy 2017). Anaphase bridge (Fig. 3d) formation could be due to chromosomes fusion or due to dicentric chromosome or changing activation of replication enzymes (El-Ghamery et al. 2000). Increased chromosome aggregation, extra chromosomal intertwining of the chromatin fibers or depolymerization of DNA can cause the stickiness (Fig. 3c) of chromosomes (El-Ghamery and Mousa 2017; Türkoğlu 2015).

The results of DNA damage shown by the exposure of SiO₂NPs in nuclei of *A. cepa* root meristems are presented in Table 4. The significant DNA damage was induced by the all concentrations of SiO₂NPs compared to control group. The increase DNA damage showed a direct dose-response relationship ($r = 0.974$, $p = 0.01$). The highest DNA damage was observed by the positive control (153.33 ± 1.53) followed by

the 100 µg/mL (149.67 ± 1.15) dose of SiO₂NPs. While the least was observed by the negative control (2.67 ± 0.58) followed by the 12.5 µg/mL (122 ± 1.73) concentration.

The literature states that SiO₂NPs can induce oxidative stress and formation of 8-OH-dG in a dose dependent manner. Which consequently results in the oxidative stress induced DNA damage (Gong et al. 2012). It is suggested that NPs induced DNA damage can be caused by the reactive oxygen species (ROS). Another suggestive mechanism of NPs mediated DNA damage is the creation of oxidants/genotoxic compounds by stimulating the target cells (Nel et al. 2006). In present study, the genotoxic effects of SiO₂NPs in the *A. cepa* cells could be due the ROS production. Different in vitro studies demonstrated size- and dose dependent cytotoxicity, enhanced production of ROS and pro-inflammatory stimulation by the nano-silica particles (Chen and von Mikecz 2005; Eom and Choi 2009). Different sizes of Silica NPs also showed the oxidative DNA damage in *Drosophila* haemocytes by the comet assay and *Drosophila* wing somatic mutation and recombination test (Demir et al. 2015).

Abnormal clusters of topoisomerase I (topo I) in the nucleoplasm, fibrogenesis and pro-inflammatory stimulation of endothelial cells was observed by the nano-silica in the Wistar rats (Chen and von Mikecz 2005; Chen et al. 2004). However, in another investigation, no toxic effects were shown by silicon NPs on mouse (Xue et al. 2006). Similarly, SiO₂NPs did not cause the DNA damage in *Daphnia magna* and *Chironomus riparius* but caused mortality of these both species (Lee et al. 2009). In cultured mammalian cells, nano-silica also indicated the primary genotoxic and cytotoxic effects but not mutagenicity (Choi et al. 2011). Amorphous silica NPs (20–240 nm) showed no genotoxic effects on A549 human lung epithelial carcinoma cells and 3T3-L1 fibroblasts by alkaline comet assay (Chen et al. 2004; Park et al. 2009). Therefore, further studies are still needed to evaluate the genotoxic effects and mechanisms of silica NPs in different organisms along with different test systems.

It was revealed by the current study that SiO₂NPs cause the cytotoxic and genotoxic effects on the root meristem cells of *A. cepa*.

Compliance with Ethical Standards

Conflict of interest All authors show no conflict of interest.

References

- Arnold M, Badireddy A, Wiesner M, Di Giulio R, Meyer J (2013) Cerium oxide nanoparticles are more toxic than equimolar bulk cerium oxide in *Caenorhabditis elegans* Arch Environ Contam Toxicol 65:224–233

- Baalousha M et al (2012) Characterization of cerium oxide nanoparticles—Part 2: nonsize measurements. *Environ Toxicol Chem* 31:994–1003
- Barbe C et al (2004) Silica particles: a novel drug-delivery system. *Adv Mater* 16:1959–1966
- Brunner TJ et al (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol* 40:4374–4381
- Caritá R, Marin-Morales MA (2008) Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. *Chemosphere* 72:722–725
- Chang J-S, Chang KLB, Hwang D-F, Kong Z-L (2007) In vitro cytotoxicity of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environ Sci Technol* 41:2064–2068
- Chaparro T, Botta C, Pires E (2010) Biodegradability and toxicity assessment of bleach plant effluents treated anaerobically. *Water Sci Technol* 62:1312–1319
- Chen Y, Chen J, Dong J, Jin Y (2004) Comparing study of the effect of nanosized silicon dioxide and micro-sized silicon dioxide on fibrogenesis in rats. *Toxicol Ind Health* 20:21–27
- Chen M, von Mikecz A (2005) Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO₂ nanoparticles. *Exp Cell Res* 305:51–62
- Choi H-S, Kim Y-J, Song M, Song M-K, Ryu J-C (2011) Genotoxicity of nano-silica in mammalian cell lines. *Toxicol Environ Health Sci* 3:7
- Çiğerci İH, Liman R, Özgül E, Konuk M (2015) Genotoxicity of indium tin oxide by *Allium* and Comet tests. *Cytotechnology* 67:157–163
- Cvijetko P et al (2017) Toxicity of silver ions and differently coated silver nanoparticles in *Allium cepa* roots. *Ecotoxicol Environ Saf* 137:18–28
- De A, Chakrabarti M, Ghosh I, Mukherjee A (2016) Evaluation of genotoxicity and oxidative stress of aluminium oxide nanoparticles and its bulk form in *Allium cepa*. *Nucleus* 59:219–225
- Demir E et al (2013) Genotoxicity of different nano-sizes and ions of silica nanoparticles. *Fresen Environ Bull* 22:2901–2909
- Demir E, Kaya N, Kaya B (2014) Genotoxic effects of zinc oxide and titanium dioxide nanoparticles on root meristem cells of *Allium cepa* by comet assay. *Turk J Biol* 38:31–39
- Demir E, Aksakal S, Turna F, Kaya B, Marcos R (2015) In vivo genotoxic effects of four different nano-sizes forms of silica nanoparticles in *Drosophila melanogaster*. *J Hazard Mater* 283:260–266
- EI-Ghamery AA, EI-Nahas AI, Mansour MM (2000) The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Vicia faba*. *Cytologia* 65:277–287
- EI-Ghamery A, Mousa M (2017) Investigation on the effect of benzyldene on the germination, radicle growth and meristematic cells of *Nigella sativa* L. and *Allium cepa* L. *Ann Agric Sci* 62:11–21
- Eom H-J, Choi J (2009) Oxidative stress of silica nanoparticles in human bronchial epithelial cell Beas-2B. *Toxicol In Vitro* 23:1326–1332
- Gerloff K, Albrecht C, Boots AW, Förster I, Schins RP (2009) Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. *Nanotoxicology* 3:355–364
- Ghosh M, Chakraborty A, Bandyopadhyay M, Mukherjee A (2011) Multi-walled carbon nanotubes (MWCNT): induction of DNA damage in plant and mammalian cells. *J Hazard Mater* 197:327–336
- Ghosh M, Bhadra S, Adegoke A, Bandyopadhyay M, Mukherjee A (2015) MWCNT uptake in *Allium cepa* root cells induces cytotoxic and genotoxic responses and results in DNA hyper-methylation. *Mutat Res* 774:49–58
- Gong C, Tao G, Yang L, Liu J, He H, Zhuang Z (2012) The role of reactive oxygen species in silicon dioxide nanoparticle-induced cytotoxicity and DNA damage in HaCaT cells. *Mol Biol Rep* 39:4915–4925
- Gonzalez L et al (2010) Exploring the aneugenic and clastogenic potential in the nanosize range: A549 human lung carcinoma cells and amorphous monodisperse silica nanoparticles as models. *Nanotoxicology* 4:382–395
- Gupta K, Mishra K, Srivastava S, Kumar A (2018) Cytotoxic assessment of chromium and arsenic using chromosomal behavior of root meristem in *Allium cepa* L. *Bull Environ Contam Toxicol* 100:803–808
- Hidalgo A, Gonzalez-Reyes J, Navas P, Garcia-Herdugo G (1989) Abnormal mitosis and growth inhibition in *Allium cepa* roots induced by protham chlorpropham. *Cytobios* 57:7–14
- Hirsch LR et al (2003) Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc Natl Acad Sci USA* 100:13549–13554
- Hochella MF, Lower SK, Maurice PA, Penn RL, Sahai N, Sparks DL, Twining BS (2008) Nanominerals, mineral nanoparticles, and earth system. *Science* 319:1631–1635
- Jordan MA, Toso RJ, Thrower D, Wilson L (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc Natl Acad Sci USA* 90:9552–9556
- Kaewamatawong T, Shimada A, Okajima M, Inoue H, Morita T, Inoue K, Takano H (2006) Acute and subacute pulmonary toxicity of low dose of ultrafine colloidal silica particles in mice after intratracheal instillation. *Toxicol Pathol* 34:958–965
- Kaygisiz ŞY, Çiğerci İH (2017) Genotoxic evaluation of different sizes of iron oxide nanoparticles and ionic form by SMART, *Allium* and comet assay. *Toxicol Ind Health* 33:802–809
- Kim Y-J, Yu M, Park H-O, Yang SI (2010) Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by silica nanomaterials in human neuronal cell line. *Mol Cell Toxicol* 6:336–343
- Kumari M, Khan SS, Pakrashi S, Mukherjee A, Chandrasekaran N (2011) Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. *J Hazard Mater* 190:613–621
- Lee S-W, Kim S-M, Choi J (2009) Genotoxicity and ecotoxicity assays using the freshwater crustacean *Daphnia magna* and the larva of the aquatic midge *Chironomus riparius* to screen the ecological risks of nanoparticle exposure. *Environ Toxicol Pharm* 28:86–91
- Liman R (2013) Genotoxic effects of Bismuth (III) oxide nanoparticles by *Allium* and Comet assay. *Chemosphere* 93:269–273
- Liman R, Çiğerci İH, Öztürk NS (2015) Determination of genotoxic effects of Imazethapyr herbicide in *Allium cepa* root cells by mitotic activity, chromosome aberration, and comet assay. *Pest Biochem Physiol* 118:38–42
- Lin W, Huang Y-w, Zhou X-D, Ma Y (2006) In vitro toxicity of silica nanoparticles in human lung cancer cells. *Toxicol Appl Pharmacol* 217:252–259
- Lin Y-S, Haynes CL (2010) Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *J Am Chem Soc* 132:4834–4842
- Mangalampalli B, Dumala N, Grover P (2018) *Allium cepa* root tip assay in assessment of toxicity of magnesium oxide nanoparticles and microparticles. *J Environ Sci* 66:125–137
- Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. *FASEB J* 19:311–330
- Mu Q, Hondow NS, Krzemiński Ł, Brown AP, Jeuken LJ, Routledge MN (2012) Mechanism of cellular uptake of genotoxic silica nanoparticles. *Part Fibre Toxicol* 9:29
- Napierska D, Thomassen LC, Lison D, Martens JA, Hoet PH (2010) The nanosilica hazard: another variable entity. *Part Fibre Toxicol* 7:39

- Nel A, Xia T, Mädler L, Li N (2006) Toxic potential of materials at the nanolevel. *Science* 311:622–627
- Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113:823–839
- Park MV et al (2009) In vitro developmental toxicity test detects inhibition of stem cell differentiation by silica nanoparticles. *Toxicol Appl Pharmacol* 240:108–116
- Rahman MM, Rahman MF, Nasirujjaman K (2017) A study on genotoxicity of textile dyeing industry effluents from Rajshahi, Bangladesh, by the *Allium cepa* test. *Chem Ecol* 33:434–446
- Rajeshwari A, Roy B, Chandrasekaran N, Mukherjee A (2016) Cytogenetic evaluation of gold nanorods using *Allium cepa* test. *Plant Physiol Biochem* 109:209–219
- Rodea-Palomares I, Boltes K, Fernández-Pinas F, Leganés F, García-Calvo E, Santiago J, Rosal R (2010) Physicochemical characterization and ecotoxicological assessment of CeO₂ nanoparticles using two aquatic microorganisms. *Toxicol Sci* 119:135–145
- Rothen-Rutishauser BM, Schürch S, Haenni B, Kapp N, Gehr P (2006) Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. *Environ Sci Technol* 40:4353–4359
- Saxena PN, Chauhan LK, Gupta SK (2005) Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: Spectroscopic basis of chromosome damage. *Toxicology* 216:244–252
- Sayes CM, Reed KL, Warheit DB (2007) Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. *Toxicol Sci* 97:163–180
- Sharma S, Vig AP (2012) Genotoxicity of atrazine, avenoxan, diuron and quizalofop-*P*-ethyl herbicides using the *Allium cepa* root chromosomal aberration assay. *Terrest Aquat Environ Toxicol* 6:90–95
- Singh D, Roy BK (2017) Evaluation of malathion-induced cytogenetical effects and oxidative stress in plants using *Allium* test. *Acta Physiol Plant* 39:92
- Slowing II, Vivero-Escoto JL, Wu C-W, Lin VS-Y (2008) Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carrier. *Adv Drug Deliv Rev* 60:1278–1288
- Sudhakar R, KN NG, Venu G (2001) Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia* 66:235–239
- Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 35:206–221
- Türkoğlu Ş (2015) Evaluation of genotoxic effects of five flavour enhancers (glutamates) on the root meristem cells of *Allium cepa*. *Toxicol Ind Health* 31:792–801
- Wagner S, Münzer S, Behrens P, Scheper T, Bahnemann D, Kasper C (2009) Cytotoxicity of titanium and silicon dioxide nanoparticles. *Journal of physics: conference series*, vol 1. IOP Publishing, Bristol, p 012022
- Xue Z et al (2006) Biototoxicology and biodynamics of silica nanoparticle. *J Cent South Univ Med Sci* 31:6–8

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