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Acute neuro-biochemical changes induced by nitrogen-tungsten co-doped titanium dioxide nanoparticles

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Abstract

Nitrogen-tungsten co-doped titanium dioxide nanoparticles (W-N-doped TiO_2 NPs) are employed for the photocatalytic degradation of environmental pollutants. However, the potential impact of these nanoparticles on the central nervous system remains a subject of concern. This study aimed to evaluate the effects of W-N-doped TiO_2 NPs on neurophysiological and biochemical parameters of healthy rat brains, including behavioral monitoring, electroencephalogram analysis, and oxidative stress markers quantification. Intraperitoneal administration of W-N-doped TiO_2 NPs to rats revealed abnormal brain electrical activity and an altered sense of balance in the treated rats. The ability of W-N-doped TiO_2 NPs to cross the blood–brain barrier and accumulate in the brain leads to oxidative stress damage, supported by the elevated levels of reactive oxygen species (ROS), nitrite concentration, and malondialdehyde levels. Additionally, exposure to W-N-doped TiO_2 NPs significantly reduced the antioxidant enzyme levels, such as catalase and superoxide dismutase, impacting a significant decrease in dopamine and acetylcholinesterase within the rat neural tissue. Furthermore, the inflammatory biomarker tumor necrosis factor-alpha and 8-hydroxy 2-deoxyguanosine significantly increased in response to W-N-doped TiO_2 NPs. The findings revealed the adverse effects of W-N-doped TiO_2 NPs on the electrical activity of rat brains and the altered concentration of various neuro-biomarkers, highlighting their potential neurotoxicity.

Keywords Doped nanoparticles, Titanium dioxide, Rat brain, Electroencephalogram, Oxidative stress

Introduction

Doping has emerged as a neoteric modality to enhance the electronic, physiochemical, biological, and optical properties of oxides and semiconductor-based nanoparticles (NPs). It is an intentional physiochemical process of inserting foreign substances into the empty lattice of another pure material to alter its characteristics [1]. Several rare earth, metallic or transition, and non-metallic elements are widely employed as a dopant that influences the structural, electrical, and chemical nature of the NPs [2]. Sol–gel, pulsed laser ablation in liquid (PLAL), sonochemical, microwave irradiation, and green synthesis are the most common methods for doping NPs. Doping has been proven to enhance the antibacterial, antifungal,

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anti-cancer, photocatalytic, oxidative, and electrolytic properties of NPs [3].

Recently, titanium oxide nanoparticles (TiO_2 NPs) have been declared the second most widely used commercial NPs globally in the food, cosmetics, paints, biomaterials, and paper industries [4]. Likewise, due to their excellent photocatalytic, high thermal stability, biocompatibility, cost-effectiveness, and ultraviolet (UV) absorption properties, TiO_2 NPs are now employed in wastewater management for organic and inorganic pollutants [5]. However, being a semiconductor, TiO_2 NPs can only utilize light at a wavelength less than 400 nm (UV light), limiting their pollutant degradation efficacy. TiO_2 NPs cannot use the visible light spectra for the photodegradation of the pollutants [6]. Hence, doping TiO_2 NPs with nitrogen was introduced by replacing the oxygen in the lattice of TiO_2 with nitrogen, and a better photocatalytic activity was achieved than the application of pure TiO_2 NPs [7]. The bandgap construction of nitrogen-doped TiO_2 NPs is still insufficient to take full advantage of visible light. Co-doping of TiO_2 NPs with tungsten, which decreases the electron recombination process, has been utilized and found to enhance the photodegradation rate to counter this limitation. Extensive studies have evaluated and characterized the Tungsten-Nitrogen co-doped Titanium dioxide (W-N-doped TiO_2) NPs [8]. However, the biological impact of such co-doped TiO_2 NPs has yet to be studied.

W-N-doped TiO_2 NPs are semiconductors and can be a potential source of neural toxicity by interfering with the electrical conduction in the brain tissue. Yongbin et al. investigated the in vitro and in vivo toxicity of semiconductor NPs, which induced cytotoxicity in mammalian cell culture and exhibited adverse locomotor activity in rat models [9]. Similarly, Maria et al. studied the impact of rod-shaped semiconductor nanoparticles on *cnidaria Hydra Vulgaris*. It has been found that semiconductor NPs were associated with eliciting neuronal activity by ion channel activation in neurons and neuronal stimulation [10]. Yan et al. applied semiconductor NPs for controlled modulation of the ion channels in neurons by the photothermal conversion and proved that thermosensitive ion channels could activate the calcium channels for the intracellular influx [11]. However, this mechanism, if uncontrolled, can induce a negative impact on neuronal activity. Incorporating doping agents in TiO_2 NPs may increase their efficiency but can also provoke negative alternations at cellular and tissue levels, leading to a toxicological risk for humans. [12]

Therefore, the industrial and biomedical advantages and the toxicological risks of applying W-N-doped TiO_2 NPs are explicitly required to be investigated. Hence, this study aimed to examine the impact of W-N-doped TiO_2 NPs on rat neural tissue. The NPs were synthesized, characterized, and inoculated to rats for assessing the acute in vivo response. Electroencephalography (EEG) was carried out, and behavioral analysis was performed while evaluating oxidative stress in the brain tissue. Additionally, several inflammatory and non-inflammatory biomarkers were measured (Fig. 1).

Materials and methods

Preparation of W-N-doped TiO_2 NPs and characterization

TiO_2 NPs were prepared by the sol–gel method. In a typical procedure, 13.3 ml titanium isopropoxide (TTIP) was added dropwise in 100 ml propanol and magnetically stirred at room temperature for 20 min. After that, 4 ml acetic acid was added as a catalyst into the solution and heated at 70 °C followed by ethylene glycol as coolant. The sediments of TiO_2 were collected and washed a few times with DI water and dried at 100 °C. Finally, the TiO_2 NPs were obtained at 500 °C for 2 h after calcination.

At the same time, nitrogen and tungsten dopant solutions were prepared to get doped W–N doped TiO_2 NPs. Solution A was prepared for nitrogen doping by mixing urea in DI water. Its pH was adjusted to 2 using HNO_3 as-synthesized TiO_2 NPs were added into solution A. Solution B was prepared for tungsten doping by mixing WO3 in a mixture of DI water and propanol, then by adding a few drops of HCl and sonicated for 10 min. The solutions A and B were mixed and stirred for 1 h and dried in an oven at 100 °C overnight. Finally, the dried powder was calcinated at 450 °C for 4 h to obtain purified W-N-doped TiO_2 NPs.

The prepared nanoparticles were then characterized by Field Emission Scanning Electron Microscopy (FESEM), X-ray diffraction (XRD) analysis, Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transforms Infrared (FTIR) spectroscopy, and Ultraviolet–Visible (UV–vis) spectroscopy.

Animals and W-N-doped TiO_2 NPs administration

The animals used in this experimental study were nine male adult Wistar rats (200–240 g) obtained from Cronex Labs, Seoul, South Korea, and utilized following the Jeju National University Institutional Animal Care and Use Committee (IACUC). All the procedures and animal testing were carried out by following the ethical guidelines for animal studies [13]. The rats were housed in the animal house at a temperature of 22 ± 2 °C, relative humidity of 60–70%, a light–dark cycle of 12:12 h, and standard animal feed. After one week of housing, the animals were randomly divided into three groups (each group contained 3 rats: one control group (C) and two treatment groups received a subsequent dose of NPs in 24 h (T1 = 100 mg/kg of W-N-doped TiO_2 NPs, T2 = 200 mg/

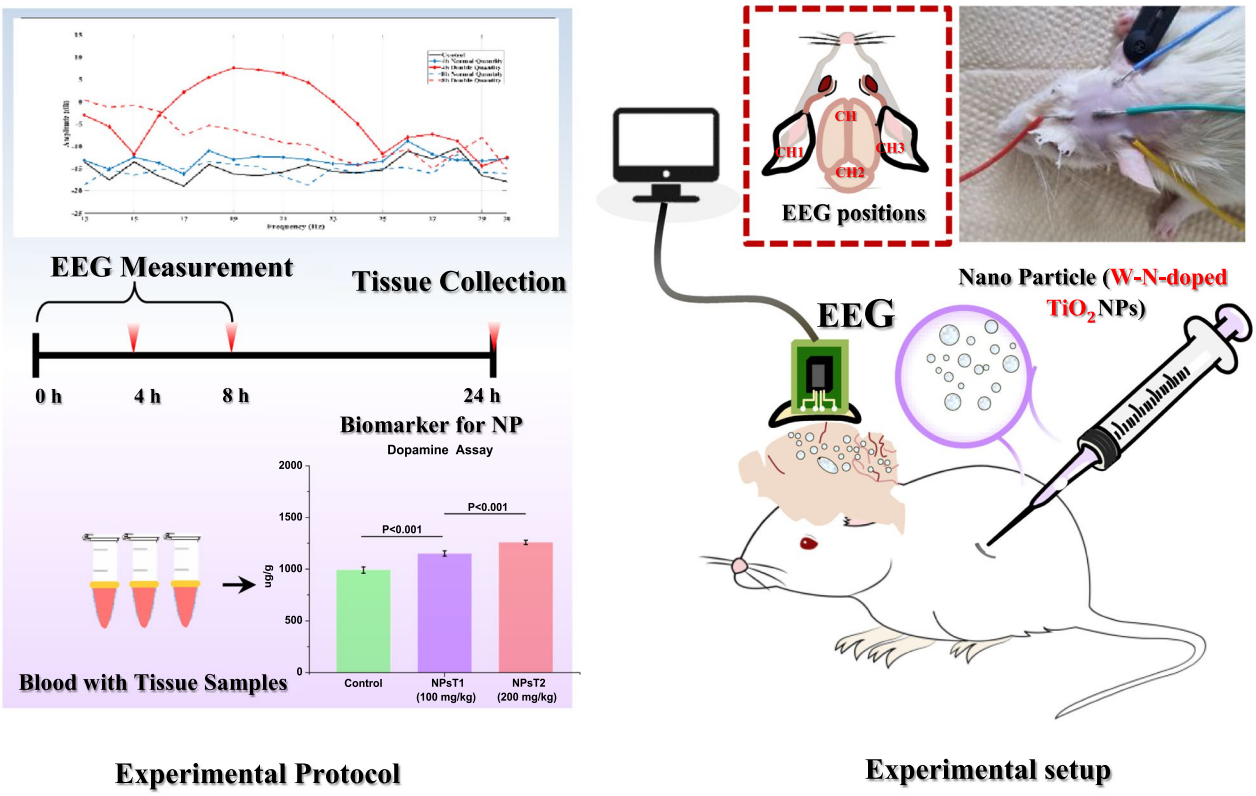

Fig. 1 The graphical illustration of the study showing the experimental protocol; rat inoculation of W-N-doped TiO_2 NPs via intraperitoneal injection (right) and subsequent exploratory testing (left)

kg of W-N-doped TiO_2 NPs). The dose selection of W-N-doped TiO_2 NPs was based on the previous literature on TiO_2 NPs' biological characterization in rats [14–16]. The NPs were dispersed in the normal saline and sonicated for 30 min to get a homogenous solution. The vortex stirring was also applied before each peritoneal injection to maintain the homogeneity of the W- N-doped TiO_2 NPs solution.

Rat behavioral analysis

A beam balance test evaluated the effect of W-N-doped TiO2 NPs on the behavior of the rats [17]. The beam walking test assessed the rat's motor coordination and balance assessment. The animal was made to walk on a suspended beam between a starting point and a cage at a particular height (50 cm). A total of 9 rats were utilized for this purpose. The data was collected for the control group before the injection of nanoparticles, and the result of performing the beam test 1 h after the injection of nanoparticles is shown in Fig. 5. The experiment was repeated 3 times for each case, and the experimental result was evaluated as a score. The beam balance test commences with a box positioned at each extremity of the beam. The goal is to assess whether the rat successfully reaches the box from the starting point to the ending point. The maximum achievable score in this evaluation is 5 points. The scoring criteria involve assigning 3 points for crossing the beam at 50 cm, 1 point for entering the box, and 1 point for accomplishing the task within 10 s.

EEG measurement setup

The electrodes of the EEG measurement system were placed on the appropriate anatomical structures considering the size of the animal skull, as described previously [18]. A commercial signal acquisition system, OpenBCI Ganglion board, was employed for recording EEG channels (200 samples per second), and data were collected in a computer via a Bluetooth device. The reference data for EEG was measured for 10 min after giving the general anesthesia to the animals. Each rat was given different W- N-doped TiO_2 NPs doses and EEG data were collected for 3 min at specific intervals of 4 h and 8 h. EEG signals were obtained for 3 rats in each group. The beta band, associated with consciousness,

brain activity, and motor behavior, was selected among the EEG frequency bands for analysis. The power spectral density (PSD) in the 12–30 Hz frequency band was examined before and after the injection of W-N-doped TiO_2 NPs.

Tissue sampling, oxidative stress assessment, neurotransmitter, and inflammatory biomarkers estimation

The brains of the animals were quickly removed from the skulls by excisional biopsy after general anesthesia and cervical decapitation. The whole brains were rinsed promptly with ice-cold phosphate buffer saline (PBS), and oxidative stress-related biomarkers were estimated by preparing the brain tissue in 50 mM Tris–HCl and 300 mM sucrose, as described previously [19]. The concentration of the tissue lysate protein was quantified using Pierce BCA Protein Assay Kit (Cat# 23227, ThermoFisher, USA). DCFDA-Cellular ROS Assay Kit (Cat# ab113852, abcam, USA), Nitric Oxide Assay Kit (Cat# ab65328, abcam, USA), Superoxide Dismutase (SOD) Activity Assay Kit (Cat#ab65354, abcam, USA), Catalase (CAT) Activity Assay Kit (Cat# ab83464, abcam, USA), Lipid Peroxidation or malondialdehyde (MDA) Assay Kit (Cat# ab233471, abcam, USA), Dopamine (DA) Assay Kit (Cat# ab285238, abcam, USA), Glutathione (GSH) Assay Kit (Cat# ab239709, abcam, USA) and Acetylcholinesterase Assay Kit (Cat #ab138871, abcam, USA) were utilized for estimating the biomarkers by following the manufacturer's instructions. At the same time, TNF- α Kit (Cat#ab236712, abcam, USA) and 8-hydroxy 2- deoxyguanosine (8-OHDG) ELISA Kit was used for quantifying the inflammatory biomarkers.

Statistical analysis

The data were presented as mean ± SD and analyzed using one-way ANOVA; simultaneously, the statistical significance was set at $P < 0.001$.

Results and discussion

Physiochemical characterization of the W-N-doped TiO_2 NPs

The morphology of the W-N-doped TiO_2 NPs was analyzed by taking field emission scanning electron microscopy (FESEM) images, as shown in Fig. 2, taken at a different resolution. These images represent that the size and shape of W-N-doped TiO_2 NPs are homogenous, although some large chunks can be seen due to agglomeration. These images also prove the efficacy of the sol–gel method adopted in this research works to synthesize doped nanoparticles.

The crystal structure and phase analysis were performed using a powder X-ray diffractometer. Figure 3a represents the X-ray diffraction (XRD) patterns demonstrating the characteristic atomic planes at 2θ for W-N-doped TiO_2 NPs successively 25.2° (101), 33.28° (103), 34.14° (004), 37.8° (112), 48.06° (200), 54.01° (105), 54.96° (211), 62.74° (204), 68.82° (116), 70.28° (220), and 75.08° (215). These peaks with their corresponding planes show the anatase crystalline phase of W- N-doped TiO_2 NPs, and no other impurities are found in the sample. These results are in good agreement with our previously reported results [20].

The energy dispersive X-ray (EDX) spectroscopic analysis confirmed the doping of nitrogen and tungsten into the crystal matrix of TiO_2. Figure 3 (b) shows the EDX spectra of W-N-doped TiO_2 NPs, indicating the presence of elements like titanium (Ti), oxygen (O), nitrogen (N), and tungsten (W), which confirms the doping of N and W into the matrix of TiO_2 and the purity of the sample.

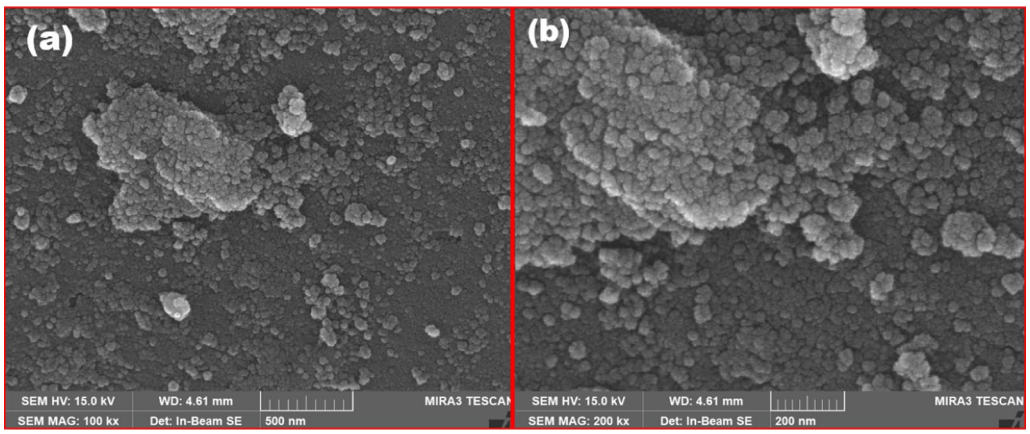

Fig. 2 **a**, **b:** FESEM images taken at different resolutions showing homogeneity in size and shape of as-synthesized W-N-doped TiO_2 NPs.

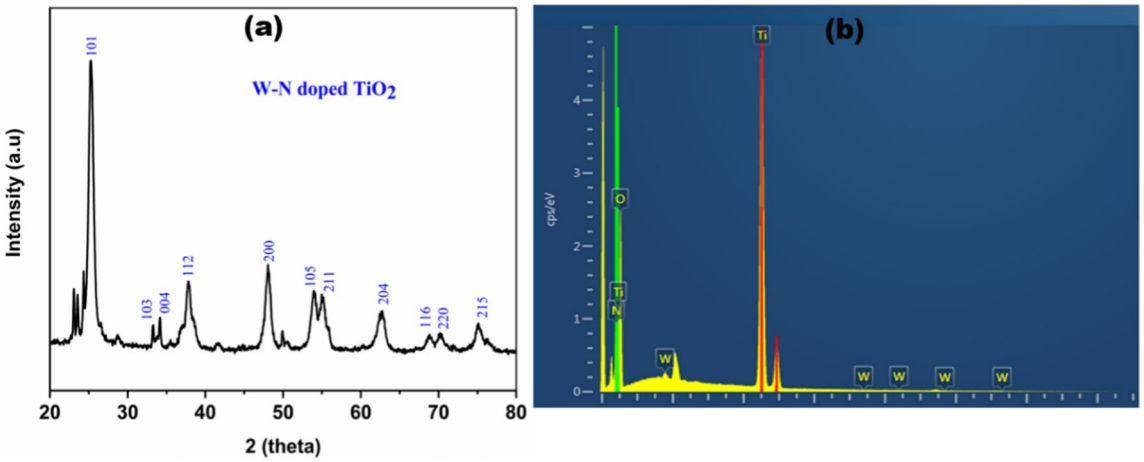

Fig. 3 **a** XRD spectra of W-N doped TiO_2 NP showing the anatase phase of TiO_2 with their characteristic atomic planes, **b** EDX spectra showing the presence of Ti, O, W, and N elements into the matric of TiO_2

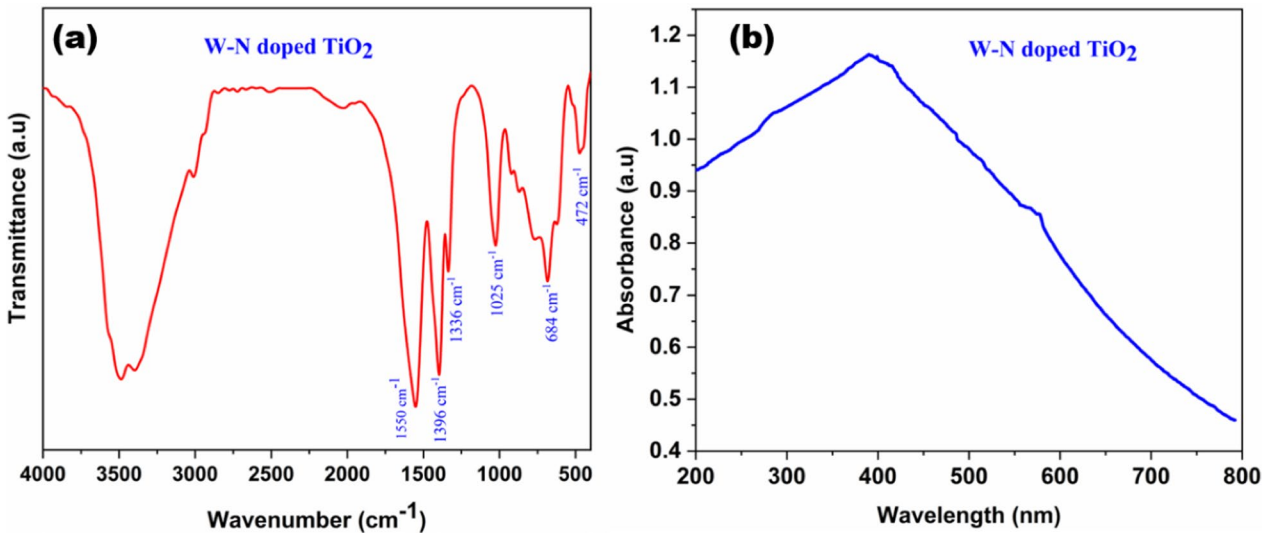

Fig. 4 **a** FTIR spectra of W-N-doped TiO_2 NPs exhibiting the characteristic peaks of corresponding bond vibrations, **b** UV/Vis spectra show the maximum absorbance peak in the visible range around 400 nm

Figure 4a shows the Fourier transform infrared (FTIR) analysis to evaluate the chemical composition of W-N-doped TiO_2 NPs. The broad peak in the 3400–3500 cm^{-1} indicates the presence of moisture in the sample, which exhibited an O–H bond. The characteristic peak at 1550 cm^{-1} showed the N–H bond vibration and indicated the presence of nitrogen. The 1000–1400 cm^{-1} peaks correspond to the absorbed molecular oxygen in the sample. The peak around 472 cm^{-1} usually demonstrates the Ti–O bond, but its intensity also indicates the presence of tungsten doping [21].

Figure 4b depicts the Ultraviolet–visible (UV/Vis) spectra of W-N-doped TiO_2 NPs measured in the range of UV and visible (200–800 nm). The maximum absorbance peak was obtained around 400 nm showing that W-N-doped TiO_2 NPs can interact with the light in the visible range. It also confirms the doping of impurities into the matrix of TiO_2, which causes the shifting of absorbance peak towards a longer wavelength, while undoped TiO_2 shows absorbance in the UV range [22].

EEG measurement and effect of W-N-doped TiO_2 NPs on the neural activity of rat

EEG offers a non-invasive procedure for acquiring brain electrical signals [23–25]. TiO_2 is a semiconductor that can potentially interfere with brain electrical signals

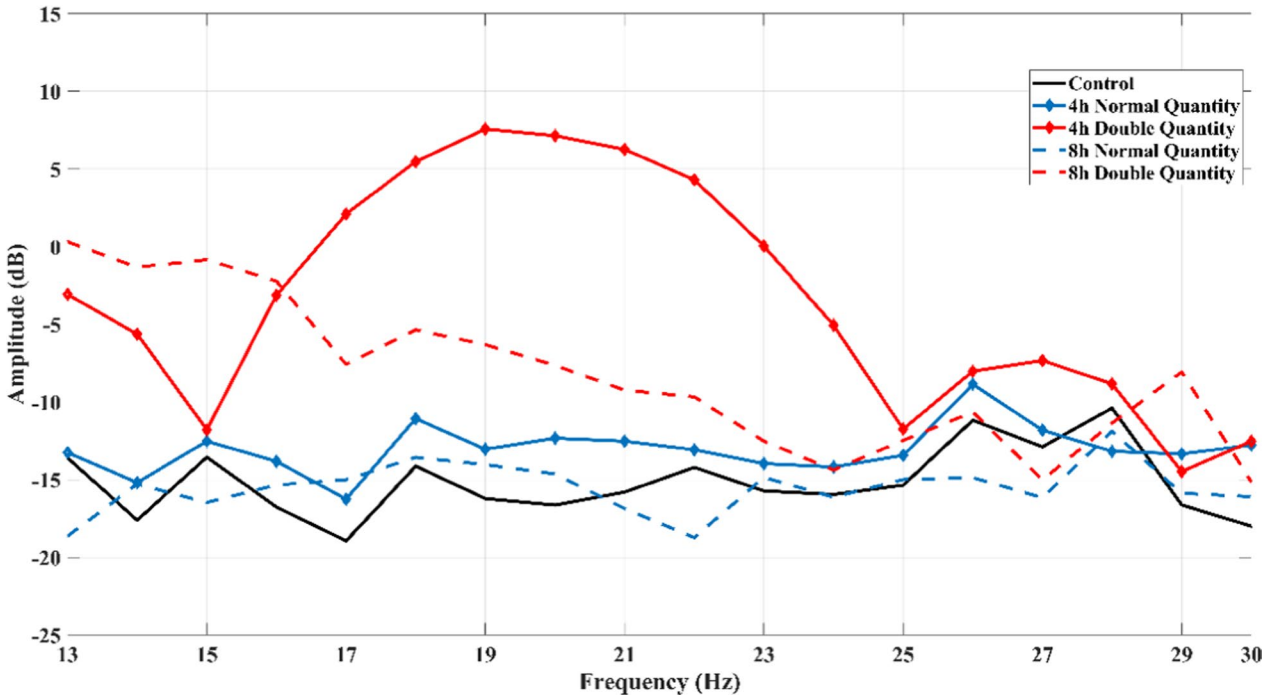

Fig. 5 EEG measurement of rat's brain in the control group and W-N-doped TiO_2 NPs treated groups, including 4 h Normal Quantity (T1), 4 h Double Quantity (T2), 8 h Normal Quantity (T1), and 8 h Double Quantity (T2). The 4 h Normal Quantity group showed the highest activity

and alter the rat's neural activity [9, 11]. Our findings, as shown in Fig. 5, reveal that W-N-doped TiO_2 NPs significantly affect rat brain electrical activity, with the double quantity (T2) W-N-doped TiO_2 NPs exhibiting a more substantial effect than standard quantity (T1) W-N-doped TiO_2 NPs. Specifically, the beta band of the EEG signal increased by 23 dB after 4 h of injecting double quantity (T2) W-N-doped TiO_2 NPs; in contrast, the standard quantity (T1) nanoparticles resulted in a minor increase of 4.3 dB compared to the control group. These changes were accompanied by a subsequent decrease in amplitude to a value similar to that of the control group in the band after 25 Hz.

At 8 h after injection, we observed a further increase of 16.2 dB in the beta band amplitude of double quantity compared to the control group. Interestingly, the amplitude converged to a value like that of the control group in the band after 25 Hz, indicating that the effect of nanoparticles decreased with time. In contrast, the standard quantity showed little change in amplitude compared to the control group, confirming that the standard amount did not affect brain activation 8 h after injection. Our results highlight the importance of nanoparticle quantity on brain activation, with double quantity exhibiting a more substantial effect than standard quantity. These EEG changes are attributable to the alterations in post-synaptic-neuronal potentials caused by the exposure to W-N-doped TiO_2 NPs.

Metal nanoparticles can interfere with brain activity, as supported by other research. A study by Jung et al. (2014) revealed that gold nanoparticles (AuNPs) increased the generation of action potential in CA1 neurons in the hippocampus by altering the electrophysiological properties within the mouse brain [26]. The principal mechanism involved is ionic flow disruption, which alters the membrane potential of neurons. Gramowski et al. (2010) also reported severe changes in electrical activity in the cortical networks in a concentration-dependent manner after exposure to different nanoparticles, including TiO_2 NPs in vitro, confirming the neurotoxic effects of nanoparticles [27].

Furthermore, our findings suggest that the effect of nanoparticles decreases with time after W-N-doped TiO_2 NPs administration. Further experiments are required to determine why the double quantity (T2) beta band signal was measured to be low at 15 Hz and maximum at 19 Hz at 4 h. The EEG signals were higher at 13 ~ 16 Hz but gradually decreased at 8 h. Overall, this study provides important insights into the effects of nanoparticle injection on brain activation and lays the foundation for future research in this area.

Assessment of W-N-doped TiO_2 NPs effect on balance sense in rats

A horizontal beam test was conducted on three groups of rats to investigate the effects of W-N-doped TiO_2 NPs on the sense of balance and locomotor activity of rats (n = 3 each). The beam test is a widely used assessment tool that measures the ability of animals to maintain balance and navigate a narrow beam. NPs T1 and NPs T2 groups were injected with W-N-doped TiO_2 NPs in standard quantity (NQ), i.e., 100 mg/kg or T1 and double quantity (DQ) 200 mg/kg or T2, respectively, while the control group was not given any treatment [14]. One hour after the W-N-doped TiO_2 NPs administration, the beam test was conducted thrice for each case and evaluated the experimental result as a score. As shown in Fig. 6, the results revealed an apparent difference between NQ and DQ cases. After nanoparticle injection, test scores increased in NQ and DQ cases compared to the control, confirming that nanoparticles impacted the sense of balance in rats.

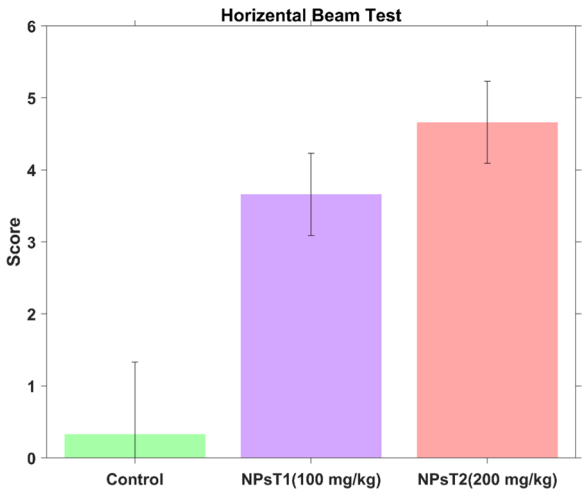

Fig. 6 Horizontal beam test results in the control group and W-N-doped TiO_2 NPs treated groups, showing higher test scores in NPsT1 and NPsT2 compared to the control. NPsT1 and NPsT2 were given 100 mg/kg and 200 mg/kg of W-N-doped TiO_2 NPs, respectively

Specifically, the beam test score in the control group was just 0.2 points, but the score in NQ and DQ after W-N-doped TiO_2 NPs administration increased to 3.50 and 4.66 points, respectively. The score of DQ is 1.16 points higher than that of NQ, suggesting that the quantity of W-N-doped TiO_2 NPs injected significantly impacts the sense of balance in rats.

This study sheds light on the effects of W-N-doped TiO_2 NPs on the sense of balance in rats. It highlights the importance of nanoparticle quantity in determining these effects. Further research is needed to elucidate the underlying mechanisms of these findings and potential applications.

Oxidative stress caused by W-N-doped TiO_2 NPs

Various semiconductor nanoparticles such as TiO_2 NPs [28, 29], Zinc oxide (ZnO) NPs, [30], and Iron oxide (Fe_2O_3) NPs [31] have been reported to alter cellular redox state. This study assesses and quantifies the alteration of different oxidative stress and inflammatory biomarkers in neural cells caused by W-N-doped TiO_2 NPs. Rats were divided into three groups, the control group, the NPs T1 group, and the NPs T2 group; the former was not given any treatment, while the latter two were injected intraperitoneally with 100 mg/kg and 200 mg/kg W-N doped TiO_2 NPs respectively. After that, oxidative stress biomarkers, including ROS, nitrite concentration, MDA, CAT activity, SDO, GSH levels, acetylcholine esterase activity, and dopamine levels, and two inflammatory biomarkers, including TNF-α and 8-OHDG, were measured using the brain lysate.

TiO_2 NPs injected into the abdominal cavity can translocate into the brain [29]. Research conducted by Grissa et al. (2019) [28], Valentini et al. (2018) [32], Song et al. (2016) [33] and Linglan et al. (2010) [31] have reported the induction of oxidative stress in the brain caused by TiO_2 NPs. The data illustrated in Fig. 7a-j demonstrate that W-N-doped TiO_2 has significantly increased brain ROS concentration, nitrite concentration, DA level, and MDA level. On the other hand, W-N-doped TiO_2 has caused a substantial decrease in brain GSH

(See figure on next page.)

Fig. 7 Biomarker estimation for assessing oxidative stress in the control group and two treatment groups: NPs T1 and NPs T2, administered with W-N-doped TiO_2 NPs at dose of 100 mg/kg and 200 mg/kg respectively, by applying one way ANOVA test **a** Reactive oxygen species levels depicting a significant dose dependent increase in treatment groups **b** Nitrite concentration in control and W-N-doped TiO_2 NPs treated groups showing a significant concentration dependent increase in treatment groups **c** Malondialdehyde assay depicting a significant dose dependent increase in treatment groups **d** Catalase activity assay in control and W-N-doped TiO_2 NPs treated groups illustrating a significant dose dependent decrease in both the treatment groups **e** Super oxide dismutase activity assay showing a significant decrease in treatment groups **f** Glutathione assay showing a significant dose dependent decrease in treatment groups **g** Acetylcholinesterase assay in control and W-N-doped TiO_2 NPs treated groups showing a significant concentration dependent decrease in both the treatment groups **h** Dopamine assay showing a significant increase in both the treatment groups **i** Pro-inflammatory cytokine, Tumor necrosis factor alpha assay in control and W-N-doped TiO_2 NPs treated groups showing a significant concentration dependent increase in both the treatment groups **j** 8-hydroxy 2- deoxyguanoise depicting a significant dose dependent increase in both the treatment groups. $p < 0.001$

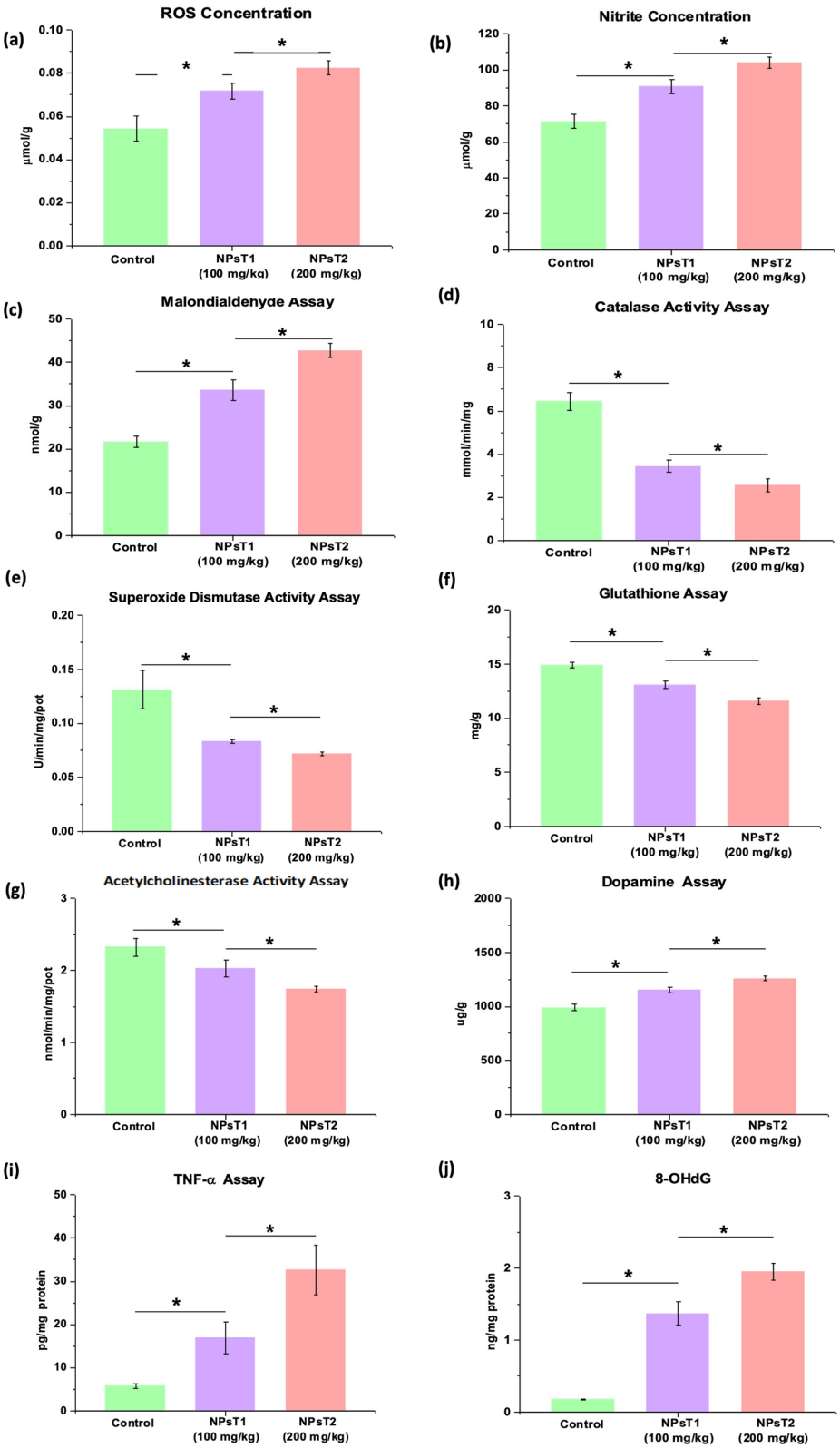

Fig. 7 (See legend on previous page.)

levels, acetylcholine esterase levels, CAT activity, and SOD activity. The inflammatory biomarkers, TNF-α and 8-OHDG, showed a significant increase.

In the present study, W-N-doped TiO_2 NPs administration induced a significant imbalance between the oxidant and antioxidant system in the rat's brain as compared to the control group, resulting in oxidative stress surpassing the antioxidant's capacity. As shown in Fig. 7a, the concentration of ROS significantly increased in both treatment groups compared to the control group. In the control group, ROS concentration was 0.055 µmol/g, while in NPs T1, it was 0.072 µmol/g, which increased to 0.083 µmol/g in NPs T2 group. *In vitro* studies have demonstrated that TiO_2 NPs induce the microglia cells to produce ROS, damaging neuronal cells [34]. Similarly, nitrite concentration also increased significantly in both the groups treated with the nanoparticles; the concentration was higher in NPs T2 than in NPs T1, which can be attributed to the higher dose administered. Figure 7c shows a statistically significant increase in MDA levels among the NPs T1 and NPs T2 groups as compared to the control group ($p < 0.01$). This mainly occurs due to the production of free radicals, which reacts with the cell membrane leading to lipids peroxidation and increasing MDA levels [28].

Major antioxidant enzymes, including SOD and CAT, act as scavengers of ROS, as SOD converts the superoxide radicals into oxygen and hydrogen peroxide (H_2O_2). In contrast, CAT catalyzes the conversion of H_2O_2 into water and molecular oxygen [35]. However, in the present study, W–N doped TiO_2 NPs caused an increase in lipid peroxidation leading to the significantly lower activity of CAT and SOD in a dose-dependent manner, both in the NPs T1 group and NPs T2 group compared to the control. Glutathione, an antioxidant, plays a vital role as the first line of defense against damage caused by oxidative stress [36]. In the present study, glutathione levels exhibited a statistically significant decrease ($p < 0.001$) in NPs T1 and NPs T2 groups compared to the control group. The control group showed glutathione levels of 15 mg/g, while NPs T1 group and NPs T2 had lower levels of 13 mg/g and 11.5 mg/g, respectively, as demonstrated in Fig. 7(f). The decline in GSH level can be attributed to its excessive utilization in scavenging reactive free radicals. A statistically significant decrease of $p < 0.001$ can be seen in the activity of acetylcholinesterase, a cholinergic neurotransmitter, in both the NPs treatment groups compared to the control. Acetylcholinesterase hydrolyzes the acetylcholine, which is released into the synapses between neurons [37]. Fig. 7(h) shows a dose-dependent increase in dopamine levels after administering W-N-doped TiO_2 NPs. Dopamine is a monoaminergic neurotransmitter in the brain, and reduced levels of DA can lead to behavioral changes in the NPs treated rats [38].

Studies have shown that oxidative stress in the brain may activate the release of pro-inflammatory cytokines like TNF-α that damage the blood–brain barrier, leading to increased permeability to the nanoparticles in a dose-dependent manner [4]. The inflammatory marker, TNF-α, showed a 5 pg/mg concentration, which increased to 17 pg/mg in NPs T1 and in NPs T2, almost double to NPs T1. The levels of 8-OHDG increased from 0.2 ng/mg in the control group to 1.4 ng/mg in NPs T1 and 1.95 ng/mg in NPs T2, having a p-value of < 0.001. The increase in 8-OHDG is primarily due to the DNA damage caused by ROS [38].

In summary, it was observed that TiO_2 NPs significantly increased the expression of antioxidant enzymes and caused lipoperoxidation and alterations in the neural redox state. According to a previous study, oxidative stress caused by TiO_2 NPs led to the intracellular generation of ROS in neuronal cells in a concentration-dependent manner [27]. Other authors have demonstrated the ROS-mediated oxidative stress induced by TiO_2 NPs in various cell types, including mouse macrophages [39], human HaCaT keratinocytes [40, 41], human lung fibroblasts (IMR-90) [42], human endothelial cells [43], and human embryonic kidney cells [44]. In addition, oxidative stress caused by TiO_2 NPs has been linked to changes in the expression of antioxidant enzymes like SOD, and lipid peroxidation, which damages mitochondrial DNA, malondialdehyde (MDA) oxidation, and mitochondrial membrane potential [40, 41]. Our results are in comparison with another study, which reported a decrease in SOD level and an increase in malondialdehyde (MDA) after treatment with TiO_2[8]. Variations in any of the results can be attributable to the different methods of analysis used. So far, most of the literature available is on TiO_2; this study has provided insight into neurobiochemical changes caused by W-N-doped TiO_2 NPs.

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Author contributions
HMUF and JHL performed laboratory experiments. CHL, SUI MAF and GUS provided significant input on the experimental design. HMUF and DGP. conceived the idea of this study and partly funded the study. HMUF and JHL analyzed the data and prepared the manuscript; all authors contributed substantially to the drafting and editing of the manuscript.

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Availability of data and materials
The data supporting this study are available from corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare no competing interest.

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