Neurotoxic effects of titanium dioxide nanoparticles on the brain of male sprague dawley rats

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Abstract: Titanium dioxide nanoparticles have diverse applications in many fields and are used in cosmetics, sterilization, paints, tooth paste, food products, air cleaning, sunscreens and waste water treatment plants due to their unique properties. But on the other hand, the wide use of titanium dioxide nanoparticles raises concerns to the upcoming challenges of human health. This study assessed the neurotoxic effect of titanium dioxide nanoparticles by histology, cell viability, oxidative stress and acetylcholine esterase level. For this purpose, 28 days old, post weaning male Sprague Dawley rats (n=25) were procured from the animal house of the Government College University, Faisalabad. Rats were randomly divided into five groups with five rats in each group and designated as Control (C) without treatment, Placebo group (S) treated with 0.9% normal saline and three nanoparticles treated groups (Group 1 of 80 mg/kg, Group 2 of 120 mg/kg and Group 3 of 160 mg/kg body weight of rat). Nanoparticles were injected intraperitonealy on alternate days for 28 days. On 29th day, rats were sacrificed and brain was isolated from all groups. Accumulation of titanium in the brain was assessed by inductively coupled plasma mass spectrometry (ICP-MS) and its increase in concentration was observed in a dose dependent manner. Cell viability, acetylcholine esterase and glutathione activities were significantly ($P<0.05$) decreased in Group 2 and Group 3 treated groups as compared to control and placebo groups. Histological alterations were also reported in brain exposed to titanium dioxide nanoparticles treated groups. This study revealed that titanium dioxide nanoparticles are neurotoxic as expressed by histological alterations, reduced cell viability, reduced acetylcholine esterase activity and induced oxidative stress by reducing glutathione activity in male Sprague Dawley rats.

Keywords: Cell viability, glutathione, acetylcholine esterase, histological alteration, Ti-Accumulation

INTRODUCTION

Nanotechnology deals with nano structures to reduce not only particle sizes, but also benefit biological sector by increasing cellular efficiency for biomedical research. The important applications of nanotechnology are in therapy, agriculture and industrial sector (Bartlomiejczyk *et al*., 2013; Singh, 2017). Other fields, including communication, electronics and medication cannot deprive the importance of nanotechnology. Outstanding applications of nanotechnology are due to nano structures having a size of about 100 nm or less. Though nanotechnology plays an important role in society by increasing benefits, but its harmful effects cannot be ignored. Reactivity of nanoparticles affects cells, tissues and organs (Jain *et al*., 2018; Weir *et al*., 2012).

Titanium is a transitional metal having white to silver color with a stable oxidation state of +4 (Bakare *et al*., 2016). Titanium is lustrous and corrosion-resistant. Titanium di-oxide in nano form lies in the top five positions of nanoparticles. TiO_2 nanoparticles (NPs) are used in cosmetics, sterilization, paints, toothpaste, food products, air cleaning, sunscreens and waste water treatment plants (Shakeel *et al*., 2018). However, unique properties of TiO_2 NPs raise concerns to the upcoming challenges of human health. From the scientific community, there is a little knowledge regarding the toxicological effects of these nanoparticles as well as safety and ecological harms (Chaturvedi *et al*., 2012).

Physiologically, TiO_2 nanoparticles are inert to humans, but numerous studies on brain reveal its toxicological effects on mice and rats. Brain is highly susceptible to oxidative damage due to high oxygen consumption rate, high gratified peroxidizable unsaturated fatty acids and relative insufficiency of antioxidant enzymes compared with other organs (Oberdorster *et al*., 2009). Nanoparticles may enter the brain and damage its internal structure (Lankveld *et al*., 2010, Yuan *et al*., 2015). Hippocampus of mouse respond to TiO_2 nanoparticles results in oxidative damage, decreased numbers of nissl bodies, increase glial fibrillary acidic protein expression, acetyl cholinesterase activity, glutamate and cause cellular degradation (Song *et al*., 2015). Nano TiO_2 disrupts acetylcholine esterase, enzymes and cause calcium deposition in neurocytes of mice brain. Memory may also be affected (Hong *et al*., 2017, Hong *et al*., 2018).

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The wide applications of TiO_2 nanoparticles and their toxicity in living organisms focused our attentions to explore their adverse effects in male Sprague dawley rats by assessing the cell viability, oxidative stress, acetylcholine esterase activities and histology of brain.

MATERIALS AND METHODS

Chemicals

Titanium dioxide nanoparticle (TiO_2anatase, 30-50nm), (US research), Nitric acid (Merck), Per chloric acid (Merck), Cell viability kit (abcam-112118), Sodium Phosphate (BDH), Triton X (BioLab), Acetylcholine iodide (Sigma), 5,5'-Dithio-Bis 2-Nitrobenzoic Acid (Sigma), Sodium Dodecyl Sulfate (Merck), Trichloroacetic Acid (Sigma), Formaldehyde (BioLab), Ethanol (BioLab), Glacial Acetic Acid (BDH), Benzol (BioLab), Paraffin wax (Merck), Haematoxylin (Sigma) and Eosin (Sigma), and Phenyl methyl sulphonylfloride (Sigma) were used in the study.

Experimental setup and administration

The study involved male Sprague Dawley rats of 200-220g body weight procured from the animal house of the Government College University Faisalabad. Before the commencement of the experiment, the rats were housed in steel cages and acclimatized for two weeks under standard laboratory conditions at room temperature (20-23°C) with 12:12h light: dark cycles and humidity (approximately 5%) after approval from the ethical committee of Government College, University Faisalabad on animal experimentation (approval No.GCUF-115). The rats were distributed randomly into five different groups having 5 rats in each group and designated as control (C) without any treatment, placebo group (S) treated with 0.9% normal saline and three TiO_2 NPs treated groups i.e., G1 of 80mg/kg, G2 of 120mg/kg and G3 of 160mg/kg body weight of rat. Rats were provided with commercial rodent feed, 19% crude protein (Kent) and distilled water ad libitum throughout the study. The rats were treated with the designated doses intraperitoneally for 28 days on alternate day.

Sample collection

At the end of the experimental period, on 29th day animals were fast overnight, anesthetized with ketamine hydrochloride (30mg/kg bw and scarified. The brain was collected, weighed with the help of Sartorius weighing balance (Shimadzu, BL-2200H). Half portion of the brain was stored in plastic bags and stored at -80^{o}C for Ti accumulation analysis, cell viability, acetylcholine esterase and glutathione activities and remaining portion of the brain was separately immersed in fixative sera (formaldehyde 35 ml, glacial acetic acid 10 ml, ethanol 55ml) for further process of histology (by haematoxylin eosin staining method).

Ti accumulation (ICP-MS)

A portion of the brain (100mg) was taken and digested in digesting solution (3ml nitric acid and 1.5ml per chloric acid) in heating digester (VelpScientifica- D-6). In start the color of fumes was yellow. Digestion process was continued till the fumes become colorless and solution remain 1ml. Distilled water was added in digested solution, made it up to 10 ml and filtered. The sample was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Perkinelmer) (Shakeel *et al*., 2016, Vasantharaja and Ramalinggam, 2018).

Cell viability

A 0.2mg sample of the brain was taken and homogenized thoroughly in a homogenized solution (100mMTris, PH 7.4, 150mM Nacl, 1mM EGTA, 1mM EDTA, 1%Triton X, 0.5% Sodium deoxycholate, 1mMPMSF) for 10 mint in bullet blender (Advance-BBY5E-CE). Supernatant was taken in eppendorf tube (1.5ml). Tags the well with sample name and control in 96 well plates. 100µl sample was poured with micropipette in sample tags wells of 96 well plate except control tag well. 20 µl assay solution was poured with micropipette in each well. Mix the reagent by shaking the plate gently for 30 minutes. The plate containing reagent was incubated for 2hr at 37ºC in 5% CO_2 incubator (MCO-18AIC). After 2 hrs the sample containing plate was removed from CO_2 incubator and rinsed from wells. Absorbance was taken at ration of 570 nm and 605 nm on the micro plate reader (BioBase-EL10A) (Yu *et al*., 2013). Percentage of cell viability for samples and controls was calculated by following formula:

$$\%\,\text{Cell viability} = 100 \times \frac{(R_{sample} - R_o)}{(R_{ctrl} - R_o)}$$

R sample is the absorbance ratio of OD570/OD605 in the presence of the test compound.
Rctrl is the absorbance ratio of OD570/OD605 in the absence of the test compound (vehicle control).
Ro is the averaged background (non-cell control) absorbance ratio of OD570/OD605

Acetylcholine esterase activity

Spectrophotometric method was used to calculate the acetylcholine esterase level in the brain. For the discharge of membrane bound enzyme a portion of the brain with homogenize solution (100mMTris, PH 7.4, 150mMNaCl, 1mM EGTA, 1mM EDTA, 1% Triton X, 0.5% Sodium deoxycholate, 1mMPMSF) was added in falcon tube of bullet blender and homogenized it for 15mint. After that 0.1mM acetylcholine iodide solution was added in each of the test tubes. The reaction mixture was incubated for 15 mint with constant shaking at 37^{o}C after 15 minutes the test tube were removed from the incubator. In the next step, both DTNB and SDS were mixed in a flask (0.04 and 44%, respectively) 0.5ml solution of DTNB and SDS was put into each test tube. The absorbance of each test

tube was noted at 412 nm at spectrophotometer (Hitachi, U-2800) (Grissa *et al*., 2016).

Estimation of glutathione activity

Homogenate (0.05 ml) of treated and untreated brain was taken in a test tube with 1.5 ml of distilled water. Each test tube was put in the shaker to shake the sample in order to mix it well. After that, 10 gram of TCA was mixed with 100 ml of distilled water. In the next step, 2ml of 10% TCA solution was added in each of the test tube and test tubes were centrifuged at 2000 rpm for 15 minutes, after that 1ml of supernatant was taken from 4 ml solution. Phosphate buffer was added in the sample to maintain 7.4 pH of the solution. In last 0.1ml (0.04% of DTNB solution) was added to phosphate buffer and absorbance was noted at 412nm (Gawryluk *et al*., 2011).

Histology

A small portion of the brain was fixed in sera (formaldehyde: 30 ml, ethanol: 60 ml & glacial acetic acid: 10ml) after fixation dehydration was done by using different grades of ethanol (Grade 1, 70% ethanol for 12 hrs, Grade 2, 80% ethanol for 2hrs, Grade 3, 90% ethanol for 2hrs and Grade 4, 100% ethanol for 2hrs at 25ºC). After dehydration, brain tissue was dipped in cedar wood oil until tissues become transparent at 25ºC. Embedding process was done after dehydration in different steps (Step 1:Benzol 1 for 15 min, Step 2:Benzol 2 for 15 min, Step 3:Benzol 1+ paraplast 1, Step 4:Paraplast for 15hrs at 50ºC, Step 5:Paraplast 2 for 15 hrs at 50ºC & Step 6. Paraplast 3 for 15hrs at 50ºC). After embedding tissues were removed from melted wax, embedded tissue was placed in plastic moulds and melted wax was poured allowed to harden. After that block was mounted on microtome (Histo-line MR 2258) and 5µm thin section of tissue was cut. To observe the histological alteration in the Brain, sections were finally stained in haematoxylin and eosin (Vasantharaja and Ramalinggam 2018), observed under the microscope (Nikon E200POL) and photographs were taken using a digital camera fitted with the microscope.

STATISTICAL ANALYSIS

The data were analyzed by one-way Analysis of Variance in Minitab17 software. The treatment means were compared with the post hoc Tukey's test. The differences between means were considered significant if $p<0.05$.

RESULTS

Ti-Accumulation by ICP-MS

Fig. 1 shows the Titanium dioxide concentration in different treated groups and control group. There was no significant difference in the Ti concentration of control and placebo group while highly significant differences were observed among control and G2 and G3 groups ($p<0.05$). Maximum titanium was accumulated in the brain was 5.7µg/kg in higher dose treated group (160mg/kg) as compared to control group.

Cell viability

The percentage cell viability of the brain treated with titanium dioxide nanoparticles (TiO_2-NPs) in male Sprague Dawley rats was decreased with the increased dose of TiO_2 NPs (fig. 2). Significant differences in the percentage cell viability were found in the groups exposed to 120 and 160 mg/kg as compared to control and placebo group ($p<0.05$).

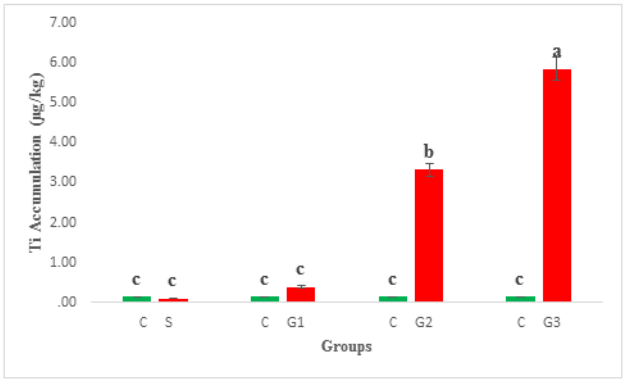


Fig. 1: Ti accumulation (µg/kg) in brain of male Sprague Dawley Rats in control and treated groups. (Bars indicating similar letters are not significantly different at $p< 0.05$).

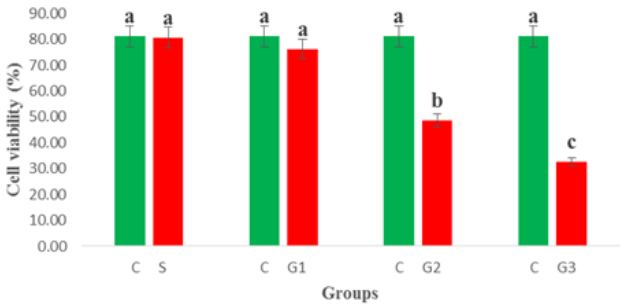


Fig. 2: Cell Viability (%) in brain of male Sprague Dawley Rats in control and treated groups (bars indicating similar letters are not significantly different at $p<0.05$).

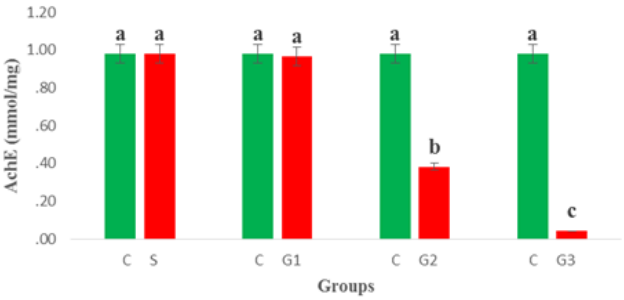


Fig. 3: Acetycholine esterase activity (mmol/mg) in brain of male Sprague Dawley Rats in control and treated

groups (bars indicating similar letters are not significantly different (P<0.05).

Acetylcholine esterase activity

Interaction between acetyl choline estrase (AChE) and TiO_2 NPs in brain of rats was assessed by measuring the concentration of acetycholine esterase. Substantial decrease in the level of acetylcholine esterase was observed at 120 and 160 mg/kg body weight as shown in (fig. 3).

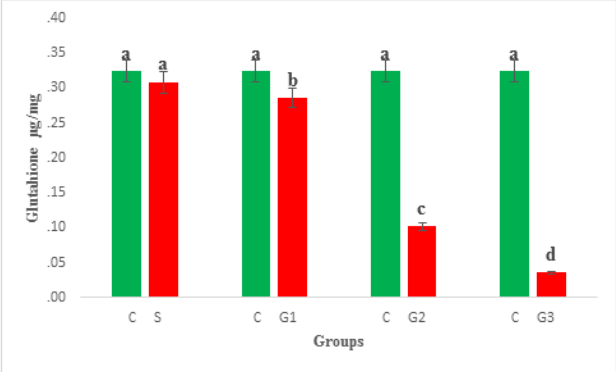


Fig. 4: Glutathione (ug/mg) in Brain of male Sprague Dawley Rats in control and treated groups (bars indicating similar letters are not significantly different (p<0.05).

Glutathione

The effect of TiO_2 NPs on glutathione activity in the brain of rats was significantly decreased with the increase of TiO_2 NPs concentration. Fig. 4 shows a significant reduction in glutathione activity in G1 and G2 groups (120 and 160 mg/kg) as compared to G1, control and placebo groups (P<0.05).

Histological observations

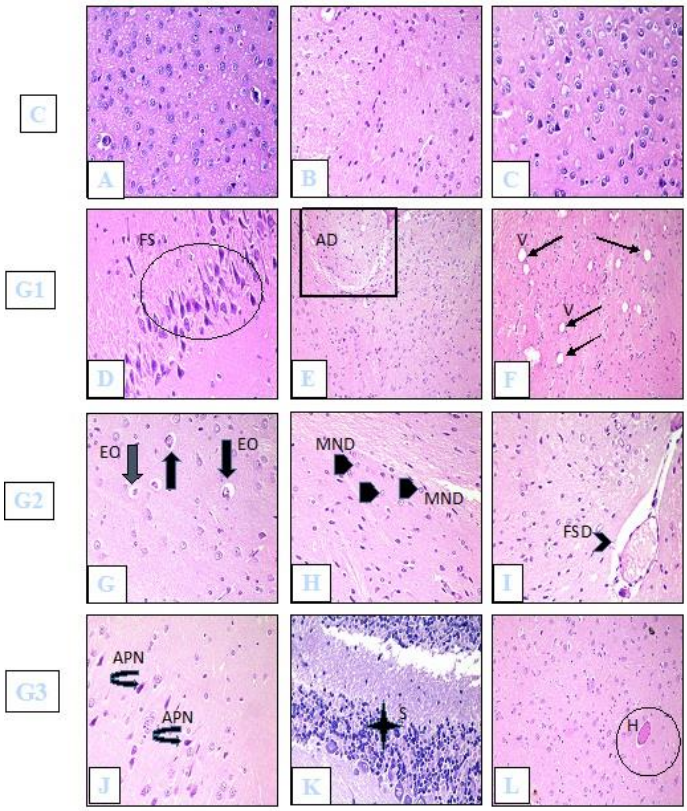
Fig. 5 represents the photomicrograph of brain tissues of control and treated groups. Histology of control group showed normal neuron cells (A-C). TiO_2 NPs treated group of 80 mg/kg BW showed slightly damage in neuron cells like neurons cleaved into filamentous shape, Axonal dystrophy (triangle), Vaculation and calcification (thin arrow) (D-F). Exposure to TiO_2 NPs of 120 mg/kg, showed moderate alterations, i.e., external edema (thick arrow), motor neuron disease (pentagon shape) and flask shaped depression (G-I). TiO_2 NPs treated group of 160 mg/kg showed high alterations like asymmetrical pyramidal nucleus, Spongiocyte proliferation and hemorrhage in local area (J-L).Thus, these results showed that exposure to higher doses of TiO_2 NPs caused severe brain injury. This may be ascribed to ROS generation, which was confirmed by further assays of ROS production in the brain.

DISCUSSION

The current study was carried out to assess the titanium dioxide nanoparticles induced brain toxicity in male sprague dawley rats by various markers. The Nanoparticles primarily affect the mammalian brain, because they have the capacity to cross the blood brain barrier, translocate into central nervous system of exposed animal and caused perilous neurotoxicity (Ze *et al*., 2014). In the present study, bioaccumulation of Ti, cell viability, acetylcholine esterase, glutathione and histological alteration in brain were studied in male Sprague Dawley rats that were intraperitonealy exposed with anatase TiO_2 NPs (30-50nm particle size) of 80 or 120 or 160mg/kg body weight for 28days on alternate day. Results of present study revealed that bioaccumulation of Ti and histological alteration were increased in higher doses of toxicant. Similarly, cell viability, acetylcholine esterase and glutathione activity in brain were significantly decreased in higher doses as compared to control and placebo group. The findings of current study are in accordance with the study of Geraets *et al.* (2014), who studied the tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles (NM101, NM102, NM103 & NM104) in rats for 90 days. Vasantharaja and Ramalingam (2018) studied the neurotoxicity by orally administrated rutile TiO_2 NPs (80nm of 50mg/kg & 100mg/kg on 250-260g weight rats for 14 days and Devoy *et al*. (2016) also used TiO_2 NPs in the form of mixture of anatase and rutile (81: 19) with respect to bioaccumulation of Ti in brain and these studies were in line with the present study and revealed that Ti accumulate in CNS and caused significant increase in Ti content in brain.

Work of Ma *et al*. (2010) supported the present study as groups were treated with 5 or 10 or 50 or 100 or150 mg/kg and two doses of TiO_2 NPs i.e., 80 or 120mg/kg used in our study comes in range of doses used by Ma *et al*. (2010) study. The experiment animal and exposure period was CD-1mice and 14 days, respectively while, we used spargue dawley rats and exposure period was 28 day. In spite of the exposure time and experimental rat breed difference, Ma *et al*. (2010) results concluded that exposure of TiO_2 NPs for 14 days showed significant decrease in AChE with increase of dose in the brain and these result are in accordance with our results. Results of Ahmad and Tarannum (2009) and Grissa *et al*. (2016) also supported our study, they also exposed male Sprague Dawley rats with same toxicant (TiO_2 NPs with particle size of 21nm) to assess the level of AChE in brain and concluded that the level of AChE decreases with the increase of dose.

The blood barrier in brain is the membrane which has an important role in acetylcholine esterase activity. TiO_2 NPs not only cross the blood brain barrier rather it reaches to CNS, accumulate and affect the function of the brain. The glial cells are very important for the repair of neurons. TiO_2 NPs not only damage the glial cells but also leads to axonal dystrophy. In the present study as compared to



G1 group (80 mg/kg BW) (D-F) indicates slightly damage in neuron cells. D) Shows neurons locally cleavage into filamentous shapes (FS=Oval), E) shows Axonal dystrophy (AD=triangle), F) shows Vaculation and calcification (thin arrow).
G2 group (120 mg/kg) (G-I) indicates moderate alterations in brain tissues. G) indicates external edema (EO=thick arrow), H) Motor neuron disease (MND=pentagon shape). I) represents flask shaped depression (FSD=Chevron).
G3 group (160 mg/kg) (J-L), indicates severe alterations in brain tissues. J) curved arrow indicates an asymmetrical pyramidal nucleus (APN) found in local area, K) star designate Spongiocyte proliferation (S), L) circle shows Hemorrhage (H) in local area.

Fig. 5: Photomicrograph (H&E; X 400) of the brain tissues in male Sprague Dawley rats: Control (C) group (without any treatment) showing normal neuron cell (A-C).

control (0.00mg/kg BW), low dose treated groups (80mg/kg bw) showed slight histological changes in brain tissue i.e., neurons locally cleavage into filamentous shapes, axonal dysthrophy and vaculation while external edema, motor neuron disease and flask shaped depression were seen in 120mg/kg BW treated groups rats. Furthermore, asymmetrical pyramidal nucleus, spongiocyte proliferation and hemorrhage in brain tissue were observed at 160mg/kg body weight. Ze *et al*. (2014) conducted an experiment in which mice were exposed to TiO_2 NPs of 2.5, 5, or 10mg/kg body weight and found denatured glial cells, necrosis, cell volume reduction and nuclear abnormality were observed, these histological changes are correlated and supported with our observations. Similarly, Mohamed Hussien (2016) reported that rats administrated with TiO_2 NPs @ (500mg/kg BW) showed intracellular and extra cellular edema in brain tissues, which is parallel to our findings. Previous studies (Ma *et al.*, 2010; Ze *et al*., 2013; Jia *et al*., 2017, Vasantharaja and Ramalingam, 2018) are also consistent with our study.

In spite of differences in nanoparticles form (Anatase or Rutile), particles size, administration method, exposure period and sampling time used in former studies, results of these studies correlates with results of present study. At the end, it was concluded that TiO_2 NPs can cause neurotoxicity that may leads to cause death of animal.

CONCLUSION

In conclusion, our study indicated that TiO_2 NPs are very toxic to rats at medium and high doses used in this study, hence anataseTiO_2 NPs induced histological alterations and reduced acetylcholine esterase concentration in the brain of male Sprague Dawley Rats. This study implies that the TiO_2NPs exposure could cause brain toxicity and risk to the human health and also the environment. Hence, there is need for further research to determine the TiO_2NPs mechanism of action on human population.

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REFERENCES

Ahmad N and Tarannum S (2009). Acetylcholinesterase activity in the brain of alloxan diabetic albino rats: Presence of an inhibitor of this enzyme activity in the cerebral extract. *Int. J. Diab. Dev. C.*, **29**(4): 174-180.

Bakare AA, Udoakang AJ, Anifowoshe AT, Fadoju OM, Ogunsuyi OI, Alabi OA, Alimba CG and Oyeyemi IT (2016). Genotoxicity of titanium dioxide nanoparticles using the mouse bone marrow micronucleus and sperm morphology assays. *J. Poll. Eff. Cont*., **4**(2): 1-7.

Bartlomiejczyk T, Lankoff A, Kruszewski M and Szumiel I (2013). Silver nanoparticles allies or adversaries. *Ann. Agric. Environ. Med.*, **20**(1): 48-54.

Chaturvedi S, Dave PN and Shah NK (2012). Applications of nano-catalyst in new era. *J. Saudi. Chem. Soc*., **16**(3): 307-325.

Devoy J, Brun E, Cosnefroy A, Disdier C, Melczer M, Antoine G, Chalansonnet M and Mabondzo A (2016). Mineralization of TiO_2 nanoparticles for the determination of titanium in rat tissues. *J. Ana. Chem*., **71**(4): 418-425.

Gawryluk JW, Wang JF, Andreazza AC, Shao L and Young LT (2011). Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *Int. J. Neuropsychopharmacol*., **14**(1): 123-130.

Geraets L, Oomen AG, Krystek P, Jacobsen NR, Wallin H, Laurentie M, Henny W, Verharen HW, Brandon EF and Jong H (2014). Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. *Part. Fibre. Toxicol*., **11**(3): 30-40.

Grissa I, Guezguez S, Ezzi L, Chakroun S, Sallem A, Kerkeni E, Elghou J, El Mir L, Mehdi M and Haouas Z (2016). The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain. *Env. Sci. Poll. Res.*, **23**(20): 20205-20213.

Hong F, Zhou Y, Ji J, Zhuang J, Sheng L and Wang L (2018). Nano-TiO2 inhibits development of the central nervous system and its mechanism in offspring mice. *J. Agric. Food Chem*., **66**(4): 11767-11774.

Hong FS, Yu XH, Wu N and Zhang YQ (2017). Progress of *in vivo* studies on the systemic toxicities induced by titanium dioxide nanoparticles. *Toxicol Res*., **6**(2): 115-133.

Jain A, Ranjan S, Dasgupta N and Ramalingam C (2018). Nanomaterials in food and agriculture: An overview on their safety concerns and regulatory issues. *Crit. Rev. Food Sci. Nutri.*, **58**(2): 297-317.

Jia X, Wang S, Zhou L and Sun L (2017). The potential liver, brain, and embryo toxicity of titanium dioxide nanoparticles on mice. *Nanoscale. Res. Lett*., **12**(1): 478-480.

Lankveld DP, Oomen AG, Krystek P, Neigh A, Troost–de Jong A, Noorlander CW, Van Eijkeren JCH, Geertsma RE and Jong W (2010). The kinetics of the tissue distribution of silver nanoparticles of different sizes. *Biomat*., **31**(32): 8350-8361.

Ma L, Liu J, Li N, Wang J, Duan Y, Yan J, Liu H, Wang H and Hong F (2010). Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. *Biomat*., **31**(1): 99-105.

Mohamed HR and Hussien NA (2016). Genotoxicity studies of titanium dioxide nanoparticles (TiO_2NPs) in the brain of mice. *Scientifica*., **20**(2): 1-7.

Oberdörster G, Elder A and Rinderknecht A (2009). Nanoparticles and the brain: Cause for concern. *J. Nanosci. Nanotechnol*., **9**(8): 4996-5007.

Shakeel M, Jabeen F, Qureshi NA and Fakhr-e-Alam M (2016). Toxic effects of titanium dioxide nanoparticles and titanium dioxide bulk salt in the liver and blood of male Sprague-Dawley rats assessed by different assays. *Biol. Trace Elem. Res*., **173**(2): 405-426.

Singh N (2017). Nanotechnology innovations, industrial applications and patents. *Envir. Chem. Lett*., **15**(2): 185-191.

Song B, Liu J, Feng X, Wei L and Shao L (2015). A review on potential neurotoxicity of titanium dioxide nanoparticles. *Nanoscale. Res. Lett*., **10**(1): 342-350.

Vasantharaja D and Ramalinggam V (2018). Neurotoxic effect of Titanium dioxide nanoparticles: Biochemical and pathological approach in male Wistar rats. *Int. J. App. Pharm*., **10**(4): 74-81.

Weir A, Westerhoff P, Fabricius L, Hristovski K and Von N (2012). Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol*., **46**(4): 2242-2250.

Yu Y, Zhang Y, Martin JA and Ozbolat IT (2013). Evaluation of cell viability and functionality in vessel-like bioprintable cell-laden tubular channels. *J. Biomech. Eng*., **135**(9): 910-914.

Yuan ZY, Hu YL and Gao JQ (2015). Brain localization and neurotoxicity evaluation of polysorbate 80-modified chitosan nanoparticles in rats. *PloSone*., **10**(8): 134-137.

Ze Y, Sheng L, Zhao X, Hong J, Ze X, Yu X, Pan X, Lin A, Zhao Y, Zhang C, Zhou Q, Wang L and Zhou Q (2014). TiO_2 nanoparticles induced hippocampal neuro-inflammation in mice. *PloSone*, **9**(3): 922-930.

Ze Y, Zheng L, Zhao X, Gui S, Sang X, Su J, Guan N, Zhu L, Sheng L, Hu R, Cheng J, Cheng Z, Sun Q, Wang L and Cheng J (2013). Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice. *Chemosphere*, **92**(9):1183-1189.