 DOI: 10.21608/adjalexu.2024.283245.1500

ASSESSMENT OF LINGUAL MUCOSA TOXICITY FOLLOWING TITANIUM DIOXIDE NANOPARTICLE EXPOSURE IN ALBINO RATS (LIGHT MICROSCOPIC STUDY)

Salma M. Noiem[1*], Hanaa M. Aly[2] PhD, Samia S. Omar[2] PhD, Mariam A. Abu Ayana[3] PhD

ABSTRACT

INTRODUCTION: Applications of nanotechnology have recently drawn more attention from a variety of disciplines. One of the most often utilized nanoparticles worldwide is titanium dioxide nanoparticles, or TiO_2 NPs because of their unique properties. Many foods, personal care, and other consumer products commonly contain them as additives. Studies revealed that TiO_2 NPs can aggregate in multiple tissues and cause oxidative stress. Gaining more knowledge about the toxicity of TiO_2 nanoparticles could improve risk assessment and safer nanomaterial application techniques. Considering the risk evaluation of consuming TiO_2 NPs, the lingual mucosa is still ignored.

OBJECTIVES: To assess the effects of TiO_2 NPs administration on the lingual mucosa of rat.

METHODOLOGY: Fourteen adult male albino rats, weighing (150–200 g) were used in the present study. Animals were randomly divided into 2 equal groups: group I (control group): 7rats received olive oil by oral gavage daily for 3 weeks. Group II (TiO_2 NPs group): 7rats received olive oil daily then 300 mg/kg TiO_2 NPs by oral gavage daily for 2 weeks. The animals were euthanized at the end of the experiment. Tongues were surgically dissected and prepared for light microscopic analysis.

RESULTS: Significant degenerative and atrophic changes were observed in the lingual mucosa of the TiO_2 NP group. These alterations included deformity of the filiform and fungiform papillae as well as vacuolations of epithelial cells. Body weight changes revealed that the group receiving TiO_2 NPs experienced an insignificant rise in body weight.

CONCLUSION: The lingual mucosa of rats exhibited significant histological alterations in the filiform and fungiform papillae due to TiO_2 NPs exposure. Consequently, future TiO_2 NPs risk assessment studies should focus on the oral cavity.

KEYWORDS: Tongue, TiO_2NPs, titanium, rats, oxidative stress

RUNNING TITLE: Titanium dioxide nanoparticles toxicity on rats' lingual mucosa.

1.Demonstrator of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt.

2.Professor of Oral Biology, Department of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt.

3.Lecturer of Oral Biology, Department of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt.

Corresponding author:

salma.noiem@gmail.com

INTRODUCTION

Recently, the application of nano-engineered materials in various sectors has become more and more popular. The physical and chemical characteristics of nanomaterials, including their large surface area, small size, photocatalytic capacity, redox potential, and quantum parameters, render them valuable tools in associated domains.(1, 2)

Titanium dioxide nanoparticles (TiO_2NPs) are being produced and applied more frequently due to their many unique and practical qualities. Owing to their superior stability, anticorrosive, and photocatalytic qualities, they have been utilized extensively as a bactericidal agent, white pigment, skincare product for individuals, and water treatment agent.(3)

These nanoparticles are used extensively in food industry. They serve a role in the manufacturing of coated sweets, chewing gum, preserved fruits, carbonated drinks, powdered drinks (in dose forms that are concentrated or unsweetened), milk and dairy products, and other food products. They have been utilized as the E171 ingredient in many different food products and have gained broad acceptance in the food industry.(4, 5)

In dentistry, the problem of caries near brackets in orthodontic treatment was proven to be resolved by the inclusion of TiO_2 NPs in the composite, which reduced the colony measures of Streptococcus mutans and Streptococcus sanguinis.(6) The risk associated with TiO_2 NPs exposure is surprisingly increased by the extensive

use of these NPs in food additives, cosmetics, and dental care products.

When titanium dioxide nanoparticles build up in various tissues, oxidative stress is induced, causing damage to the tissues. Increased exposure to TiO_2 NPs may cause chronic cytotoxicity due to their non-degradability and capacity to accumulate in a variety of tissues.(7, 8)

Studies revealed that the digestive tract and lungs are the primary entrance sites for toxins.(9) TiO_2 NPs are systemically distributed throughout the body and can potentially reach the liver, spleen, lung, brain, testis, and other organs depending on the amounts used.(10) The toxicity mechanism of TiO_2 NPs is comprised of three processes which are the attachment of TiO_2 NPs to biological micromolecules and intracellular organelles after cell membrane disruption, the lipid peroxidation of the cell membrane and the generation of reactive oxygen species (ROS).(11, 12)

One important channel for human exposure to TiO_2 NPs is through food. Food contains at least 36% of TiO_2 particles that are nanoscale in size. TiO_2 NPs with diameters less than 100 nm are found in larger concentrations in chewing gum, candies, and many other food products.(4, 9, 13) Consequently, the mentioned products expose the oral mucosa to elevated levels of TiO_2 NPs.

Rat tongue has various types of papillae on its dorsal surface. However, they differ in their distribution from human being. The filiform papillae cover the entire dorsal surface of the tongue while the fungiform papillae are located in the anterior half of the tongue. The sole circumvallate papilla, which has the maximum density of taste buds, is located along the midline of the tongue base.(15)

Therefore, the aim of the current investigation is to estimate the effect of TiO_2 NPs on the mucosa of the rat tongue. However, this study's null hypothesis suggests that no significant effect will result by administration of TiO_2 nanoparticles in tongue mucosa of rat model.

MATERIALS AND METHODS

Study sample

In this experiment, fourteen adult male albino rats, weighing (150–200 g) were used. They were obtained from The Medical Research Institute, Alexandria University and kept in its experimental animal house. The study was conducted after the research ethics committee approval in Faculty of Dentistry, Alexandria University. (IRB NO: 00010556) (IORG 0008839). Animals were kept under light/dark cycles with 12 hours of light and 12 hours of darkness in custom-designed wire mesh bottom cages kept at room temperature. Throughout the duration of the experiment, they were supplied with a regular diet.

Chemicals:

Titanium dioxide nanoparticles administration

Titanium dioxide NPs were obtained from Nano Gate Company, Cairo, Egypt. The product was packed in the form of white granules/powder. They were dissolved in distilled water to form a suspension by ultrasonication through applying sound energy to agitate particles in distilled water for allowing solubility and administrated orally using oral gavage to group II (TiO_2 NPs group).(16, 17) 300 mg/kg of TiO_2 NPs was used in order to guarantee the induction of definite acute toxicity in rats. Stock solution of TiO_2 NPs in distilled water was prepared weekly so that each 1 ml of the prepared solution contained the required dose.

Grouping

Fourteen adult male albino rats were randomly assigned by using computer generated random numbers to one of 2 equal groups:

Group I (Control group): 7rats received olive oil by oral gavage daily for 3 weeks.

Group II (TiO_2 NPs group): 7rats received olive oil for 1 week then they received 300 mg/kg TiO_2 NPs by oral gavage daily for 2 weeks.(3, 4)

At the start of this study, animals were weighed to calculate the dosage of TiO_2 NPs. During the experiment, the rats' body weights were recorded weekly in order to detect any weight changes.

Diethyl ether overdoses were used to euthanize the animals in each group after three weeks, the dorsal segments of rat tongues were divided sagitally into right and left halves and prepared to be examined histologically. The procedures of histological examination were performed in the laboratory of the Oral Biology Department at Alexandria University.

Characterization of Titanium Dioxide Nanoparticles (5)

Transmission Electron Microscope (TEM) was used to observe the particle shape and to measure particle size of Titanium Dioxide Nanoparticles suspension. A little drop of the TiO_2 NPs suspension was placed on a TEM grid, covered with a thin layer of carbon, allowed to evaporate, then electron micrographs of several spots on the grid were taken. These nanoparticles appeared spherical-shaped formed electron dense aggregates of atypical sizes with their diameter less than 100 nm (Fig. 1).

Histological procedures(18)

Specimens were preserved in a 10% neutral buffered formalin solution in labeled containers. The dorsal segments of rat tongues were divided sagitally into right and left halves. These halves were then washed, dehydrated in increasing alcohol concentrations, cleared with xylene, infiltrated, and

embedded in paraffin blocks. Five micrometers thickness sections were cut by the microtome rotary device. The obtained sections were stained with Hematoxylin and Eosin stains (H&E) then examined by Optika light microscope to investigate their histological structure.

Statistical Analysis

Normality was checked using Shapiro Wilk test and Q-Q plots. Normal distribution was confirmed thus data were presented using mean and standard deviation. Independent t test was used to assess differences between groups and Repeated measures ANOVA was used to analyzed difference in weight across time. All tests were two tailed and the significance level was set at p value≤0.05. Data were analyzed using IBM SPSS, version 23 for Windows, Armonk, NY. USA.

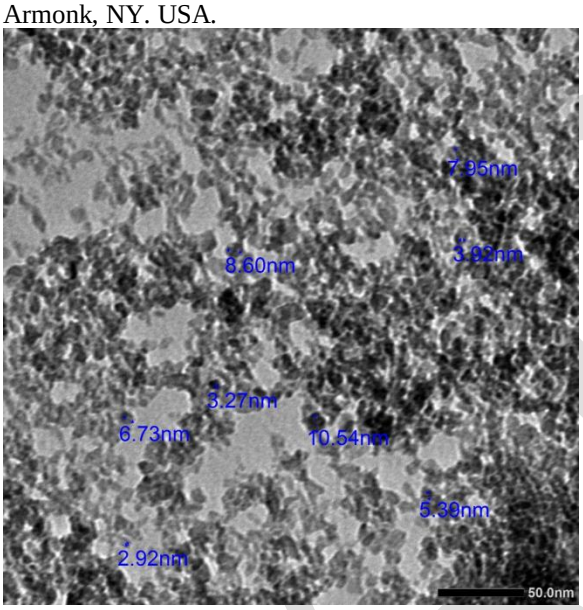

Figure 1: Transmission electron micrograph of the titanium dioxide nanoparticles dispersion showing the spherical-shaped nanoparticles. (Scale bar 50 nm)

RESULTS

Body weight changes results:

Table 1 and Figure 2 illustrate the changes in rats' body weight (g/week) of both groups throughout the time of the experiment.

After three weeks, the animals' final body weights increased in both groups. Rats that received TiO_2 NPs (group II) gained less weight than control rats. This increase in weight was insignificant in comparison to control group.

Histological results

Group I (control group)

The tongue's dorsal surface of the control group under a light microscope revealed that the fungiform and filiform papillae were arranged and structurally normal. Cone-shaped filiform papillae with keratinized stratified squamous epithelium were visible. (Fig.3a&b). Dispersed clear cells were observed (Fig.3b). Normal cellular and fibrous elements were present in the underlying lamina propria, which blended with the adjacent tongue muscles without a distinct demarcation line (Fig.3a). The fungiform papillae, which exhibit a mushroom-like appearance, were observed to have a normal stratified squamous epithelium with a vascular connective tissue core and a thin, uniform layer of keratin covering them. (Fig.4a). At the superior surface, a solitary barrel-shaped taste bud with peripherally organized cells was visible. (Fig.4b).

Group II (TiO_2 NPs group)

Examination of TiO_2 NPs group revealed marked alteration of the shape of the tongue papillae. The filiform papillae exhibited a significant decrease in height and a visible deformity in their structure, with blunt-ended tips. Also, papillae were completely absent in several regions. Focal keratin layer detachment from the underlying epithelial cells was also observed (Fig. 5a). Additionally, the cytoplasm of some basal and suprabasal epithelial cells was vacuolated. Numerous clear cells were seen (Fig. 5b). Also, lack of the typical connective tissue papillae was observed with separation between tongue muscle fibers (Fig. 5a).

Moreover, fungiform papillae manifested slight distortion losing its typical mushroom shaped. There was some distortion and separation in the overlying keratin layer. (Fig. 6a). Degeneration of the taste buds were also observed (Fig. 6b).

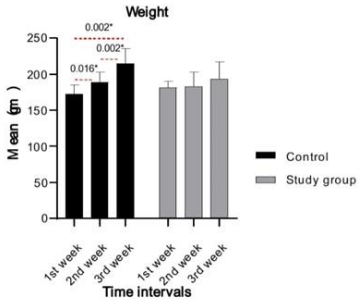

Figure 2: Comparison of animals' body weight among the two groups across time intervals represented in bar chart with plotted data points.

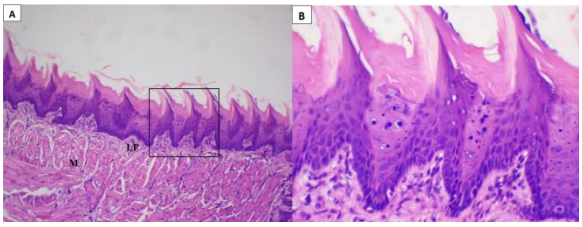

Figure 3(A&B): Photomicrograph of control group showing the dorsal surface of the rat tongue,

demonstrating the filiform papillae. A&B: Cone shaped filiform papillae covered by keratinized epithelium with a normal architecture of the lamina propria (LP) and tongue muscles (M) can be seen. [H & E stain, A: x100, B: x400]

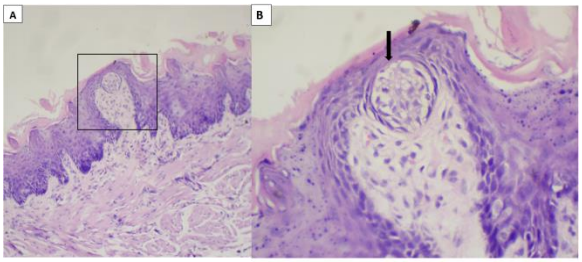

Figure 4(A&B): Photomicrograph of control group showing the dorsal surface of the rat tongue, demonstrating the fungiform papilla. A: Fungiform papilla with normal mushroom shape is covered by a thin keratinized epithelium. B: On the superior surface of the papilla, a barrel-shaped taste bud (arrow) is seen. [H & E stain, A: x100, B: x400]

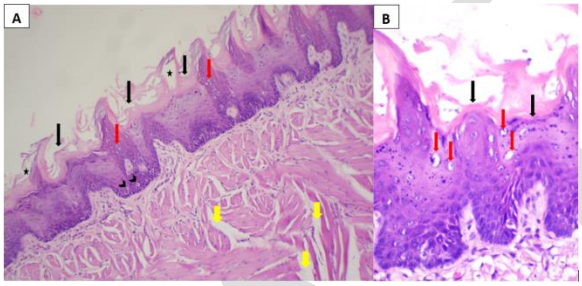

Figure 5(A&B): Photomicrograph, (TiO_2 NPs group) showing marked alteration of the shape of the filiform papillae. Some papillae exhibited blunt-ended tops with a marked reduction in their height (black arrows), some areas revealed a complete loss of the papillae, focal separation of the keratin layer from the underlying epithelial cells was also observed (black stars), some of the basal and suprabasal epithelial cells had vacuolated cytoplasm (red arrows), numerous clear cells (arrow heads), Loss of the normal connective tissue papillae and separation between tongue muscle fibers was also noted (yellow arrows). [H & E stain, A: x100, B: x400]

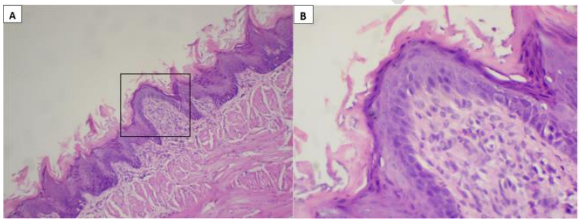

Figure 6(A&B): Photomicrograph, (TiO_2 NPs group) showing fungiform papillae lost their characteristic mushroom shape. There was a little distortion and layer separation in the overlying keratin layer. Absence of the taste buds were also observed. [H & E stain, A: x100, B: x400]

Table 1: Comparison of animals' weight among the study groups across time intervals

	Group I (n=7)	**Group II (n=7)**	***p* value**
1st week	172.43 ±12.69	181.14 ±9.43	0.170
2nd week	188.56 ±14.55	183.27 ±19.06	0.571
3rd week	215.06 ±20.27	193.14 ±23.79	0.088
***p* value**	<0.0001*	0.333	
Pairwise comparisons			
1st vs 2nd week	0.016*	-	
1st vs 3rd week	0.002*	-	
2nd vs 3rd week	0.002*	-	

*Statistically significant difference at *p* value≤0.05

DISCUSSION

Titanium dioxide nanoparticles are commonly used in the paint, food, cosmetics, medicinal, and sterilization industries. The use of these materials is growing across many industries because of some of the special physicochemical characteristics of their nanoparticles. Even if there are advantages, prolonged exposure to nanoparticles can be harmful to humans.(19) It has been established that toxicity may result from exposure to TiO_2 NPs through various processes linked to the generation of reactive oxygen species in biological systems and the subsequent onset of oxidative stress.(20, 21)

In the current study, TiO_2 powder containing anatase phase which is a tetragonal crystalline form with a mean particle size of less than 10 nm was utilized. Weir et al. (2012) observed genotoxicity induced by TiO_2 NPs in the anatase crystalline phase that have a diameter of less than 25 nm.(22) It has been proved that titanium dioxide nanoparticles' anatase form is more phototoxic and cytotoxic than other forms, such as rutile.(23, 24)

Based on earlier research, the concentration of TiO_2 NPs required to cause acute toxicity in rodents has been found to range from 50 to 500 mg/kg body weight.(9, 25) Even though some researches demonstrated that toxicity happens at lower concentrations, toxicity for a wide range of factors cannot be generalized at such levels. At 300 mg/kg, researchers reported that TiO_2 NPs could change the livers and spleens of Wistar rats in terms of

biochemical and histological markers.(16, 17, 26) Accordingly, rats were given 300 mg/kg of TiO_2 NPs to guarantee the production of definitive acute toxicity.

In this investigation, intragastric delivery was the preferred method as the capability of TiO_2 NPs to move across biological barriers, including the gastrointestinal tract, was examined in earlier research. It was established that oral TiO_2 NPs (5, 50, and 500 mg/kg body weight) accumulated in mice over time, even at low doses. This led to oxidative stress, inflammation, and apoptosis, which in turn resulted in the development of chronic gastritis.(27)
According to the statistical analysis in the current study of the rats' body weights, the group that received NPs experienced a statistically insignificant increase in body weight in comparison to the control group. This is in accordance with Ibrahim et al. who discovered that rats' body weight and feed intake were unaffected by TiO_2 NPs. (28)

Moreover, another study by Warheit and Donner revealed that neither the rat's body weight nor any nutritional parameters changed following the administration of TiO_2 NPs.(29) Contrary another investigation revealed that rats given TiO_2 NPs lost weight in contrast to the control group.(26)

The histological results of the current investigation showed that the rats' tongue mucosa in the group exposed to TiO_2 NPs had various degenerative changes compared to the control group. The structure and form of the tongue papillae were noticeably altered because of these particles. The tips of the filiform papillae appeared blunt, and their height was noticeably reduced. Moreover, in certain points, the papillae had completely disappeared.

These findings coincide with Jahangirfard et al. (2020) observations. They found that animals treated with mid- and high-dose TiO_2 NPs had shorter germinal epithelium heights than the control group.(30)

These observations could be justified by Trouiller et al. (2009) who discovered that TiO_2 NPs may increase the generation of ROS, which results in mitochondrial damage, breaking DNA and causing chromosomal disruptions. Thus, these findings are thought to indicate that repeated exposure to TiO_2 NPs may deplete the epithelium's capacity for proliferation, which would eventually result in epithelial atrophy.(31)

Furthermore, the fungiform papillae showed defective architecture. In addition, it was noted that the keratin layer and the underlying epithelial cells were focally separated. These light microscopic results coincide with the study of Shamel et al. (2022) who evaluated lingual mucosa toxicity and recovery when followed-up in rats, following sub-chronic exposure to TiO_2 NPs. They demonstrated that fungiform papillae were deformed and undersized.(14) This could be due to the oxidative stress which could act as a mediating factor in the cytotoxic effects of TiO_2 NPs on the tongue mucosa, as documented by the findings of Jing et. al.(12)

In another study, the build-up of TiO_2 NPs in the spleen, endocrine system, and reproductive sector has been demonstrated by Roberta Tassinari et al. (2014). These tissues experience acidic stress as a result of this build-up, which increases lipid peroxidation.(32) The study by Moradi et al. (2019) demonstrated that the intoxicated group that received 300 mg/kg TiO_2 NPs by gavage for two weeks showed a noticeable decrease in the activity of enzymes involved in oxidative stress protection, such as superoxide dismutase (SOD) activity compared to control groups. This suggests that oxidative reactions and reactive oxygen species caused by TiO_2 NPs were also responsible for liver toxicity.(17)

Furthermore, changes in the fungiform taste buds of the TiO_2 NPs group manifested in this study as taste bud cells degeneration that had been similarly revealed by the study of Shamel et al. (2022). They observed that exposure to TiO_2 NPs caused degenerative alterations in the orientation and morphology of the taste bud cells leading to their separation.(14)

In accordance with these findings, Hong et al. found out that exposure to TiO_2 NPs in cultured rat primary hippocampal neurons resulted in neurite growth suppression and reduction in cell viability.(33) Moreover, Halawa et al. reported that neurotoxicity-induced titanium dioxide nanoparticles in adult rat brains. Their findings showed that the TiO_2 NPs group which exhibited significant histological alterations including edematous and vacuolated neuropil, and a loss of micromorphological shapes in the majority of cerebral cortex cells, Purkinje cells, and pyramidal cells of the hippocampus.(34) According to the previous explanations, this could be attributed to neuropathy, which impairs the function of the taste nerve and causing a defect in taste receptors.

In this study, cytoplasm was vacuolated in several of the suprabasal and basal epithelial cells. The existence of these vacuoles was also observed by Jia et al. (2017), who discovered that high concentrations of TiO_2 NPs led to hepatocyte swelling in liver tissues. They concluded that TiO_2 NPs generate excess reactive oxygen species and decrease the cells' ability to produce antioxidants by causing damage to the mitochondria indicating that the appearance of these vacuolations could be a result of mitochondrial apoptosis.(35)

Finally, it was also observed in this study that the tongue muscle fibers showed areas of separations in between. This is consistent with the results of El-din et al. (2019), who observed that the cardiac muscle of rats treated with TiO_2 NPs displayed large intercellular gaps between the cardiomyocytes, with some of these cardiomyocytes exhibiting vacuolation. They explained their results, stating that exposure to TiO_2NPs was thought to reduce antioxidant activity with declines in the enzymatic antioxidant capacity and cause cellular oxidative damage in the mouse heart by means of lipid peroxidation and nucleic acid degradation. This led to myocardial cell necrosis and degeneration, inflammation, atherosclerosis, and mitochondrial injury.(36)

Therefore, it is evident from these data that prolonged exposure to TiO_2 NPs may be harmful to human health, suggesting that risk assessment guidelines should be reexamined.

CONCLUSION

Exposure to TiO_2 NPs can have cytotoxic effects on albino rats' lingual mucosa. Utilizing TiO_2 NPs in food and other aspects of daily life should be more closely monitored. Furthermore, the present findings imply that future TiO_2 NPs risk assessment research has to focus on the oral cavity.

CONFLICT OF INTEREST

We affirm that we have no conflicts of interest.

FUNDING

No specific funding was received by the authors for this study.

REFERENCES

1. Chen X, Mao SS. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chemical reviews. 2007;107(7):2891-959.
2. Brown SC, Boyko V, Meyers G, Voetz M, Wohlleben W. Toward advancing nano-object count metrology: a best practice framework. Environmental health perspectives. 2013;121(11-12):1282-91.
3. Jimenez-Cadena G, Riu J, Rius FX. Gas sensors based on nanostructured materials. Analyst. 2007;132(11):1083-99.
4. Weir A, Westerhoff P, Fabricius L, Hristovski K, Von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environmental science & technology. 2012;46(4):2242-50.
5. Chen XX, Cheng B, Yang YX, Cao A, Liu JH, Du LJ, et al. Characterization and preliminary toxicity assay of nano-titanium dioxide additive in sugar-coated chewing gum. Small. 2013;9(9-10):1765-74.
6. Sodagar A, Bahador A, Jalali YF, Gorjizadeh F, Baghaeian P. Effect of chitosan nanoparticles incorporation on antibacterial properties and shear bond strength of dental composite used in orthodontics. Iran J Ortho. 2016.
7. Hong J, Zhang Y-Q. Murine liver damage caused by exposure to nano-titanium dioxide. Nanotechnology. 2016;27(11):112001.
8. Shi H, Magaye R, Castranova V, Zhao J. Titanium dioxide nanoparticles: a review of current toxicological data. Particle and fibre toxicology. 2013;10(1):1-33.
9. Shakeel M, Jabeen F, Shabbir S, Asghar MS, Khan MS, Chaudhry AS. Toxicity of nano-titanium dioxide (TiO2-NP) through various routes of exposure: a review. Biological trace element research. 2016;2(1):1-36.
10. Elgrabli D, Beaudouin R, Jbilou N, Floriani M, Pery A, Rogerieux F, et al. Biodistribution and clearance of TiO2 nanoparticles in rats after intravenous injection. PLoS One. 2015;10(4):e0124490.
11. Aillon KL, Xie Y, El-Gendy N, Berkland CJ, Forrest ML. Effects of nanomaterial physicochemical properties on in vivo toxicity. Advanced drug delivery reviews. 2009;61(6):457-66.
12. Hou J, Wang L, Wang C, Zhang S, Liu H, Li S, et al. Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms. J Environ Sci (China). 2019;75:40-53.
13. Orazizadeh M, Fakhredini F, Mansouri E, Khorsandi L. Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats. Chemico-biological interactions. 2014;220:214-21.
14. Shamel M, Rady D, Ankily MA. Evaluation of lingual mucosa toxicity and recovery follow-up in rats, following sub-chronic exposure to titanium dioxide nanoparticles. Dent Med Probl. 2022;59(3):427-35.
15. Parker GA, Picut CA. Atlas of histology of the juvenile rat: Academic Press; 2016.
16. Khorsandi L, Orazizadeh M, Mansori E, Fakhredini F. Glycyrrhizic acid attenuated lipid peroxidation induced by titanium dioxide nanoparticles in rat liver. Bratislavske Lekarske Listy. 2015;116(6):383-8.
17. Moradi A, Ziamajidi N, Ghafourikhosroshahi A, Abbasalipourkabir R. Effects of vitamin A and vitamin E on attenuation of titanium dioxide nanoparticles-induced toxicity in the liver of male Wistar rats. Mol Biol Rep. 2019;46(3):2919-32.
18. Panzacchi S, Gnudi F, Mandrioli D, Montella R, Strollo V, Merrick BA, et al. Effects of short and long-term alcohol-based fixation on Sprague-Dawley rat tissue morphology, protein and nucleic

acid preservation. Acta histochemica. 2019;121(6):750-60.
19. Papp T, Schiffmann D, Weiss D, Castranova V, Vallyathan V, Rahman Q. Human health implications of nanomaterial exposure. Nanotoxicology. 2008;2(1):9-27.
20. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed research international. 2013;2013.
21. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases. 2007;2(4):MR17-MR71.
22. Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol. 2012;46(4):2242-50.
23. Xue C, Wu J, Lan F, Liu W, Yang X, Zeng F, et al. Nano titanium dioxide induces the generation of ROS and potential damage in HaCaT cells under UVA irradiation. Journal of nanoscience and nanotechnology. 2010;10(12):8500-7.
24. Sanders K, Degn LL, Mundy WR, Zucker RM, Dreher K, Zhao B, et al. In vitro phototoxicity and hazard identification of nano-scale titanium dioxide. Toxicology and applied pharmacology. 2012;258(2):226-36.
25. Rizk MZ, Ali SA, Hamed MA, El-Rigal NS, Aly HF, Salah HH. Toxicity of titanium dioxide nanoparticles: effect of dose and time on biochemical disturbance, oxidative stress and genotoxicity in mice. Biomedicine & Pharmacotherapy. 2017;90:466-72.
26. Afshari-Kaveh M, Abbasalipourkabir R, Nourian A, Ziamajidi N. The Protective Effects of Vitamins A and E on Titanium Dioxide Nanoparticles (nTiO2)-Induced Oxidative Stress in the Spleen Tissues of Male Wistar Rats. Biol Trace Elem Res. 2021;199(10):3677-87.
27. Mohamed HRH. Estimation of TiO2 nanoparticle-induced genotoxicity persistence and possible chronic gastritis-induction in mice. Food and Chemical Toxicology. 2015;83:76-83.
28. Ibrahim R, Elkady M, Hassanein A. Effect of Some Antioxidants on Rats Treated With Titanium Dioxide Nanoparticles. Egyptian Journal of Food Science. 2019;0(0):0-.
29. Warheit DB, Donner EM. Risk assessment strategies for nanoscale and fine-sized titanium dioxide particles: Recognizing hazard and exposure issues. Food and chemical toxicology. 2015;85:138-47.
30. jahangirfard r, raji ar, moghaddam jafari A, Nourani H. Ameliorating Effects of Vitamin E on Morphological and Histological Alterations and Oxidative Stress Factors Assessment Against Titanium Dioxide Nanoparticle-Induced in Mice Testis. Journal of Advanced Biomedical Sciences. 2020;10(2):2197-205.
31. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer research. 2009;69(22):8784-9.
32. Tassinari R, Cubadda F, Moracci G, Aureli F, D'Amato M, Valeri M, et al. Oral, short-term exposure to titanium dioxide nanoparticles in Sprague-Dawley rat: focus on reproductive and endocrine systems and spleen. Nanotoxicology. 2014;8(6):654-62.
33. Hong F, Sheng L, Ze Y, Hong J, Zhou Y, Wang L, et al. Suppression of neurite outgrowth of primary cultured hippocampal neurons is involved in impairment of glutamate metabolism and NMDA receptor function caused by nanoparticulate TiO2. Biomaterials. 2015;53:76-85.
34. Halawa A, Elshopakey G, El-Adl M, Lashen S, Shalaby N, Eldomany E, et al. Chitosan attenuated the neurotoxicity-induced titanium dioxide nanoparticles in brain of adult rats. Environmental Toxicology. 2022;37(3):612-26.
35. Jia X, Wang S, Zhou L, Sun L. The potential liver, brain, and embryo toxicity of titanium dioxide nanoparticles on mice. Nanoscale research letters. 2017;12:1-14.
36. El-Din EAA, Mostafa HE-S, Samak MA, Mohamed EM, El-Shafei DA. Could curcumin ameliorate titanium dioxide nanoparticles effect on the heart? A histopathological, immunohistochemical, and genotoxic study. Environmental Science and Pollution Research. 2019;26:21556-64.