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Biological and Histopathological Impacts of Titanium Dioxide Nanoparticles on the Freshwater Clam, *Caelatura nilotica*, and the Role of Dimercaptosuccinic acid

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ABSTRACT

Given the widespread application of nanomaterials in various domains, titanium dioxide nanoparticles (TiO_2 NPs) emerge as a potential ecological concern for aquatic ecosystems. This study aimed to investigate the impact of TiO_2 NPs on the morphometrics, biometrics, and the histological structure of the digestive gland of the *Caelatura nilotica* clams, alongside the potential ameliorative effects of the dimercaptosuccinic acid (DMSA). Clams were continuously exposed to variable concentrations of TiO_2 NPs (25 and 150µg/L) and DMSA (200µg/L) for 4 weeks. The results revealed a significant increase in body length, width, height, shell, flesh, and total body weights in response toTiO_2 NPs exposure at the end of the experiment. Histological examination of the digestive gland indicated notable changes, including necrosis and fibrous tissue formation. Ultrastructural abnormalities included irregularly shaped and detached secretory and digestive cells. Additionally, damage to some organelles was detected, such as Golgi bodies, and disrupted chromatin condensation inside the nuclei of those groups. However, DMSA administration showed a promised mitigation of the adverse effects of TiO_2 NPs, leading to a reduction of the metal accumulation and histological improvements. This study underscored the potential use of *C. nilotica* as a model for assessingTiO_2 NPs toxicity and highlighted the role of DMSA as a potential therapeutic intervention against nanoparticles induced toxicity.

INTRODUCTION

Nanomaterials are widely used across various industries due to their unique characteristics, including their small size, high surface area, mobility, penetration ability, and reactivity, which collectively contribute to their potential environmental impact **(Khashan *et al.*, 2016)**. The widespread use of nanomaterials is expected to increase the discharge into aquatic environments during manufacturing, use, and disposal phases **(Mueller & Nowack, 2008)**. Aquatic ecosystems are increasingly exposed to nanoparticles (NPs) due to their extensive use and unintentional leakage into the environment **(Shanmugam *et al.*, 2024)**.

TiO_2 NPs are widely used in food, pharmaceuticals, and personal care products due to their excellent properties, viz. high opacity, chemical inertness, and high UV absorption rate **(Jafari *et al.*, 2020; Lu *et al.*, 2021)**.There are evidences indicating that TiO_2 NPs found in aquatic ecosystems are taken up and accumulate in the organs of aquatic organisms **(Lammel *et al.*, 2019)**. These contaminants are ingested through the digestive systems of the aquatic organisms **(Benson *et al.*, 2022)**.

Dimercaptosuccinic acid (DMSA) is a chemical compound containing two (SH) groups **(Flora*et al.*, 2011)**. Renowned for its water solubility and efficacy as a chelator, DMSA has been advocated for therapeutic applications aimed at reducing metal burdens **(Miller, 1998)**. The beneficial effects of DMSA are largely attributed to its chelating properties **(Flora *et al.*, 2008)**. Chelating agents like DMSA have the capacity to bind with metal ions, creating complex molecules that can be efficiently excreted from the body **(Flora & Pachauri, 2010)**.

Bivalves, as filter feeders organisms, have the ability to accumulate nanoparticle contaminants **(Faggio *et al.*, 2018)**, making them biomonitors for the presence of those nanoparticles in their habitat **(Curpan *et al.*, 2022)**. *Caelatura nilotica*, the Egyptian freshwater clam, belongs to the Unionoidae family and is native to the Nile River in Egypt **(Ibrahim *et al.*, 1999)**. *C. nilotica* has been heavily employed as a model to analyze the adverse effects of different pollutants, such as heavy metals, on the aquatic environment **(Mohamed *et al.*, 2004; Mohamed & Sheir, 2005; Sheir *et al.*, 2020)**. Therefore, the objective of this study was to investigate the potential impacts of TiO_2 NPs on the freshwater clam *C. nilotica* and to assess the effectiveness of DMSA against TiO_2 NPs toxicity in the aquatic environment.

MATERIALS AND METHODS

The experimental design

TiO_2 NPs (with a purity 97%, CAS Number: 13463-67-7) was obtained from Sigma Aldrich, St. Louis USA. Characterization of TiO_2 NPs by TEM (electron microscope unit, Faculty of Science, Alexandria University, El-Shatby, Egypt) and by Uv-visible spectrometer (Department of Physics, Faculty of Science, Menoufia University, Egypt) showed that these nanoparticles exhibit a spherical shape, with an average diameter ranging from 30 to 50nm. These nanoparticles were dispersed in double distilled water using a water bath sonicator for 25 minutes. Prior to use, the stock solution was diluted to the appropriate concentrations. On the other hand, the dimercaptosuccinic acid (DMSA) (CAS Number: 304-55-20) was purchased from Sigma Aldrich, St. Louis USA. The concentrations utilized in this study (25 and 150µg/ L TiO_2 NPs and TiO_2, representing low and high concentrations, respectively) and one concentration of DMSA (200µg/L) were selected according to the outlines described in the studies of **Palaniappan *et al.* (2008)** and **Shi *et al.* (2019)**.

Freshwater clams, *Caelatura nilotica*, (120 clams) were collected with hands from the irrigation Canal of Gizay village, Menoufia Government, Egypt, in November 2022. The parameters of water quality were as follows: temperature 28.58± 2.08ºC; salinity levels 0.76± 0.10 PPT; pH 7.48±0.12; conductivity 364.73± 52.84µmhos/ cm, and total dissolved solids 200.67± 26.22mg/ l.

Prior to the experiment, the clams were acclimated in glass aquaria containing water from the site, and they were fed (3gm/ L) silt from the site and kept under consistent laboratory conditions for one week. The *C. nilotica* clams were then divided into eight groups (15 clams/group/3 replicates) as follows: a- Control group, b- Two groups were exposed to 25 and 150µg/ L of TiO_2, c- Two more were exposed to 25 and 150µg/ L of TiO_2 NPs, d- One group was exposed to 200µg/ L of DMSA and e- Two groups were exposed to 25 and 150µg/ L of TiO_2 NPs + 200µg/ L of DMSA. Throughout the 4 weeks of continuous exposure, the concentrations in the experimental tanks were daily fresh prepared by TiO_2, TiO_2 NPs, and DMSA (2L/ tank).

Morphometric analysis

Randomly collected samples of 3 clams/ group at time intervals (zero time, 1 day, 2 and 4 weeks) were morphologically examined. The dimensions of the clam shells were recorded by measuring the length, width, and height of each shell in millimeters using a digital caliper. The total weight of samples, such as shell, and meat (gm), was determined.

Biometric analysis

Condition index (CI):

The CI of the clams was calculated using the following formula provided by **Aguirre (1979)**:

$$\mathbf{CI} = \frac{\mathbf{MW}/(\mathbf{TW} - \mathbf{SW})}{\mathbf{100}}$$

Where,**MW**: wet meat weight; **TW**: total wet weight, and **SW**: shell weight.

Histological study

After four weeks, clams from both the control and exposed tanks underwent a histological analysis. The digestive gland was promptly fixed in 10% neutral formaldehyde for 24 hours. Subsequently, the tissues were transferred to 70% ethanol and then dehydrated using an ascending ethanol series (70, 80, 90, and 100%). Following dehydration, the tissues were embedded in paraffin wax at 60°C and allowed to cool until the wax solidified **(Romeis,1989)**. Serial sections, approximately 5- 8µm thick, were cut and stained with Eosin and Ehrlich's Haematoxylin. The stained sections were then mounted in DPX and covered with glass cover slips. Slides were examined under an Optika microscope, and photographs were captured using an Optika digital camera at desired magnifications. Scale bars were included in each photograph for reference.

Tissue ultrastructural preparations

Small blocks (~ 1mm3) of digestive gland from different groups were fixed by immediately immersing in formalin-glutaraldehyde fixative (4 parts formalin: 1 part glutaraldehyde). Post fixation 1% osmium tetroxide was used for 1-2 hours at 4°C, followed by washing the tissues with phosphate buffer solution (pH 7.2) at 4°C for 3 hours. After fixation, the tissues underwent dehydration through a graded ethanol series (30, 50, 70, 80, 90%, and two changes of absolute alcohol). Infiltration was accomplished using a series of propylene oxide and Epon mixture. Embedding and polymerization were carried out in the oven at 58°C, utilizing an Araldite Epon mixture. Semi-thin sections (1µm thick) were cut using an LKB ultra-microtome, stained with toluidine blue, and examined and photographed at desired magnifications using a light microscope. Ultra-thin sections (60-70nm thick) were cut from selected areas for ultrastructural study. These ultra-thin sections were placed on 200 mesh copper grids, which were then double stained with uranyl acetate for half an hour and lead citrate for 20- 30 minutes **(Reynolds, 1963)**. Grids were examined and photographed at desired magnifications using a JEM-1400 Plus electron microscope in the Electron Microscope unit, Faculty of Science, Alexandria University, Egypt.

Statistical analysis

All data sets were analyzed using Statgraphics Centurion XIX (Stat-Point Technologies Inc., Warrenton, VA, USA). Data were analyzed using one-way ANOVA to set the difference between control and exposed groups. Data were considered significant when $P \leq 0.05$.

RESULTS

Effect of TiO_2 NPs concentrations with/without DMSA on *Caelatura nilotica* morphometrics/ biometrics

The morphometrics/ biometrics of the *C. nilotica* clams from the control and exposed clams were recorded after various time intervals of zero time, 1 day, 2 and 4 weeks.

The body length

During the study, the body length of the *C. nilotica* clams in the control and other exposed groups increased significantly with time and different concentrations (P = 0.0001). However, when comparing the experimental groups, it's evident that exposure to different substances led to distinct outcomes. The clams of all exposed groups had relative lower body lengths compared to the control group at zero time. However after 4 weeks, the exposed clams to 150µg/ l TiO_2, 25 and 150µg/ l TiO_2 NPs have the highest body lengths. On the other hand, the exposed *C. nilotica* clams to 200µg/ l DMSA were non-significantly lower than all groups (P> 0.05). The exposed clams to 25 and 150µg/ l

TiO_2 NPs+ 200µg/ l DMSA displayed variations in body length over time, with a noticeable decrease at 2 weeks. However, this trend reversed after 4 weeks, where the body length raised to higher levels than the initial values at zero time but non-significantly (P> 0.05) (Fig. 1A).

The body width

At zero time, the control group had a body width of 17.83± 1.65mm, which increased after 4 weeks to reach 21.00±2.64mm(P> 0.05). Notably, the exposure of the *C. nilotica* clams to 25, 150µg/ l TiO_2, 25, 150µg/ l TiO_2 NPs and 200µg/ l DMSA, consistently showed an increase in body width compared to the control group, except after 1 day, with significant differences observed after 2 weeks ($P\leq$ 0.05). In contrast, the groups exposed 25, 150µg/ l TiO_2 NPs + 200µg/ l DMSA demonstrated a significant reduction in the body width, particularly at 2 weeks and 1 day, respectively ($P \leq 0.05$) (Fig. 1B).

The body height

The body height of the *C. nilotica* clams in the control and exposed groups (except 25µg/ L TiO_2) exhibited a significant increase depending on concentration and time of exposure (P= 0.0001). Initially, the control group established a baseline body height of 28.5± 1.73mm then raised to 31.00±1.73 after 4 weeks (P> 0.05). Notably, the 150µg/ L TiO_2 and 150µg/ L TiO_2 NPs groups demonstrated an elevation in their body height compared to the control group, with significantly differences observed after 2 weeks. The group exposed to 200µg/ L DMSA also experienced a slight increase in the body height over time but it was non-significant (P> 0.05) (Fig. 1C).

The shell's weight

After 2 weeks, the 25µg/ L TiO_2 NPs group showed a significant increase in the shell weight compared to the control group. The 150µg/ L TiO_2 group showed a slight decrease in the shell weight after 2 weeks, but with 4 weeks experiment, it was increased significantly compared to the control group ($P\leq$ 0.05). The 150µg/ L TiO_2 NPs group showed a significant increase in the shell weight at a 2 weeks period, but after 4 weeks, the shell weight decreased significantly compared to the control group. The 200µg/ l DMSA group showed at a 2 weeks time a significant increase compared to the control group ($P\leq$ 0.05). The groups exposed to a combination of TiO_2 NPs and DMSA (25 µg/ L TiO_2 NPs + 200µg/ L DMSA and 150µg/ L TiO_2 NPs + 200µg/ L DMSA) showed a significant decrease in the shell weight at zero time, which continued to decrease significantly at 2 weeks ($P\leq$ 0.05) (Fig. 2A).

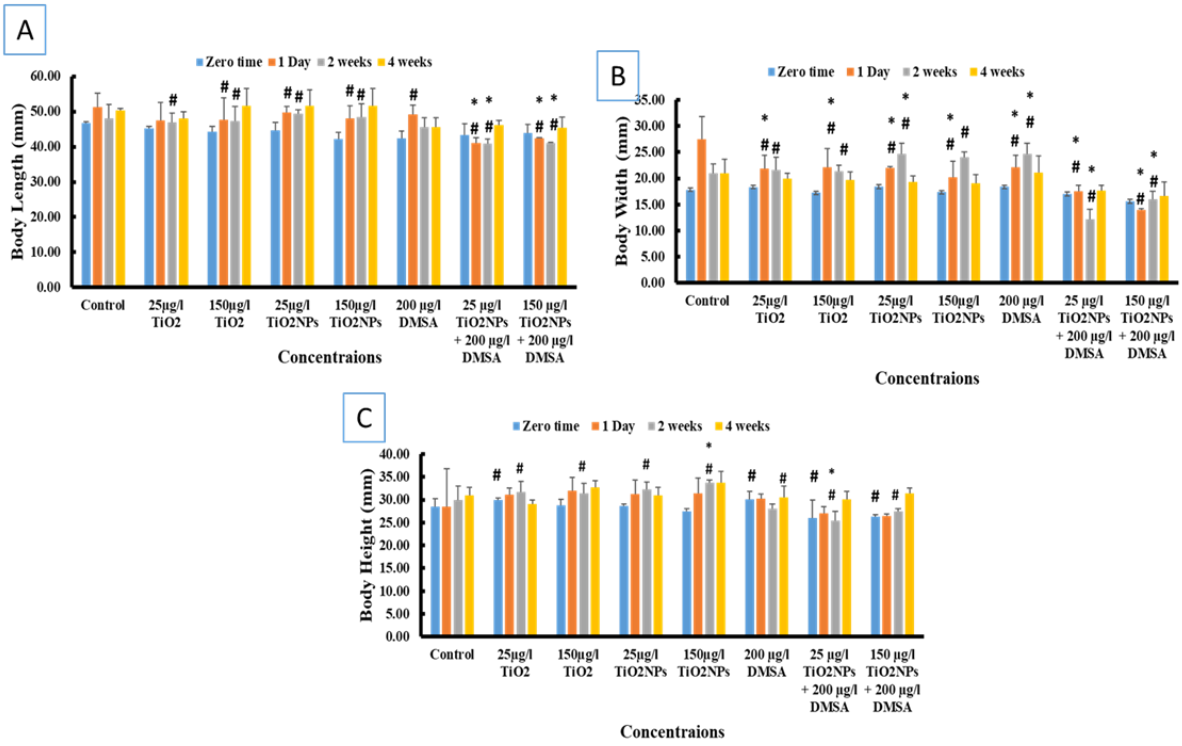

Fig. 1. The effect of different concentrations of TiO_2 NPs with/without DMSA on **(A)** the body length, **(B)** width and **(C)** height (mm) of the *C. nilotica* clams of all groups after different time intervals.

*represents a significant difference between control and exposed groups, # represents significant difference between the exposed groups at the same time interval when $P \leq 0.05$, ANOVA.

The flesh's weight

During this study, the weight of flesh increased significantly across different experimental groups and time intervals ($P=0.0001$). At zero time, the control group established the highest flesh's weight with 5.14± 1.14gm, while the lowest value (3.50±0.31gm) was recorded in the exposed clams to 25µg/ LTiO_2 NPs + 200µg/ l DMSA. As the study progressed, distinct changes in the flesh weight across different groups were detected. The groups exposed to 25µg/ L TiO_2, 150µg/ L TiO_2, 25µg/Ll TiO_2 NPs, and 150 µg/L TiO_2 NPs showed different trends in flesh weight compared to the control group, with significant differences observed at multiple time points ($P \leq$ 0.05).The group exposed to 200µg/ L DMSA showed relatively flesh weight increase over time, with minor fluctuations compared to the control group. The exposed clams to a combination of TiO_2 NPs and DMSA exhibited a significant decrease in flesh weight, particularly evident after 2 weeks ($P>$ 0.05) (Fig. 2B).

Total body weight

The total body weight (gm) of the control and exposed clams was significantly increased with time and different concentrations ($P \leq 0.05$). After 4 weeks of exposure, the total body weights of the exposed clams to 25µg/ L TiO_2 NPs were non-significantly higher than the rest groups, while in the exposed clams to 25µg/ L TiO_2, the total body weight of clams was slightly decreased from 17.01±1.01 at zero time to 16.46±2.03 after 4 weeks of exposure ($P\leq 0.05$). From the observed data, theexposure of clams to 25 and 150µg/ L TiO_2 NPs + 200µg/ L DMSA resulted in a decrease in their total body weight (Fig. 2C).

Condition index (CI)

The exposure of the *C. nilotica* clams to 25 150µg/ L TiO_2 and 200µg/ L DMSA led to a significant increase in their condition index ($P= 0.0001$). In contrast, clams exposed to 25µg/ L and 150µg/ L TiO_2 NPs, as well as 25µg/ l and 150µg/ L TiO_2 NPs + 200µg/ l DMSA, showed an increase in their condition index although it was not statistically significant ($P> 0.05$). The most significant impact on the condition index ($P=0.001$) was observed in the clams exposed to 150µg/ L TiO_2 NPs at 4 weeks time, with a value of 38.04±1.45, which was the lowest among the exposed groups (Fig. 2D).

Effect of TiO_2 NPs concentrations with/without DMSA on the histological structure of *Caelatura nilotica* digestive gland

The digestive gland of *Caelatura nilotica* (control group) is an irregular mass that ranges in color from light to dark brown and surrounds the stomach. The digestive gland consists of several tubules, each lined with a single layer of columnar epithelial cells that have differentiated into digestive and secretory cells, surrounding a central lumen. The basal parts of the cells converge, and the tubules are interconnected by connective tissue (Fig. 3A). Some histological changes, such as haemocyticin filtration and hyperplasia in some digestive tubules, have been seen in the 25µg/ L TiO_2 exposed clams. Some digestive tubules merged with one anothernotably. There was an increase in the numbe rof secretory cells compared to the control clams (Fig. 3B). In clams exposed to 150µg/ L TiO_2, fibrous tissue was detected between several digestive tubules. Some digestive tubules have been necrotic. The lumen inside the digestive tubules became larger, but the connective tissue connecting parts of the digestive tubules deteriorated (Fig. 3C).The digestive tubules of the clams exposed to 25µg/ L TiO_2 NPs were substantially close to each other. Infiltrations of haemocytes, necrosis of connective tissue, and fusion of certain digestive tubules were all detected (Fig. 3D). In the clams exposed to 150µg/ L TiO_2 NPs, all of the previously indicated signs in the 25µg/ l TiO_2 NPs clams were seen in this group, in addition to the widening of the lumen of many digestive tubules with the appearance of vacuoles in the epithelia (Fig. 3E). The histological appearance of the digestive gland in the DMSA-exposed clams appeared healthier and more intact

compared to the control clams. Specifically, the cells of the digestive gland showed a well-preserved structure with clear cell boundaries and organized cellular arrangement (Fig. 3F-H).

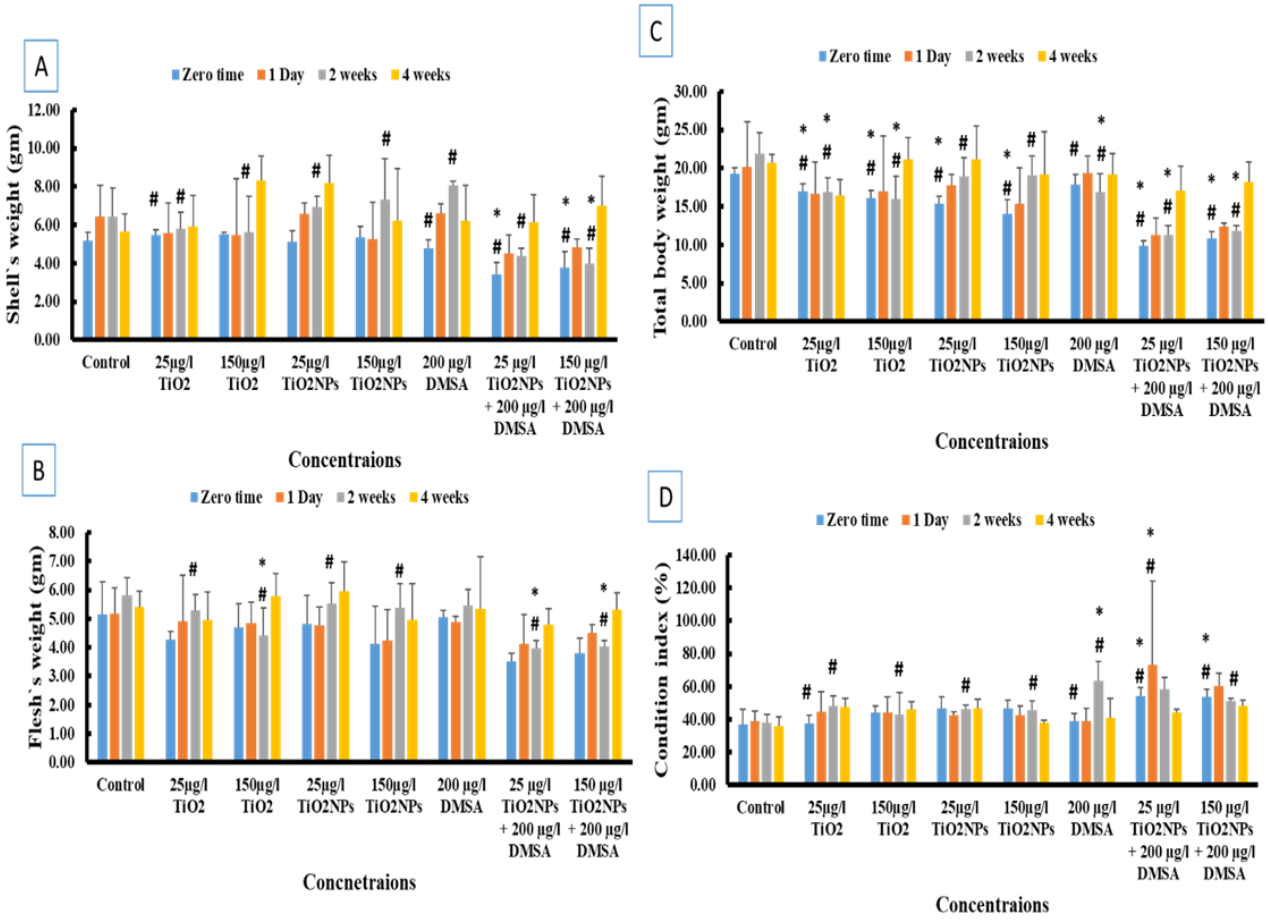

Fig.2. The effects of different concentrations of TiO_2 NPs with/without DMSA on **(A)** the shell, **(B)** flesh, **(C)** total body weights (gm) and **(D)** condition index (%) of the *C. nilotica* clams of all groups after different time intervals.

* represents significant difference between control and exposed groups, # represents significant difference between the exposed groups at the same time interval, when $P \leq 0.05$, ANOVA.

Effect of TiO_2 NPs concentrations with/without DMSA on the ultrastructure of *Caelatura nilotica* digestive gland

Examination of the digestive gland of the control group, *Caelatura nilotica*, by TEM revealed the presence of two cell types within each digestive tubule, as well as digestive & secretory cells. The surface of the digestive cells featured *microvilli*, extending from the apical membrane toward the lumen of the digestive tubule. These *microvilli* increased the membrane surface area, enhancing nutrient uptake efficiency. The secretory cells exhibited cilia that dispersed digestive secretions onto food particles. Vacuoles were observed in certain areas. The nuclei appeared rounded with clear contents and were

surrounded by a notable number of mitochondria (Fig.4A, B). In the exposed clams to 25 and 150µg/ L TiO_2, negative effects were observed on the ultrastructure of the *C. nilotica* digestive gland. Although the secretory cells still possessed cilia on their apical surfaces, they displayed irregular shapes and altered cell boundaries, indicating the detrimental effects of TiO_2. The secretory as well as digestive vesicles occupied notable large size compared to the control clams. The content of the secretory and digestive vesicles changed, becoming disintegrated and incompletely occuping the vesicles, relative to the control group. There was an observed increase in both the size and number of vacuoles. Abnormalities in the nuclei were detected, with irregular nuclear membranes exhibiting an increase in thickness. Furthermore, there was a disruption in chromatin condensation, suggesting potential DNA damage or impaired nuclear functions caused by TiO_2 exposure (Fig.4C- F). The clams exposed to 25 and 150µg/ l TiO_2 NPs experienced negative impacts, which could be summarized as disrupted cellular organization, including irregularly shaped and detached secretory and digestive cells. Cytoplasmic changes include increased vacuolation. Damage was also observed in organelles such as Golgi bodies, which compressed a rounded style with numerous large sized secretory granules. Nuclear abnormalities as irregular nuclear membranes and disrupted chromatin condensation were recorded. Membrane integrity is compromised, leading to leakage of cellular components. These effects, along with structural changes in *cilia microvilli* distribution highlight the negative impacts of TiO_2 NPs on the clams' digestive gland structure (Figs. 4G, H & 5A, B). In the exposed clams to 200µg/ l DMSA, some vesicles were incompletely occupied with their content, the number of vacuoles were detected and some mitochondria shrinked, while other became elongated. In the exposed clams to 200µg/l DMSA+ 25 and 150µg/l TiO_2 NPs, the TEM image showed revitalized of some cellular structures with well-defined boundaries and a rejuvenated cellular arrangement. The organelles within the epithelial cells exhibit a restored integrity, exemplified by normal mitochondria displaying well-organized cristae and an improved electron density. The cytoplasm characterized by an increased density and a better distribution of organelles signify a restored metabolic activity. The nuclei display signs of regeneration, including smooth and intact nuclear membranes, along with chromatin condensation. The cell membranes remain intact, ensuring the preservation of cellular compartments and preventing the leakage of cellular contents. The structural features such as *microvilli* were promoted, fostering efficient nutrient absorption. It is clear, there was a positive impact of DMSA exposure on the ultrastructure of the clams' digestive gland, reduced inflammatory responses, and promoted structural features (Fig.5C-H).

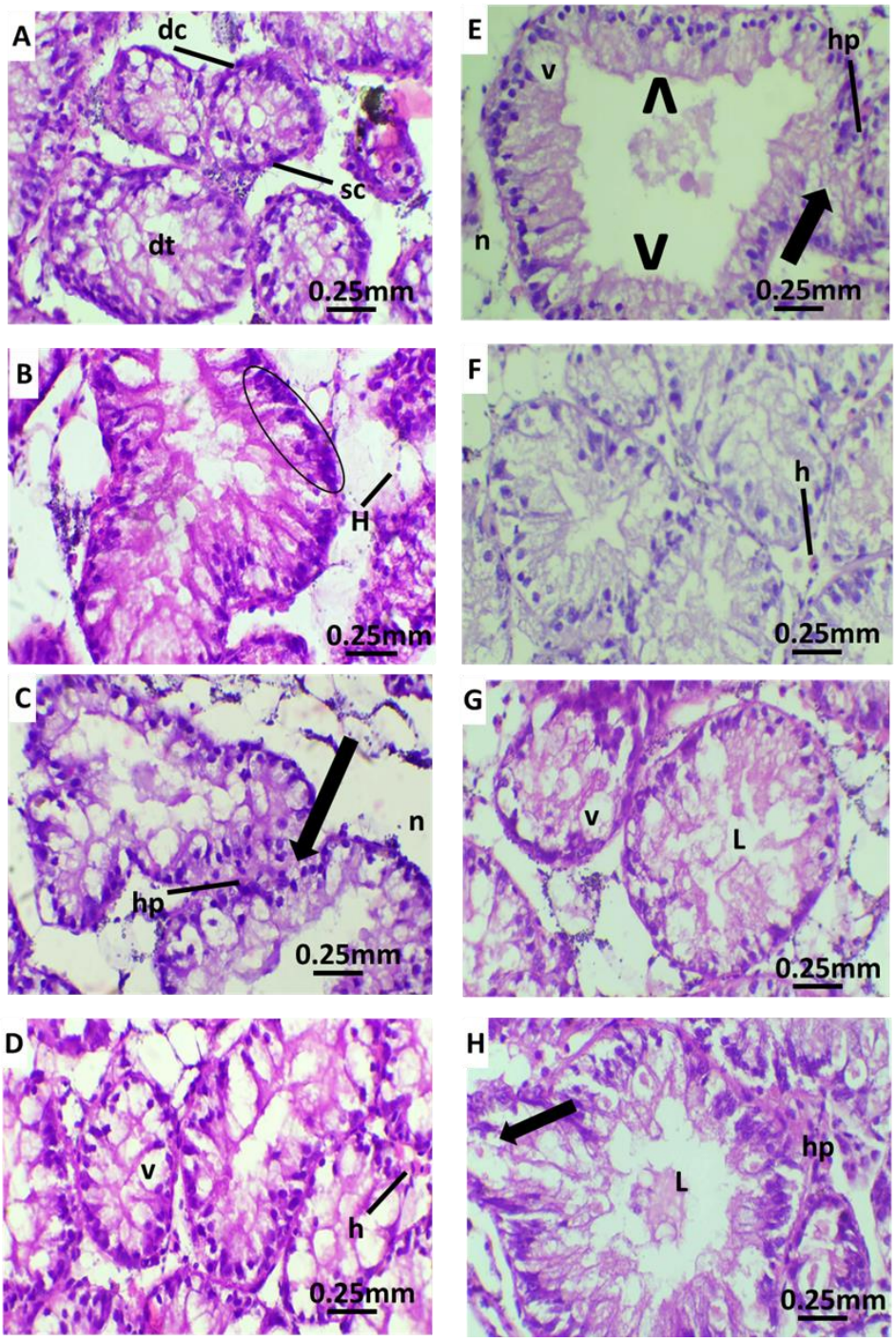

Fig. 3. Light photomicrographs of sections through the digestive gland of *C. nilotica* stained with H&E showing: **(A)** Control clams; **(B)** 25µg/ l TiO_2; **(C)** 150µg/l TiO_2;**(D)** 25µg/ l TiO_2 NPs; **(E)** 150µg/ l TiO_2 NPs; **(F)** 200µg/ l DMSA; **(G)** 25µg/ l TiO_2 NPs + 200µg/ l DMSA, and **(H)** 150µg/ l TiO_2 NPs + 200µg/l DMSA exposed clams. dt:digestive tubules; dc:digestive cells; sc:secretory cells; L:lumen; h:haemocytes infiltration; hp:hyperplasia; n:necrosis; v:vacuolization. The presence of secretory cells (black circle), some digestive tubules fused together (black arrow), and an increase in the lumen size of some digestive tubules (arrowhead).

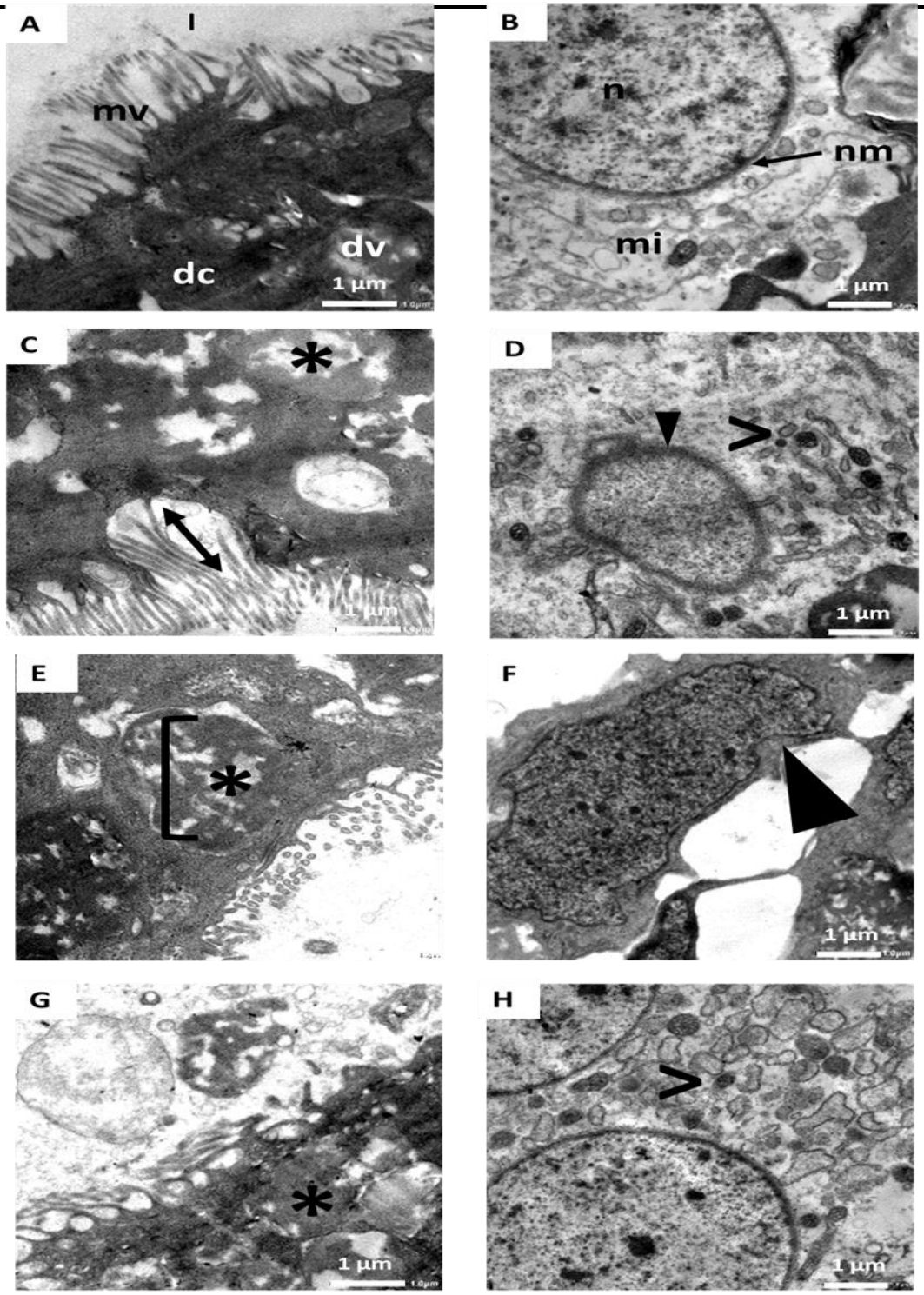

Fig.4. TEM photomicrographs of *C. nilotica* digestive gland showing:**(A, B)** Control clams; **(C, D)** 25µg/ l TiO_2; **(E, F)** 150µg/ l TiO_2; **(G, H)** 25µg/ l TiO_2 NPs exposed clams.

dc:digestive cells; dv:digestive vesicles; mv:*microvilli*; l:lumen; n: nucleus; nm:nuclear membrane; mi:mitochondria. Invagination of digestive cell surface (double head arrow); incompletely occupied vesicles (*), increased in size ([); thickening of nuclear membrane (black triangle); shrunkenmitochondria (arrowhead).

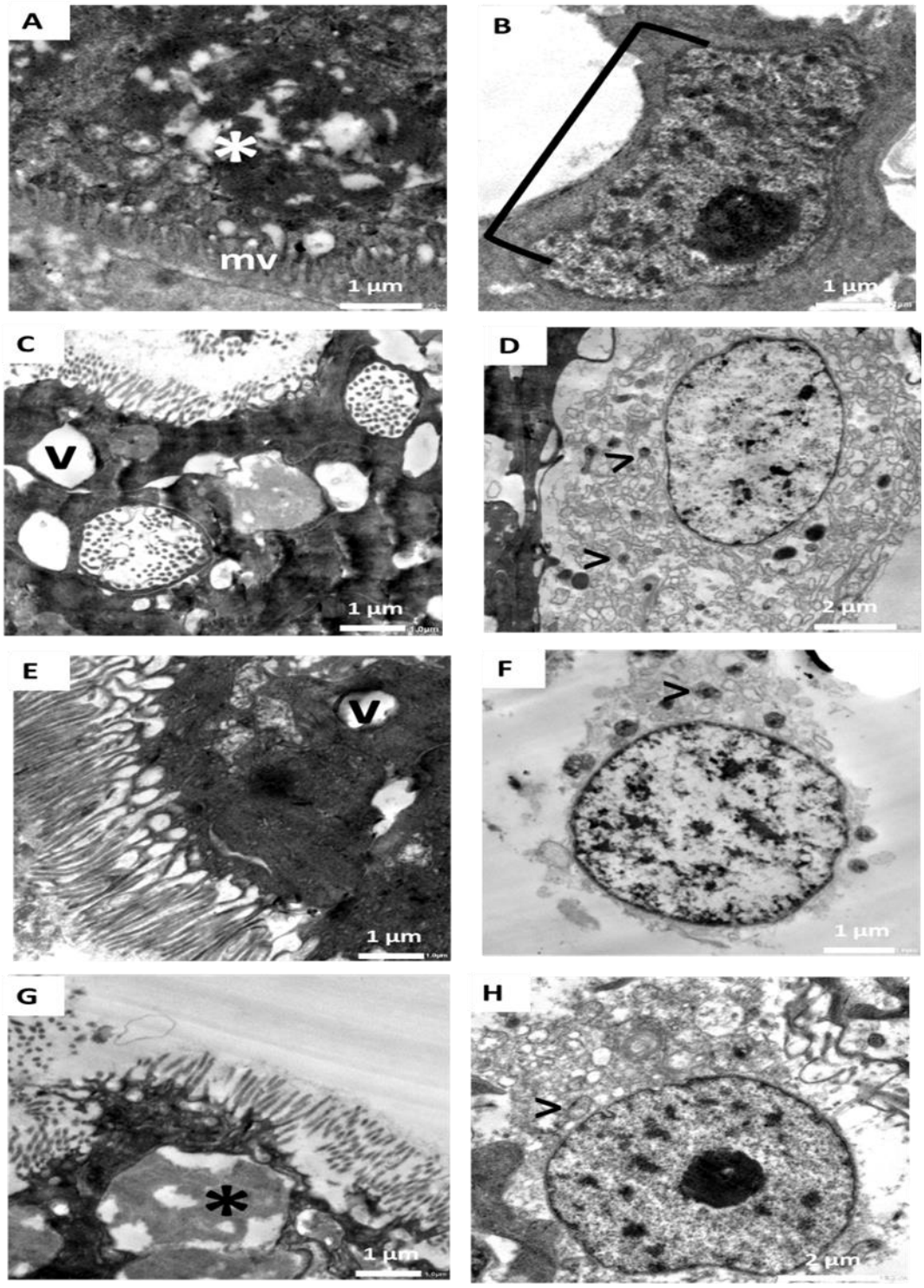

Fig. 5. TEM photomicrographs of *C. nilotica* digestive gland showing: **(A, B)** 150µg/ l TiO_2 NPs; **(C, D)** 200µg/ l DMSA; **(E, F)** 25µg/ l TiO_2 NPs + 200µg/ l DMSA, and **(G, H)** 150µg/ l TiO_2 NPs + 200µg/l DMSA exposed clams.

mv:*microvilli* (mv); v: vacuoles. Incompletely occupied vesicles (*); nucleus turned into elongated shape ([); shrunkenmitochondria (arrow head)

DISCUSSION

In the current study, the biometric parameters of the *C. nilotica* groups exposed to TiO_2 NPs revealed an increase in the body length, width, height, as well as the total body, flesh, and shell weights. This finding aligns with those of **El Morshedy (2021)**, who observed an increase in the total body and shell weights in date mussels (*Lithophaga lithophaga*) exposed to CuO NPs after 4 weeks. Similarly, **Zhu *et al.* (2021)** reported that the administration of TiO_2 NPs for eight weeks resulted in an elevation in liver and body weights, as well as the adipose tissue in mice. This was explained by that TiO_2 NPs induced disruption of the colonic mucus layer and brought about changes in the gut microbiota, with the down-regulation of the Muc2 gene being implicated. This disturbance, combined with microbiota dysbiosis, heightened a low-grade systemic inflammation, thereby worsening the induced obesity. However, **Farkas *et al.* (2015)** observed no noteworthy variations in biometric variables between exposed mussels (*Mytilus edulis*) to TiO_2 NPs and the control groups. This lack of significant differences might be attributed to the relatively short exposure duration of 96 hours chosen by those authors. This brief exposure period may not have been sufficient to elicit discernible effects on the biometric parameters measured in the blue mussels. Contrastingly, a notable decrease in the condition index (CI) of the exposed clams was observed. This coincides with the findings reported by **Kljakovic´-Gaspic *et al.*(2006)** and **Sheir *et al.* (2013)**, who documented significant negative effects of metal pollution on the CI of the *Mytilus* sp. The lower CI values in the polluted mussels, as suggested by **Sheir *et al.* (2013)**, could possibly be due to tissue damage or changes in shell and flesh weight from metal accumulation. Similarly, exposure of the clam *Macoma balthicaton* to silver nanoparticles resulted in a general decrease in their CI **(Dai *et al.*, 2013)**.

During this study, some histopathological signs were recorded in the digestive gland of the exposed *C. nilotica* to TiO_2 NPs. For example, infiltrations of haemocytes, the detection of fibrous tissue, connective tissue degradation, and fusion of certain digestive tubules, in addition to the wide lumen of certain digestive tubules. Similarly, **Vale *et al.* (2014)** found that the exposure of the freshwater bivalve (*Corbicula fluminea*) to 0.1 and 1.0mg/ l TiO_2 NPs resulted in an enlargement or widening of the lumen of digestive tubules and a reduction in the thickness of the epithelium of the digestive gland. These changes may indicate that TiO_2 NPs can cause inflammation in the bivalve digestive gland. The epithelial cells lining the tubules of the digestive gland have been shown to be susceptible to damage from various pollutants, including metals **(Ciacci *etal.*, 2012)**. **Leite*et al.* (2020)** mentioned that the digestive gland of the marine mussels *Mytilus galloprovincialis* exposed to TiO_2 NPs showed an increase accumulation of lipofuscin, atrophy and haemocytes infiltration. **D'Agata *et al.* (2014**) found that in *M. galloprovincialis* exposed to TiO_2 NPs, there was an increased vacuolization of the digestive tubules and substantial haemocytes infiltration. **Abdel-Azeem *et al.* (2023)** observed that the exposure of *Helix aspersa* to 140µg/ l TiO_2 NPs resulted in heightened

degeneration of the digestive tubule, coupled with noticeable inflammatory reactions like hemocytic infiltration. These effects manifested as tubule degradation, expansion of tubule lumens, and full necrosis of digestive cells, alongside an increasing occurrence of vacuoles. **Mansouri *et al.* (2016)** detected histological changes in the liver of the common carp (*Cyprinus carpio*) exposed to TiO_2 NPs as the lyses/breakdown of epithelial cells, cellular shrinkage, and vacuolation. These histological modifications may be attributed to the direct toxic effects of TiO_2 NPs on digestive gland cells, as this organ serves as the primary site for detoxification and therefore accumulates potential toxins like trace metals **(Jaiswal & Sanojini, 1990)**.

The clams exposed to TiO_2 NPs, the negative impacts could be summarized as disrupted cellular organization, including irregularly shaped and detached secretory and digestive cells. Cytoplasmic changes include increased vacuolation. Damage was also observed in the organelles, such as Golgi bodies, nuclear abnormalities as irregular nuclear membranes and disrupted chromatin condensation. Membrane integrity is compromised, leading to leakage of cellular components, along with structural changes in *cilia microvilli* distribution. Images of TEM of the digestive gland revealed the presence of TiO_2 NPs in digestive cells, located both around and within the *microvilli*. This is in accordance with **Barmo *et al.* (2013)**,who found the same findings when used TiO_2 NPs on digestive gland of the marine bivalve *Mytilus galloprovincialis*. This is because the digestive gland plays a crucial role in the uptake, digestion of food, and storage of nutrient reserves. Its specialized function involves intracellular lysosomal digestion of food through endocytosis, making it a key site for nutrient processing. Inside the nuclei of the digestive gland in the exposed *C. nilotica* to TiO_2 NPs, those nanoparticles were detected. **Ciacci*et al.* (2012)** found similar results, after haemocytes of marine bivalve *Mytilus galloprovincialis* incubation with TiO_2 NPs (10mg/ ml for 60min), TiO_2 NPs were agglomerated within the nuclei and the endosomes of the haemocytes. This finding might occur as a result of the ability of nanomaterials to pass through cellular/nuclear membranes due to their tiny size which doesn't exceed 100nm. **Gornati *et al.* (2016)** demonstrated the presence of multi-lamellar bodies, rough endoplasmic reticulum fragmentation, cytoplasmic vacuolization, dense granules, and residual bodies. These findings represent morphological alterations typical of an advanced apoptotic state. **Abd El-Atti *et al.* (2019)** reported that exposure of the red swamp crayfish, *Procambarus clarkii*, to TiO_2 NPs resulted in a severe degeneration of cellular organelles, including ruptured *microvilli*, lytic and vacuolated cytoplasm, and deformed mitochondria. The lyses of the cytoplasm may result from the interaction of nanoparticles with enzymes, leading to oxidative stress and the formation of reactive oxygen species, which can induce cell necrosis **(Chio *et al.*, 2010)**.

The findings of this study revealed that the utilization of DMSA significantly alleviated the adverse impacts of TiO_2 NPs on *C. nilotica*. This was supported by an improvement in morphometric/biometric measurements, as well as histological

observations, highlighting DMSA's capability to mitigate TiO_2 NPs toxicity in *C. nilotica* clams. Furthermore, the efficacy of DMSA may be attributed to its ability to reduce the concentration of TiO_2 NPs to which *C. nilotica* was exposed. **Mohamed *et al.* (2019)** stated that, DMSA was found to decrease lead and mercury concentrations in the tissues of the catfish *Clarias gariepinus* following DMSA exposure.

CONCLUSION

In conclusion, the study underscores the significant biometric and histopathological alterations induced by TiO_2 NPs exposure in *C. nilotica*, indicative of potential toxicity. These findings contribute to our understanding of the nanoparticle-induced effects on aquatic organisms and highlight the importance of assessing nanoparticle toxicity for environmental and human health. Moreover, the study demonstrates the promising efficacy of DMSA in mitigating TiO_2 NPs toxicity, suggesting its potential therapeutic application in nanoparticle-induced toxicity scenarios.

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