Nanomed Res J 11(3):308-321, Summer 2026

RESEARCH ARTICLE

Protection of L-arginine over cinnamic acid against aluminum chloride-induced toxicity in *Paramecium caudatum* in the presence of zinc oxide nanoparticles and static magnetic field

Fatemeh Asadi-Zardroodi[1], Manizheh Karami[1*], Abazar Hajnorouzi[2]

[1] Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.

[2] Department of Physics, Faculty of Basic Sciences, Shahed University, Tehran, Iran.

ARTICLE INFO

Article History:
Received: 2026-06-10
Revised: 2026-09-10
Accepted: 2026-09-19

Keywords:
Paramecium
Aluminum Chloride
Zinc Oxide Nanoparticles
Static Magnetic Field
Cinnamic Acid
L-Arginine

ABSTRACT

Objective(s): So far, the effects of aluminum chloride (Al) on *Paramecium caudatum* during challenge with cinnamic acid (CA) and L-arginine (L-Arg) in the presence of ZnO nanoparticles (NPs) under natural geomagnetic conditions (*in vitro*) and under static magnetic fields (SMF), which is our aim in this study, have not been investigated.

Methods: *P. caudatum* was obtained from water sources and cultured weekly to prepare pure culture medium. Different concentrations of CA and L-Arg (3-27 µg/µL) in the presence and absence of Al and ZnO NPs were tested on 0.1 mL samples of pure *Paramecium* culture repeatedly (25 times) in the laboratory (Lab) and SMF. Movements, avoidance, blebbing, nuclear fragmentation, density of calcium (Ca) channel, and catalase, superoxide dismutase, glutathione and nitric oxide (NO) levels were studied. All data were analyzed using analysis of variance (ANOVA).

Findings: Al alone reduced the motility of *P. caudatum* and caused nuclear fragmentation and cytotoxicity. CA had no motility-modulating effect and even caused changes in the levels of antioxidant system factors, especially in the presence of ZnO NPs in the field. However, L-Arg, except in some cases, improved mobility in the presence of NPs and in SMF due to NO levels.

Conclusions: The mechanism of Al-induced toxicity in *P. caudatum* is not only oxidative, but also depends on geomagnetic conditions.

How to cite this article
Asadi-Zardroodi F, Karami M, Hajnorouzi A. Protection of L-arginine over cinnamic acid against aluminum chloride-induced toxicity in Paramecium caudatum in the presence of zinc oxide nanoparticles and static magnetic field. Nanomed Res J, 2026; 11(3): 308-321. DOI: 10.22034/nmrj.2026.03.010

INTRODUCTION

Heavy metals (HM) in soil, water, and air can induce toxic effects and disrupt various biological pathways [1]. These natural elements have high atomic weights and densities at least five times greater than that of water [2]. HM are essential for various biological processes, but their excessive consumption is harmful. They induce oxidative stress by producing free radicals and reducing antioxidant levels. HM alter genetic material and inhibit protein function [3].

Aluminum is the third most common element in the Earth's crust [4]. It enters the body through natural processes and a number of human activities, such as mining and is known to be an important element in neurological and behavioral disorders [5]. According to previous findings, the consumption of aluminum chloride (abbreviated as Al), causes the following side effects: 1) Impairment of memory and learning, 2) Increased oxidative stress, 3) Cell damage and histopathological changes, F) Disturbance in cholinergic pathways and 5) Inflammation [6].

Unicellular animals are often used as bioindicators of chemical pollution, especially in aquatic environments. *Paramecium caudatum* is a ciliated protozoan that is widely used in laboratory

* Corresponding Author Email: *karami@shahed.ac.ir*

This work is licensed under the Creative Commons Attribution 4.0 International License.
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

research [7]. This freshwater species resembles a human footprint and is named *P. caudatum* because of the tuft of long cilia on its tail [8]. Studies show that ciliates have significantly different tolerances to HM [9]. To prevent HM toxicity, especially aluminum, the consumption of antioxidants should not be neglected. Cinnamic acid (CA), with its abundant antioxidant properties, has been shown to improve brain function and prevent the onset of Alzheimer's disease (AD). This substance protects the brain against free radicals and improves cognitive function [10]. Brains are rich in the amino acid L-arginine (L-Arg), which is a precursor of nitric oxide (NO) and has been shown to be very effective in preventing plaque buildup and vascular occlusion in tissues [11]. However, classical treatments are not very effective in improving cognitive impairment, and scientists believe that combined interventions are more effective in resolving cognitive injury. One of the new therapeutic strategies is the use of nanomaterials with beneficial effects, such as ZnO, which has a metallic nature and diamagnetic properties dependent on the magnetic field [12]. ZnO nanoparticles (NPs) have been widely used in various industries including food, pharmaceutical, rubber, electronics and the like, but its protective effect under static magnetic field (SMF) exposure in Al-induced Alzheimer-like toxicity in a unicellular organism, which is our goal, has not been studied. Also, drug interactions of antioxidants such as CA and the NO-producing amino acid, L-Arg, have been studied due to their particular importance in modern multi-pronged therapeutic strategies [13].

MATERIAL AND METHODS

Subjects

The present study was conducted on a protozoan species called *P. caudatum*, a sensitive biological model to investigate the effects of chemical and biological agents. This species of ciliates is considered one of the most suitable organisms for studying biological processes at the cellular level due to its high rate of cell division, ease of cultivation *in vitro*, and rapid response to environmental changes.

Initial samples of this species were collected from natural aquatic environments and, after key identification, were maintained in natural culture medium. Environmental conditions including temperature, pH, dissolved oxygen, and light intensity were kept constant throughout the experimental period to minimize the effect of confounding factors on the results. Also, only physiologically active and homogeneous populations were selected for the treatments to maintain comparability between groups. This research was in accordance with health principles and was approved by the local ethics committee (IR.SHAHED.AEC.1404.002).

Sampling method and sample size

The sample was 0.1 mL of pure *P. caudatum* culture, and each sampling (each experiment) was repeated 25 times conducted during 30 sec period with 5 sec intervals to ensure sufficient sample size and statistical validity of the results. For all three concentrations (3, 9, and 27 µg/µL) of L-Arg and CA, a challenge with concentration of 25 µg/µL of Al, and a concentration of 3 µg/µL of ZnO NPs, were conducted under laboratory (Lab) and field conditions.

Sonoelectrochemistry for NPs Production

In this study, we used direct sonoelectrochemistry, which involves ultrasonic erosion and oxidative heating. This is a very flexible method, and its synthesis process is based on electrochemical and ultrasonic parameters and the relationship between them. One of the most important advantages of this method is its greenness, speed, and high purity of the produced nanomaterial.

We performed X-ray diffraction (XRD) analysis on the ZnO NPs used in this study. The diffraction pattern (see Figure 1) confirms the crystalline structure of the NPs. The peaks were indexed and matched with the reference code 01-076-0704, which corresponds to the hexagonal structure with the space group $P6_3mc$ (number 186). This confirms that the NPs are phase-pure ZnO with a hexagonal crystal system.

For more information on the ZnO NPs used in this study, refer to the references [14] and [15] which provide detailed information including FE-SEM images, UV-Vis spectroscopy for optical properties, synthesis method and growth mechanism, and the effect of ultrasonic irradiation on nanostructure formation.

Hematoxylin nuclear staining of P. caudatum

Slides containing fixed samples were first placed in descending degrees of alcohols for hydration. Then, the samples were stained in 20% hematoxylin aqueous solution for 30 min, then washed twice

CC BY

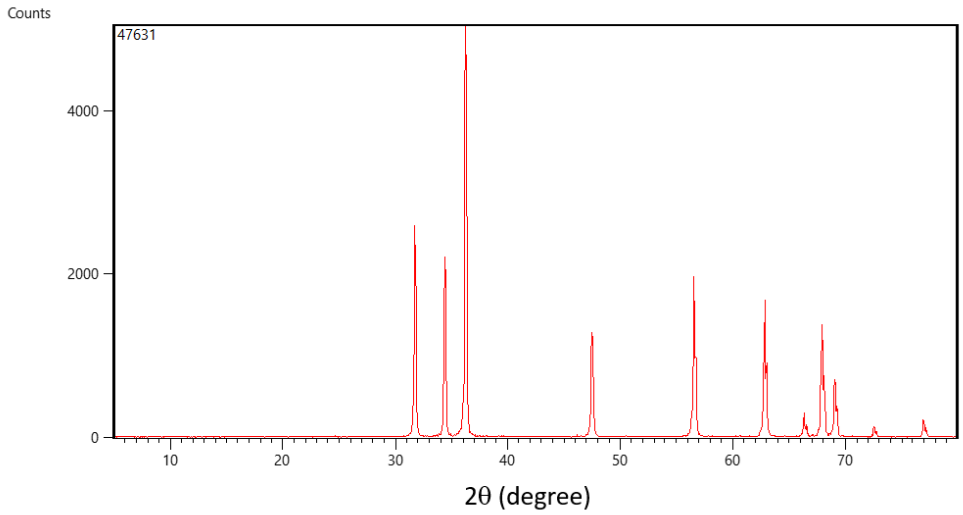


Fig. 1. X-ray diffraction (XRD) pattern of ZnO NPs used in this study. The XRD analysis of the NPs revealed characteristic peaks at 2θ values of 31.8°, 34.4°, 36.3°, 47.6°, 56.7°, 62.9°, 68.0°, and 69.1°, corresponding to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes of hexagonal ZnO, respectively. All peaks were consistent with reference code 01-076-0704 (hexagonal system, space group $P6_3mc$, No. 186), confirming the phase purity and hexagonal crystalline nature of the particles.

with distilled water (dH_2O). Next, the dehydration process was performed in alcohols with increasing degrees (50%, 70% and 96%) for 2 min each. After washing with dH_2O, the samples were placed in xylene for 3 min. In the final stage, mounting was performed using Entellan glue (Merck, Germany) and the coverslip was carefully placed on the slide.

Calcium channel-specific immunohistochemical staining

Fixed slides were first dehydrated and then incubated in phosphate buffer solution (PBS) at pH = 7.6 for 1–2 min. They were then exposed to specific primary antibody (Ab) for 60 min in a water bath at 36°C, then washed four times with PBS, and then Avidin/Biotin blocking solution was added to the samples and kept for 10 min in laboratory conditions. Next, they were exposed to chromogen for 10 min, followed by washing with PBS and then dH_2O. The dehydration process was carried out in increasing alcohol grades (1–2 min each) followed by xylene for 2 min. Finally, mounting was performed using Entellan (Merck, Germany) and the slides were coverslipped. Given the sensitivity and specificity of this staining, all steps were performed with great care.

Determination of the concentration of oxidative system factors

Initially, an appropriate volume of pure culture of the single-cell organism was centrifuged, then subjected to ultrasonication to prepare cell lysates.

All reagents, working standards, and samples were prepared according to the instructions provided in the kit.

Catalase assay

The catalase (CAT) enzyme catalyzes the decomposition of hydrogen peroxide into water and oxygen and is capable of converting millions of hydrogen peroxide molecules into end products per second. The assay method is based on the peroxidative activity of the CAT enzyme present in the sample. In this reaction, the CAT enzyme produces aldehyde (formaldehyde) in the presence of hydrogen peroxide and methanol (as a hydrogen donor). Next, potassium hydroxide terminates the reaction and the resulting formaldehyde combines with the chromogen. The colored product formed can be measured spectroscopically at a wavelength of 540–550 nm. In this study, proprietary kits from Navand Salamat (Ardabil, Iran) were used to measure CAT and other oxidative stress indicators.

Superoxide dismutase (SOD) assay

One of the most important antioxidant mechanisms of the body against reactive oxygen species (ROS) is the presence and activity of the enzyme superoxide dismutase (SOD) as a metalloprotein that catalyzes the conversion of superoxide anion

(cc) BY

to oxygen and hydrogen peroxide. Measuring the activity of this enzyme using a dedicated kit is a simple, reproducible and standardized method and can be measured in biological samples such as cell lysates. The basis of this test is based on the inhibition of the autoxidation reaction of pyrogallol. Pyrogallol undergoes spontaneous oxidation under normal conditions and in the presence of air and has a certain half-life. In this reaction, the addition of a sample containing SOD at an unknown concentration causes the inhibition of the autoxidation of pyrogallol at a certain time and by comparing the results with the control, the concentration of SOD in the sample is determined.

Nitric Oxide (NO) Assay

NO mediates many physiological processes such as vasodilation, inflammation, thrombosis, immunity, and neurotransmission. NO is synthesized from L-arginine, oxygen, and NADPH by the enzyme NO synthase (NOS) and, as a highly diffusible molecule, leads to numerous effects. Various methods have been designed to measure NO in biological systems, one of which is the use of a deionization reaction and spectrophotometric detection that measures nitrite produced by the oxidation of NO under physiological conditions. The Griess reaction can also be used to analyze nitrate through its catalytic reduction to nitrite. The Natrix kit (Ontario, Canada) is based on the Griess method, which provides a simple, reproducible, and standardized method for measuring NO in biological samples such as cell lysis. In this method, NO is measured indirectly by reacting sulfanilic acid with nitrite in an acidic solution to form an azo dye that can be measured at an optical absorption of 520 to 590 nm.

Glutathione (GH) Assay

GH is a key intracellular thiol tripeptide composed of glutamic acid, cysteine, and glycine. GH acts as an antioxidant by protecting cells from free radical damage. GH exists within cells in the reduced (GSH) and oxidized (GSSG) states. In healthy cells and tissues, more than 90% of total GH is in the reduced form, while less than 10% is in the GSSG disulfide form. High GSH concentrations are due to the highly active enzyme glutathione reductase. An increase in GSSG to GSH indicates oxidative stress.

Data Analysis

Raw data were first examined for normal distribution (using the K-S or Kolmogorov–Smirnov test) and after ensuring the normality of the distribution, one-way or two-way (if needed) analysis of variance was performed. Qualitative data were examined using ImageJ software (Java free, 1.41 version). $P<0.05$ was considered significant.

RESULTS

The effect of Al, nano ZnO, and different concentrations of CA and L-Arg (3, 9, and 27 µg/µL) on the locomotor activity of P. caudatum under Lab conditions (in vitro) and a SMF

See Figure 2. The results show that the locomotor activity of *Paramecium* was significantly reduced in the presence of Al (in the Lab and field) and especially ZnO NPs (in the field) compared to the control group receiving only one microliter of distilled water (marked with * and ** in the Figure 2). This reduction indicates the toxic effect of these substances on the cell's locomotor capacity under the aforementioned conditions.

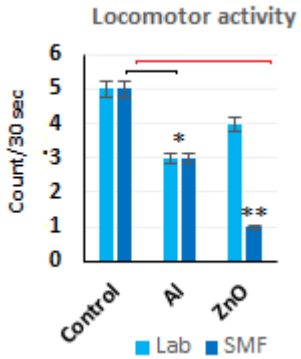


Fig. 2. Comparison of motor activity in different experimental groups including control, Al treatment, and ZnO treatment under laboratory conditions (Lab) and under a SMF. A significant decrease in motor activity was observed in the Al group and especially in the ZnO group compared to the control.

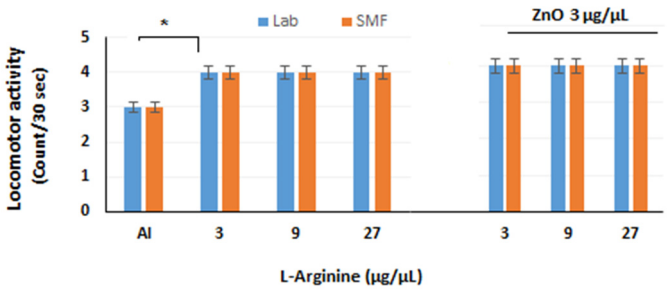


Fig. 3. Comparison of locomotor activity in the presence of Al and different concentrations of L-Arg alone and in combination with ZnO NPs under Lab and under SMF. L-Arg, either alone or in the presence of the nanomaterial, restored the decrease in locomotion caused by Al, regardless of the type of environment (Lab or field). Between-group comparisons were shown with Tukey's *post hoc* test with 95% confidence (*p<0.05).

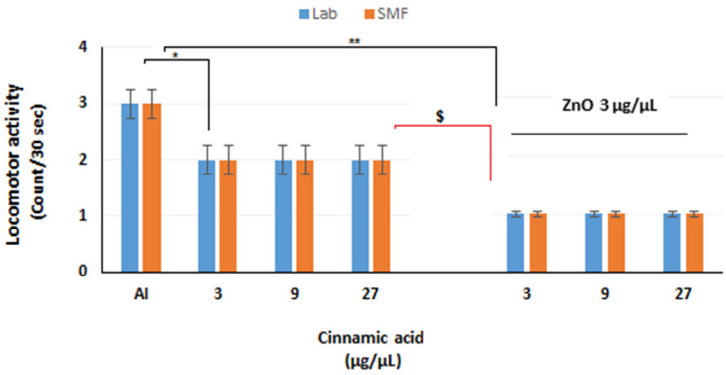


Fig. 4. Comparison of locomotor activity in the presence of Al and different concentrations of CA, either alone or in combination with ZnO NPs, *in vitro* (Lab) and under a SMF. The effect of CA was particularly reduced in the presence of ZnO NPs. The between-group comparison with the Tukey *post-hoc* test with 95% (*p<0.05) and 99% confidence is shown for the Al groups and with 95% confidence between the drug alone compared to when the nanomaterial was added (p<0.05).

The effect of L-Arg at different concentrations in the challenge with Al, whether alone or in the presence of the nanomaterial

This substance was able to significantly increase locomotor activity, which was reduced in the presence of Al. However, apart from this increasing effect, there was no significant difference between the two environments (Lab and SMF) and it was also not dependent on the L-Arg concentration (Figure 3).

Effect of CA in the interference process

A significant decrease in locomotor activity was observed and in combination with ZnO NPs a further decrease in the locomotion was achieved (Figure 4). The response was neither affected by the Lab or field environment and was independent on the dose of CA.

Changes in the Avoiding Response in P. caudatum to Al under treatment with L-Arg and CA alone or in combination with nanomaterials, under Lab conditions and SMF

Effect of L-arginine on the avoidance response of P. caudatum

The highest avoidance response is observed in the Al treatment. With the intervention of L-Arg, at low concentrations, this effect was improved, and with increasing its concentration, the response rate intensified and reached a stable trend at higher concentrations. The presence of nano ZnO at a concentration of 3 µg/µL next to L-Arg at low concentrations significantly reduced that response, but under a SMF, the amount of the avoidance response under the highest concentration of L-Arg (27 µg/µL) was much higher than in the Lab conditions, which indicates that the effect of a SMF proportional to the concentration of L-Arg can be protective or destructive (Figure 5). Conversely, increasing the dose reduced blebbing.

Effect of CA on the avoidance response of P. caudatum

After initial treatment with Al, with increasing

(cc) BY

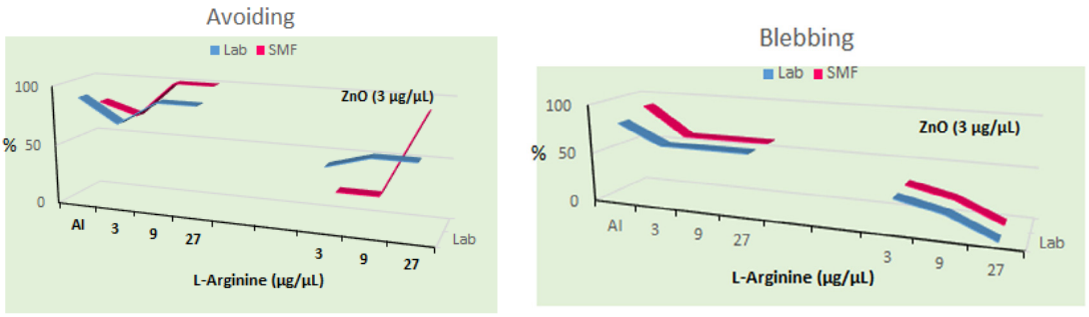


Fig. 5. Avoidance and blebbing responses of *P. caudatum*, ratio of the interference effect of different concentrations of L-Arg, either alone or in combination with nanomaterial, with Al under Lab conditions and under a SMF. The highest response was observed in Al treatment. The avoiding response increased with increasing L-Arg concentration in the presence of nano ZnO under the field. Increasing the dose reduced blebbing.

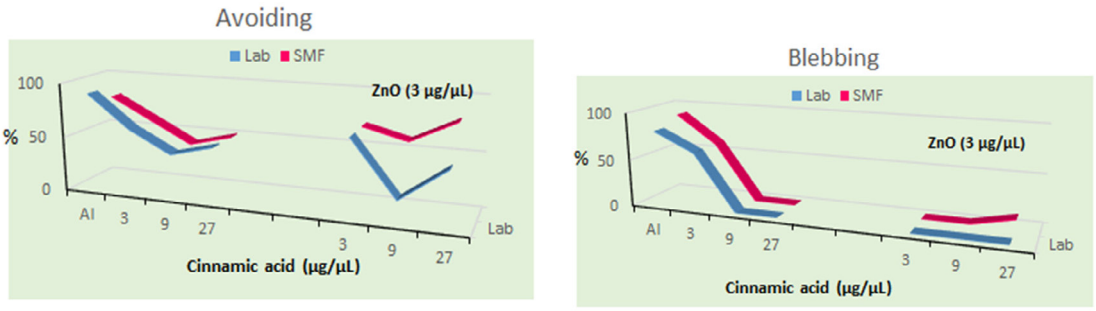


Fig. 6. Changes in the avoidance response of *Paramecium* at different concentrations of CA in two Lab conditions and under a SMF in the challenge with Al. The response had a high intensity in Al, but in the interference of L-Arg with this substance, the response was reduced. Treatment with nano ZnO caused a further reduction in the response, but in the SMF, this protective interference response was reduced compared to the Lab. Increasing the dose reduced blebbing.

CA concentration, the percentage of avoidance response decreased at lower concentrations but increased at higher concentrations. At concentrations combined with nano-ZnO, the avoidance response showed a more pronounced decrease, especially in Lab conditions, although the response was intensified with increasing CA concentration, especially under the field. Again, the intensity of the avoidance response under a magnetic field was slightly higher than under Lab conditions; this probably indicates a decrease in the tolerance threshold of *Paramecium* in a SMF. Again, increasing the dose reduced blebbing (Figure 6).

Changes in reduced glutathione (GSH) levels in Paramecium under challenge between Al with L-Arg and CA, either alone or in combination with nanomaterials, in vitro (Lab) and in a SMF

Effect of L-Arg treatment on GSH levels in vitro and under magnetic field

In Lab, GSH levels in the Al group were around 1 µM/mL. At low concentrations, L-Arg significantly enhanced the antioxidant status of the cell. However, when the L-Arg concentration was increased, GSH levels returned to Al levels (Figure 7).

Changes in GSH levels in the presence of different concentrations of CA

In Lab, CA at a concentration of 3 µg/µL had a relative increasing effect on GSH, but this effect was not as significant as that of L-Arg (Figure 8).

Changes in CAT activity in Paramecium under challenge between Al with L-Arg and CA, either alone or in combination with nanomaterials, in vitro and in a SMF

Effect of L-Arg on CAT activity in two environments Lab and SMF

In Lab, the baseline value of CAT activity in the reference group (Al) was about 20-22 nMol/min/mL

Although under the lowest concentration of L-Arg we did not see a significant change in this challenge, with increasing concentration in combination with nanomaterial, even under normal conditions (Lab), a significant decrease of

CC BY

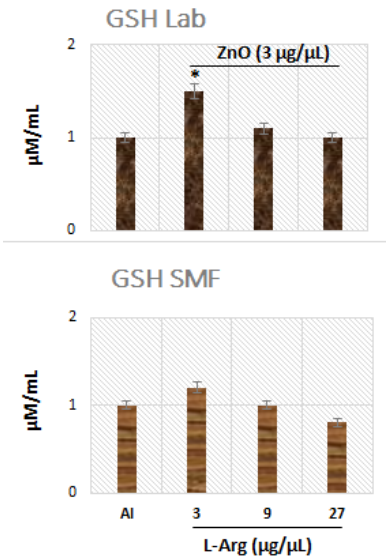


Fig. 7. Changes in the level of GSH *in vitro* and in the field, either alone or in the presence of ZnO NPs in the challenge between L-Arg and Al. In the presence of a magnetic field, the level of GSH increased slightly under the challenge of a very low concentration of NO precursor. The magnetic field decreased the effect of L-Arg on the antioxidant system.*P<0.05 shows difference to the reference group (Al) based on Tukey's *post hoc* test.

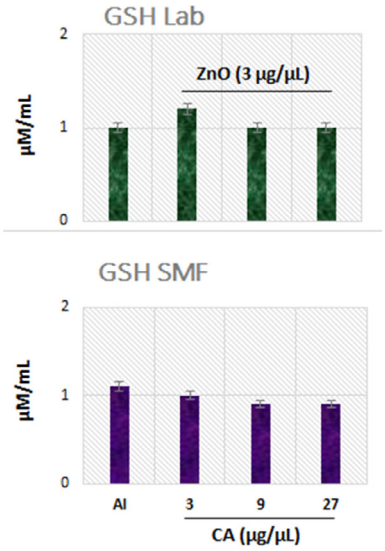


Fig. 8. Changes in the level of reduced glutathione *in vitro* (Lab) and under field conditions.

this enzyme occurred (Figure 9).

Effect of CA on CAT Activity in Lab and SMF Environments

In Lab, the CAT level in the reference group was about 20 nMol/min/mL

Addition of CA at a concentration of 3 µg/µL caused a slight increase in enzyme activity, but at concentrations of 9 and 27 µg/µL, CAT activity returned to the baseline range. Consequently, under Lab conditions, treatment with CA did not significantly change CAT activity. However, a different trend is observed under SMF. With increasing CA concentration, CAT activity gradually decreased. This graph shows that a field in combination with high concentrations of CA had a potent inhibitory effect

(cc) BY

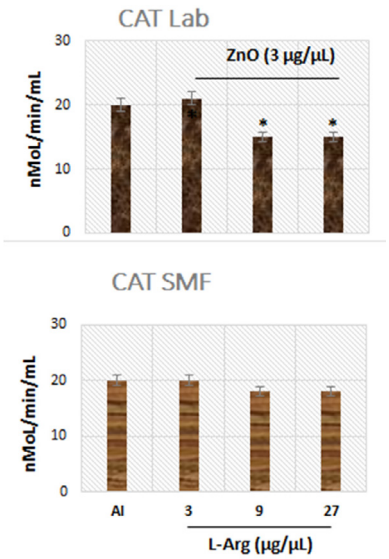


Fig. 9. Changes in CAT levels *in vitro* and in the field, either alone or in the presence of ZnO NPs, in the challenge between L-Arg and Al. *P<0.05 shows difference to the reference group (Al) based on Tukey's *post hoc* test.

on the antioxidant pathway of CAT (Figure 10).

Changes in superoxide dismutase (SOD) activity in Paramecium under challenge between Al with L-Arg and CA, either alone or in combination with nanomaterials, in vitro (Lab) and in a SMF
Effect of L-Arg on SOD activity in two environments Lab and SMF

The results show that the differences were not significant. This could indicate that nano ZnO did not cause a significant interference effect (Figure 11).

Effect of CA on SOD activity in two environments Lab and SMF

In lab, the applied treatments did not cause significant changes in SOD activity and the antioxidant system remained stable. In a SMF compared to the Lab, CA, especially at concentrations of 9 and 27 micrograms/microliter, caused a decrease in SOD activity, which is probably due to the presence under a SMF (Figure 12).

Changes in nitric oxide (NO) levels in Paramecium under challenge between Al with L-Arg and CA, either alone or in combination with nanomaterials, under Lab conditions and SMF
Effect of L Arginine on NO levels in both Lab and SMF environments

In lab, the reference group shows a NO level of about 10 µg/µL. In the ZnO-treated group, a significant increase in NO was observed, and the presence of a (*) sign above the columns indicates a statistically significant difference compared to the Al groups, regardless of the L-Arg concentration. Therefore, the nanoparticles have significantly increased ZnO production *in vitro.*

In a field, with increasing L-Arg concentration (NO precursor), a concentration-dependent increasing trend was observed. At the highest concentration (27 µg/µL) a completely significant response was observed (Figure 13).

Effect of CA on NO levels in two environments: Lab and SMF

The study shows that CA treatment did not cause a significant change in NO levels in any of the considered concentrations, in both Lab conditions and SMF (Figure 14).

Nuclear status

The vegetative nuclear fragmentation was not observed under these studies except in the case of the Al group alone compared to the control that received only one microliter of distilled water. This means that the statistical difference in this case did not occur in the other cases and each cell maintained the integrity of the nuclear structure (to see this preserved structure, refer to the Figure 15, which, except for one Al group compared to the control,

CC BY

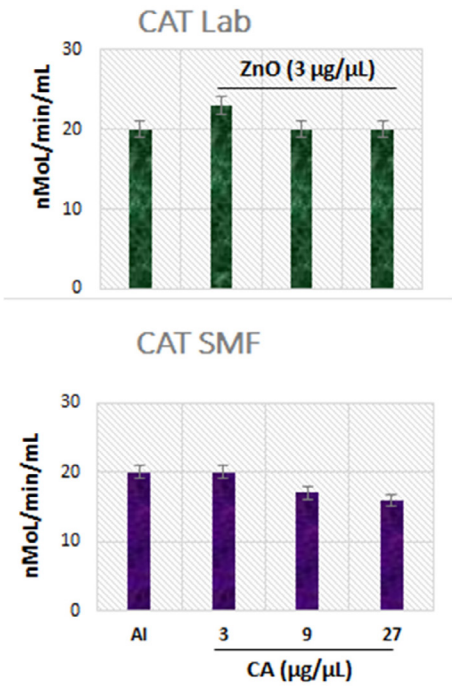


Fig. 10. Changes in CAT levels *in vitro* and in the field, either alone or in the presence of ZnO NPs, in the challenge between CA and Al.

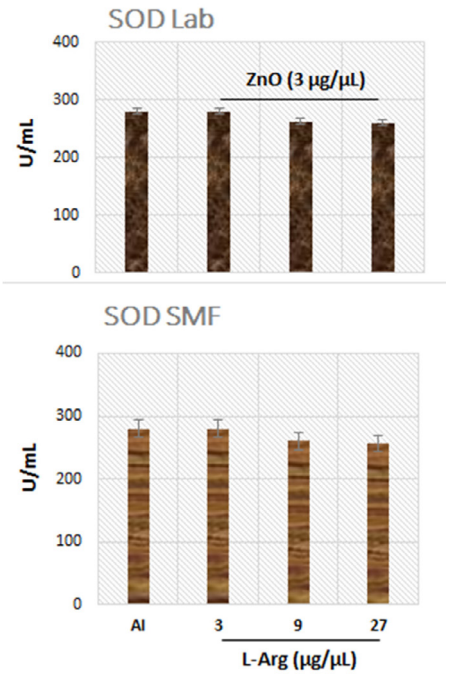


Fig. 11. Changes in SOD levels *in vitro* and in the field, either alone or in the presence of nano ZnO, in the challenge between L-Arg and Al.

does not differ significantly from any other sample).

The status of calcium (Ca) channels under immunohistochemical examination

The change in the density of these channels located at the base of the cilia was only significantly observed in the Al group compared to the control (receiving distilled water), but the density was preserved during the interventions (to see the

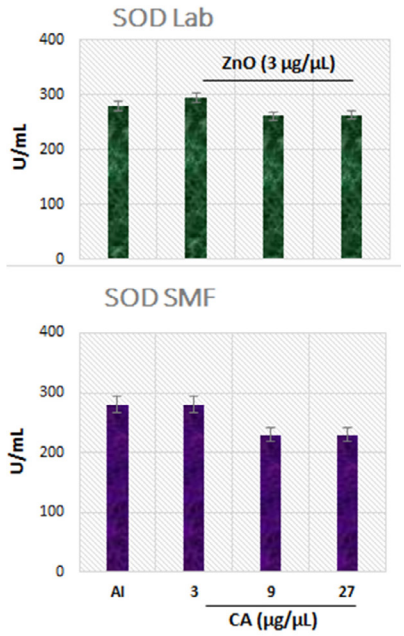


Fig. 12. Changes in SOD levels *in vitro* and in the field, either alone or in the presence of ZnO NPs, in the challenge between CA and Al.

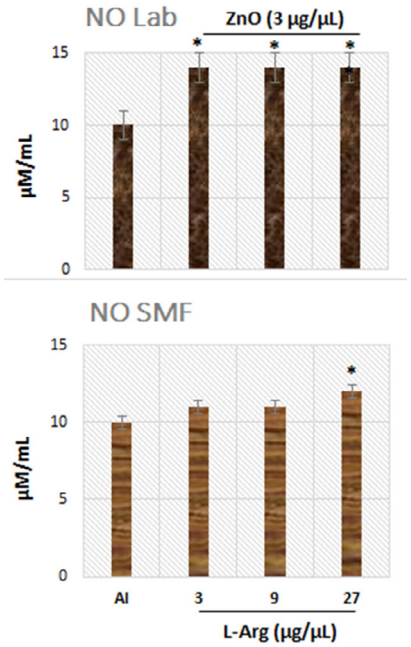


Fig. 13. NO levels *in vitro* and in the field, both alone and in the presence of ZnO NPs in the challenge between L-Arg and Al.

difference in this density between the Al group and the control, refer to the Figure 16).

DISCUSSION

Effect of CA on biological and oxidative responses induced by Al in *P. caudatum*.

CA alone causes a significant decrease in the mobility of *Paramecium*. On the other hand, ZnO NPs cause a more severe decrease in the mobility of *Paramecium*. As a result, the effect of these two substances (CA and nanomaterial) is synergistic, and the role of the third factor (field compared

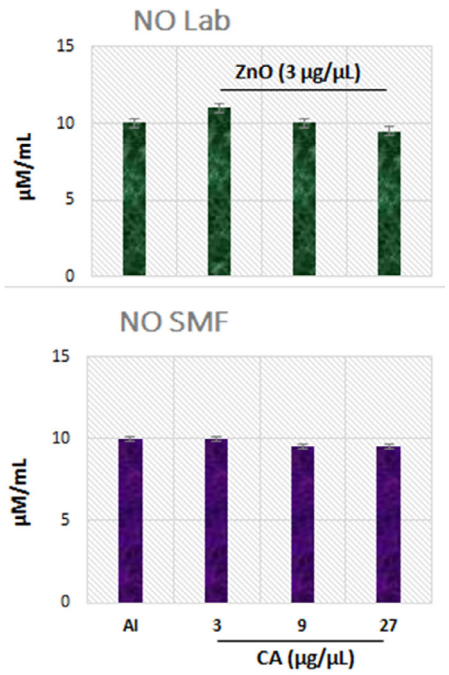


Fig. 14. NO levels *in vitro* and in the field, either alone or in the presence of ZnO NPs, in the challenge between CA and Al.

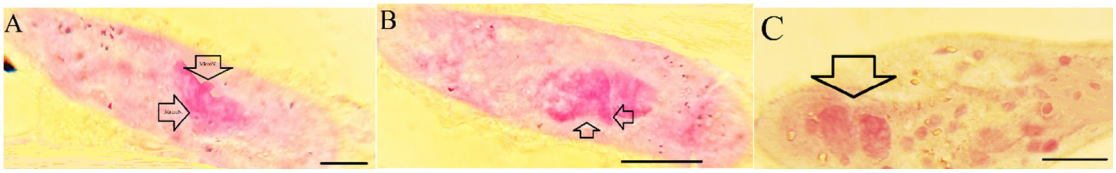


Fig. 15. Vegetative nucleus under nuclear specific staining with hematoxylin. The nucleus is almost kidney-like. Scale bar is 50 µm. Control sample (A), intervention sample (B), and Al only sample (C). The difference between the Al sample (C) and the control (A) is noticeable. Scale bar is 50 µm.

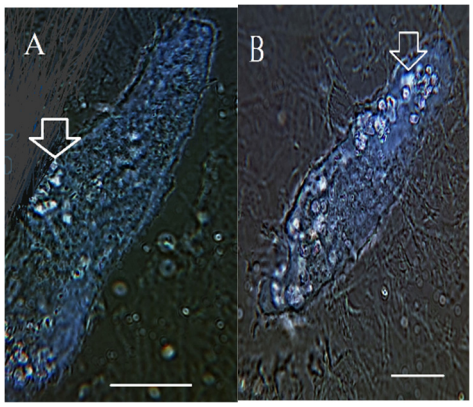


Fig. 16. Density of the ciliary base Ca channel in a specific immunohistochemical study. The difference in density of this channel between the Al sample (B) and the control (A) is noticeable. The scale bar is 50 µm.

to natural geomagnetic conditions, which is the Lab) is also unaffected by the concentration of CA. Cinnamic acid is also a phenolic compound that can change its role from being protective to being cytotoxic and inhibitory depending on the concentration and conditions; at higher concentrations, this substance can cause changes in permeability or interaction with metabolic pathways

(cc) BY

and ultimately slow movement [11]. However, the simultaneous presence of ZnO NPs and CA exposes *Paramecium* to double chemical pressure; as a result, *Paramecium*'s regulatory systems are less successful in maintaining locomotor behavior. *In vitro*, CA at a concentration of 3 µg/µL has a mild enhancing effect on the glutathione enzyme, but at higher concentrations, the antioxidant system does not show an enhancing response. However, in the presence of a SMF and concentrations of 3, 9, and 27 µg/µL, the level of reduced glutathione showed a decreasing trend. The same substance *in vitro*, at a concentration of 3 µg/µL, caused a slight increase in CAT enzyme activity, but at concentrations of 9 and 27 µg/µL, CAT activity returned to the baseline range. In a SMF, a different trend was observed and at the lowest concentration, the amount of CAT remained similar to the control, but with increasing concentration to 9 and 27 µg/µL, CAT activity gradually decreased. CA *in vitro* did not cause a significant change in SOD activity and the antioxidant system remained stable. However, in a field, CA, especially at concentrations of 9 and 27 µg/µL, decreased SOD activity, indicating increased oxidative stress in the presence of a SMF. CA did not change NO levels *in vitro* at all three concentrations, suggesting that it is probably not able to affect the NO system at these concentrations.

Effect of L-arginine on biological and oxidative responses induced by aluminum chloride in *Paramecium*.

L-Arg is a substance that stimulates ciliary movement in *Paramecium* by increasing access to the NO pathway. In fact, L-Arg significantly increased the motility of *Paramecium*, which is likely to occur through activation of the NO pathway and enhancement of ciliary activity (the results of this study also showed that it has a stimulating effect on NO levels).

Because NO plays a key role in regulating ciliary activity, Ca ion transport, and membrane potential. On the other hand, a SMF or nanomaterial did not significantly affect this interfering effect of L-Arg. According to previous reports, the toxicity of ZnO NPs is strongly dependent on concentration, particle size, contact time, and surface properties [16]. At lower concentrations, these NPs may stimulate adaptive responses and cause mild oxidative stress without leading to obvious motor or cellular damage. However, in the study of glutathione in the presence of L-Arg at low concentrations and *in vitro* conditions, L-Arg acts as a stimulating antioxidant and enhances the cell defense system.

However, when the concentration of L-Arg increases, the GSH level decreases again and returns to the control level; therefore, at high concentrations, the body system probably consumes more GSH (to neutralize the increased oxidizing agents) or its production pathway is inhibited. In the presence of a field, the GSH level in the L-Arg challenge is weakened. Therefore, the magnetic field probably caused the positive effect of L-Arg on the antioxidant system to decrease. In another study, CAT levels were examined. At higher doses of L-Arg, due to increased oxidative stress or consumption of intracellular antioxidants, the activity of the CAT enzyme decreased. In fact, high concentrations may increase the production of hydrogen peroxide (H_2O_2), which inhibits CAT activity in the long term [17]. The effect of L-Arg on SOD activity in both Lab and SMF environments showed that *in vitro* conditions and in the presence of ZnO NPs at a concentration of 3 µg/µL, no significant change in the enzyme activity was observed; also, the values recorded in all sections were in a relatively similar range and the difference between them was small. This could indicate that the antioxidant system was able to maintain oxidative balance. Under conditions of exposure to a field and challenge treatment with L-Arg at concentrations of 3, 9, and 27 µg/µL, no change in SOD activity was observed.

The effect of L-Arg on NO activity in both Lab and SMF environments is such that ZnO NPs increased its production in laboratory conditions better than in the field, regardless of concentration. Research shows that the increase in NO can be interpreted as a cellular response to ZnO NPs or its signaling role in regulating oxidative stress [18]. This is probably why a significant effect was observed in a magnetic field with an increase in the concentration of L-Arg (a precursor of NO), (*i.e.*, at a concentration of 27 µg/µL). Regarding important cellular mechanisms, the effect of Al on vegetative nucleus fragmentation can be mentioned, which can reveal the functional effect of this substance on the cell (since this nucleus controls the actions under study). But even more fundamental is the effect of the substance on the density of membrane Ca channels, which in this study was still observed to increase their density compared to the control. This could explain a key mechanism in the locomotor-reducing effect of Al, which is the Al-

CC BY

induced toxicity, which has even been attributed to Ca-induced cytotoxicity (increased intracellular Ca levels) in other organisms [19].

These explanations could indicate the precise mechanisms for the effects of Al in this eukaryotic model, which, as mentioned, is consistent with the findings of a multicellular organism. This in itself has a very high value in animal modeling in animal studies that question previous possibilities based on the evolutionary distance between *Paramecium* and humans and the inability to generalize the findings of this organism to humans. It should also be acknowledged that in these challenging effects (between L-Arg and CA and nanomaterials, whether under natural conditions or in the presence of the field), the functional effect of the nucleus and channels cannot be considered unlikely, just because we did not achieve such an achievement in this study.

In future studies, more repetitions will be performed to clarify the dimensions and details. This may be due to experimenter error. In this study, Al acted as an oxidative stress inducer. Aluminum is one of the metals that can disrupt the cell's oxidation-reduction balance, increase the production of reactive oxygen species (ROS), and cause damage to membrane lipids, proteins, and nucleic acids [20].

On the other hand, several studies have shown that aluminum accumulation in the nervous system can cause neuronal dysfunction, increased lipid peroxidation, and decreased antioxidant enzyme activity. These processes are among the mechanisms involved in neurodegenerative diseases such as Alzheimer's disease (AD). It has also been reported that aluminum can play a role in the accumulation of beta-amyloid and abnormal phosphorylation of tau protein, which are pathological features of AD [21].

In the study of the effect of CA, it was found that this compound significantly reduced the mobility of *Paramecium* at the concentrations studied, and this effect was intensified in the presence of ZnO NPs, especially under the field. This situation was almost consistent with the reduction in the level of some antioxidant indicators such as glutathione and the reduction in the activity of enzymes such as SOD and CAT, which indicates a weakening of the cell's antioxidant defense system. On the other hand, L-Arg, as a precursor of NO, showed a different role in the biological responses of *Paramecium*. The results showed that this compound significantly increased the locomotor activity of *Paramecium*.

This effect is likely due to increase in NO production and regulation of Ca-dependent signaling pathways in cilia, which play a key role in the movement of this unicellular organism (although molecular findings related to the channel were not observed in this study to confirm the aforementioned statement). Regarding this controversial statement, to express other possibilities, it should be noted that L-Arg at low concentrations caused a relative strengthening of the antioxidant system and increased glutathione levels, while at higher concentrations a decrease in the activity of some antioxidant enzymes was observed, which could be due to increased oxidative effects with increasing concentration [22]. The results also showed that the SMF did not have a significant effect on the motility of *Paramecium* in relation to this precursor, but as mentioned, changes were observed in some oxidative indices. This indicates that magnetic fields can affect the redox balance of the cell and the activity of antioxidant enzymes in some conditions. In general, the findings of this study indicate that Al is able to act as an oxidative stress inducer and compounds such CA, L-Arg and ZnO NPs can change the biological responses of *Paramecium* depending on the concentration and environmental conditions.

Since oxidative stress is considered one of the key factors in the occurrence of neurodegenerative diseases, including AD, the results of this study can be useful in better understanding the effects of heavy metals and chemical compounds on oxidative pathways and cellular mechanisms related to these diseases (20).

CONCLUSION

The results of this study showed that the physiological and oxidative responses of *Paramecium caudatum* are affected by chemical factors including Al, ZnO NPs, CA, and L-Arg, as well as the physical factor of a SMF, and these responses can depend on the concentration of substances and environmental conditions.

ACKNOWLEDGMENTS

We would like to thank Shahed University for supporting this research.

CONFLICT OF INTEREST

The authors of this article have no conflict of interest.

(cc) BY

FUNDING

We did not receive any special funding.

AVAILABILITY OF DATA

All data are included in this article.

AUTHOR CONTRIBUTIONS

MK designed the experiments. FA-Z completed the research. MK and FA-Z analyzed the data. FA-Z provided an initial draft of the manuscript. MK edited the article. All authors reviewed and approved the final manuscript before submission.

REFERENCES

1. Abdel-Megeed RM. Probiotics: a Promising Generation of Heavy Metal Detoxification. Biol Trace Elem Res. 2021;199(6):2406-2413. https://doi.org/10.1007/s12011-020-02350-1
2. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metals toxicity and the environment. EXS. 2012;101:133-164. https://doi.org/10.1007/978-3-7643-8340-4_6
3. Kim JJ, Kim YS, Kumar V. Heavy metal toxicity: An update of chelating therapeutic strategies. J Trace Elem Med Biol. 2019;54:226-231. https://doi.org/10.1016/j.jtemb.2019.05.003
4. Rahman A, Banu Z. Impact of Aluminium Chloride ($AlCl_3$) on brain function: a review of neurotoxic mechanisms and implications for Alzheimer's disease. Trends Pharm Sci. 2024;10(4):355-366.
5. Ghahramani P, Eshagh-Harooni H, Fatemi-Tabatabaei SR, Moazedi AA. Effect of zinc chloride on the passive avoidance memory of female rats ovariectomized in the pre-pubertal stage. Feyz J Kashan Univ Med Sci. 2019;23(2):216-222.
6. Zhu X, Hao W, Liu Z, Song Y, Hao C, Wu S, et al. Aluminum induces neuroinflammation via P2X7 receptor activating NLRP3 inflammasome pathway. Environ Res. 2022;215(Pt 1):114373. https://doi.org/10.1016/j.ecoenv.2022.114373
7. Rao JV, Gunda VG, Srikanth K, Arepalli SK. Acute toxicity bioassay using Paramecium caudatum, a key member to study the effects of monocrotophos on swimming behaviour, morphology and reproduction. Toxicol Environ Chem. 2007;89(2):307-317. https://doi.org/10.1080/02772240601010071
8. Karami M. Zoology (1) Invertebrates. 2nd ed. Tehran: Shahed University; 2004.
9. Vilas-Boas JA, Cardoso SJ, Senra MVX, Rico A, Dias RJP. Ciliates as model organisms for the ecotoxicological risk assessment of heavy metals: A meta-analysis. Ecotoxicol Environ Saf. 2020;199:110669. https://doi.org/10.1016/j.ecoenv.2020.110669
10. Yi J, Horky LL, Friedlich AL, Shi Y, Rogers JT, Huang X. L-Arginine and Alzheimer's Disease. Int J Clin Exp Pathol. 2008;2(3):211-238.
11. Pritam P, Deka R, Bhardwaj A, Srivastava R, Kumar D, Jha AK, et al. Antioxidants in Alzheimer's Disease: Current Therapeutic Significance and Future Prospects. Biology (Basel). 2022;11(2):212. https://doi.org/10.3390/biology11020212
12. Yi J, Horky LL, Friedlich AL, Shi Y, Rogers JT, Huang X. L-Arginine and Alzheimer's disease. Int J Clin Exp Pathol. 2008;2(3):211-238.
13. Lokman M, Ashraf E, Kassab RB, Abdel Moneim AE, El-Yamany NA. Aluminum chloride-induced reproductive toxicity in rats: the protective role of zinc oxide nanoparticles. Biol Trace Elem Res. 2022;200(9):4035-4044. https://doi.org/10.1007/s12011-021-03010-8
14. Hajnorouzi A, Afzalzadeh R, Ghanati F. Ultrasonic irradiation effects on electrochemical synthesis of ZnO nanostructures. Ultrason Sonochem. 2014;21(4):1435-1440. https://doi.org/10.1016/j.ultsonch.2014.01.013
15. Hajnorouzi A, Afzalzadeh R, Ghanati F. Investigation on the effects of power amount and pulse time of ultrasound on the energy gap of the synthesized ZnO colloid. J Mod Res Phys. 2017;1(1):1-12. https://doi.org/10.52547/jmrph.1.1.1
16. Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch Toxicol. 2013;87(7):1181-1200. https://doi.org/10.1007/s00204-013-1079-4
17. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Life Sci. 2004;61(2):192-208. https://doi.org/10.1007/s00018-003-3206-5
18. Ardebili Z, Danaei E. The efficacy of nitric oxide and zinc oxide nanoparticles in improving growth, nutrition, metabolism and reproductive development in tomato (Solanum lycopersicum). Plant Prod. 2024;47(3):337-353.
19. Najafi F, Mohammadi F. The effect of indoleacetic acid on growth rate, protein, catalase and peroxidase enzyme activities in soybean (Glycine max L.) under aluminum chloride stress. Plant Res (Iran Biol). 2015;28(1):186-198.
20. Kumar V, Gill KD. Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: A review. Neurotoxicology. 2014;41:154-166. https://doi.org/10.1016/j.neuro.2014.02.004
21. Morris G, Puri BK, Frye RE. The putative role of environmental aluminium in the development of chronic neuropathology in adults and children: how strong is the evidence and what could be the mechanisms involved? Metab Brain Dis. 2017;32(5):1335-1355. https://doi.org/10.1007/s11011-017-0077-2
22. Iwadate Y, Nakaoka Y. Calcium regulates independently ciliary beat and cell contraction in Paramecium cells. Cell Calcium. 2008;44(2):169-179. https://doi.org/10.1016/j.ceca.2007.11.006
23. Hajnorouzi A, Afzalzadeh R, Ghanati F. Ultrasonic irradiation effects on electrochemical synthesis of ZnO nanostructures. Ultrason Sonochem. 2014;21(4):1435-1440. https://doi.org/10.1016/j.ultsonch.2014.01.013

CC BY