Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 15, Issue 1, 2026, 43
https://doi.org/10.33263/LIANBS151.043

Quinoa and Moringa Seeds Extracts Mitigated Endoplasmic Reticulum Stress, Mitochondrial Dysfunction, and Neuronal Toxicity Induced by TiO_2-Nanoparticles in Rats

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Received: 30.04.2025; Accepted: 5.02.2026; Published: 30.03.2026

Abstract: Titanium dioxide nanoparticles (TiO_2-NPs) can harm the brain, causing neurotoxicity. Functional foods such as quinoa and moringa seeds have many pharmacological benefits and neuroprotective effects. This study investigates the impact of quinoa and moringa seeds ethanolic extracts (QSE and MSE) administration on neurotoxicity induced by TiO_2-NPs in rats. TiO_2-NPs were synthesized and characterized by several methods. Sixty adult male rats were categorized into six groups: two control groups designated as healthy, and TiO_2-NPs-intoxicated groups. The other groups received the same dose of TiO_2-NPs with either low (100 mg/kg) or high (200 mg/kg) doses of QSE or MSE orally. HPLC analysis of the phytochemical components of each extract was performed. Several behavioral and biochemical measures were assessed. In addition, the gene expression of endoplasmic reticulum stress (ERS) and apoptosis markers, cerebral mitochondrial activity, and DNA fragmentation were also evaluated. The results indicated a higher total phenolic and flavonoid content in red QSE than in MSE. The study reported the potential neuroprotective effects of QSE and MSE against TiO_2-NPs neurological intoxication, targeting neuronal ERS, apoptosis, and restoring cerebral mitochondrial activity.

Keywords: titanium dioxide nanoparticles; neurotoxicity; quinoa seeds extract; Moringa seeds extract; ERS.

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1. Introduction

Because of their small size and large surface area, nanoparticles can have harmful effects despite their many benefits and wide range of uses. Damage to the blood-brain barrier (BBB) can increase permeability and allow nanoparticle entry into the central nervous system (CNS), potentially causing neurotoxicity [1]. By bypassing the blood-brain barrier (BBB), titanium dioxide nanoparticles (TiO_2-NPs), the widely used white-pigmented industrial additive (E171), cause oxidative damage and neuroinflammation [2]. The buildup of TiO_2-NPs

in the brain is linked to oxidative stress. It causes the brain's activated microglial cells to release proinflammatory cytokines, leading to the development of several neurological disorders [3].

Furthermore, TiO_2-NPs may also induce necrosis, cytotoxicity, genotoxicity, and carcinogenesis [4]. TiO_2-NPs produce oxidative stress and the accumulation of unfolded or misfolded proteins, which results in endoplasmic reticulum stress (ERS). Additionally, their accumulation damages mitochondria by inhibiting mitochondrial respiration and promoting cytochrome release, apoptosis, and an inflammatory cascade [5].

Endoplasmic reticulum stress (ERS) occurs when unfolded or misfolded proteins accumulate, which may be induced by ROS, leading to activation of the apoptotic machinery and loss of cell viability. The ERS-dependent feedback loops are the PERK/ATF/CHOP pathway [4,5].

Because they contain many bioactive compounds, functional foods, such as fruits, seeds, cereals, and vegetables, may help preserve neurons by scavenging free radicals and restoring antioxidant defenses in cells [6].

Quinoa (*Chenopodium quinoa Willd.*), a grain that belongs to the *Chenopodiaceae* family, is high in phenolic acid content, including p-coumaric, vanillic, protocatechuic, ferulic, caffeic, and 4-hydroxybenzoic acids [7]. Quercetin, kaempferol, orientin, vitexin, rutin, morin, hesperidin, neo-hesperidin, and phytoecdysteroids are the most abundant phytochemicals found in quinoa [8]. Quinoa seeds have anti-inflammatory, antioxidant, antibacterial, antithrombotic, antidiabetic, antiulcer, anticancer, neuroprotective, and immunomodulatory properties, among other pharmacological benefits [9].

Moringa (*Moringa oleifera*), a member of the family *Moringaceae*, possesses many therapeutic properties [10]. Flavonoids, including quercetin-3-O-glucoside, rhamnetin, apigenin, kaempferol, myricetin, moringin, and glucosinolates, are abundant in moringa seeds. These seeds also include benzyl isothiocyanate, β-sitosterol-3-O-β-D-glucopyranosides, and pterygospermin [11]. Analgesic, antipyretic, antibacterial, antidiabetic, cardioprotective, hepatoprotective, antirheumatic, antiviral, antiulcer, anti-inflammatory, anti-cancer, antidepressant, and antiepileptic properties are only a few of the pharmacological activities that these phytochemicals provide [12].

The overarching goal of this study is to investigate the potential neuroprotective effects of quinoa and moringa seeds' ethanolic extracts in rats neuro-intoxicated with TiO_2-NPs. It also studies both extracts' phenolic content and their potential neuroprotective impact on our model. The study, for the first time, shed light on the role of QSE and MSE in tackling endoplasmic reticulum stress in the TiO_2-NPs neuro-intoxication model.

2. Materials and Methods

2.1. Chemicals.

Titanium (IV) isopropoxide ($Ti(OC_3H_7)_4$, Purity: 98%, Molecular weight: 284.22) was purchased from Sigma Company for Chemicals, Cairo, Egypt. Ethyl Alcohol (70%) and hexane were purchased from El-Gomhoria Company for Chemicals and Drugs, Cairo, Egypt.

2.2. Synthesis and Characterization of TiO_2 nanoparticles.

TiO_2 nanoparticles were synthesized using a modified sol-gel method with controlled hydrolysis conditions. Titanium (IV) isopropoxide (TTIP) served as the precursor in anhydrous ethanol under a nitrogen atmosphere. A TTIP solution (1:40 molar ratio in ethanol) was

prepared with 30 minutes of stirring. Separately, a hydrolysis solution of deionized water and ethanol (1:5) was prepared at pH 2.0 using nitric acid and maintained at 4°C. The TTIP solution was added dropwise (1 mL/min) to the hydrolysis solution while stirring at 800 rpm. The resulting solution. was aged for 24 hours at room temperature with continuous stirring. Thermal treatment followed a three-step process: 8 hours at 80°C for gel formation, 12 hours at 120°C for drying, and calcination at 450°C for 4 hours (2°C/min heating rate) to obtain crystalline TiO_2 nanoparticles with spherical morphology [13].

Characterization included Transmission Electron Microscopy (TEM, JEOL JEM-2100F) at 200 kV, Atomic Force Microscopy (AFM, Bruker Dimension Icon) in tapping mode, X-ray Diffraction (XRD, Bruker D8 Advance) with Cu Kα radiation, Dynamic Light Scattering (DLS, Malvern Zetasizer Ultra), and zeta potential measurements at various pH values [14-18].

2.3. Plant.

The red Quinoa seeds *(Chenopodium quinoa Willd)* and Moringa Seeds *(Moringa oleifera)* were obtained from the Ministry of Agriculture, Giza, Egypt.

2.4. Preparation of quinoa seed extract (QSE).

The dried red quinoa seeds were ground into a fine powder. Using a Soxhlet apparatus, two kilograms of powdered seeds were extracted using 600 milliliters of hexane at 60°C for two hours. The residue was then extracted using 600 milliliters of 70% ethanol at 60°C for three hours. The ethanolic extracts were evaporated at a regulated temperature of 75°C while under reduced pressure (400 mmHg) [9].

2.5. Preparation of Moringa seed extract (MSE).

Moringa seeds were cleaned, dried, and finely powdered using a grinder. Six liters of 70% ethanol were used to extract two kilograms of dried powdered moringa seeds, which were then left at room temperature for seventy-two hours. The extract was filtered, and a rotary evaporator (WHEATONSP35) was used to evaporate the extract in a water bath at 40°C [19].

2.6. Determination of total phenolic content in QSE and MSE.

The total phenolic content was determined using the Folin-Ciocalteu procedure [20]. After dissolving 1 gram of the extract in 10 milliliters of distilled water, 100 microliters of the resulting solution was diluted to 3.5 milliliters with distilled water, and 250 microliters of Folin-Ciocalteu reagent was added. After five minutes, the mixture is neutralized using 1.25 milliliters of a 20% aqueous sodium carbonate solution. The absorbance at 725 nm was then measured against the solvent blank after 40 minutes. Using a calibration curve made with gallic acid, the total phenolic content was ascertained.

2.7. Determination of total flavonoid content of QSE and MSE.

The total flavonoid content was determined in QSE and MSE using an aluminum chloride ($AlCl_3$) colorimetric assay [20]. In short, 100 µl of dissolved extract (one gram of the extract was dissolved in 10 ml of distilled water) was mixed with 300 µl of 5% sodium nitrite solution. After six minutes, 300 µl of a 10% $AlCl_3$ solution was added, and distilled water was used to adjust the volume to 2.5 mL. 1.5 mL of 1 M NaOH was added after 7 minutes, and the

mixture was centrifuged for 10 minutes at 5000 rpm. At 510 nm, the supernatant's absorbance was measured. A calibration curve made with catechin was used to calculate the total flavonoid concentration.

2.8. Determination of phenolic compounds in QSE and MSE by high-performance liquid chromatography (HPLC).

Agilent Technologies' 1100 series liquid chromatograph, which has a diode-array detector and an autosampler, was used for the High-performance liquid chromatography analysis. Eclipse XDB-C18 (150 x 4.6 µm: 5 µm) with a C18 guard column (Phenomenex, Torrance, CA) served as the analytical column. Acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B) made up the mobile phase. The gradient that was programmed was 100% B to 85% B in 30 minutes, 85% B to 50% B in 20 minutes, 50% B to 0% B in 5 minutes, and 0% B to 100% B in 5 minutes. The flow rate was maintained at 0.8 ml/min for a total run period of 70 minutes. Peaks for the benzoic acid and cinnamic acid derivatives were concurrently observed at 280 and 320 nm, respectively, using an injection volume of 50 µl. Prior to injection, each sample was passed through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI). Congruent retention durations and UV spectra were used to identify the peaks, which were then compared to the standards [21].

2.9. Experimental design.

All experimental measures were performed according to the recommendations for the care and use of laboratory animals and following the local animal ethics committee of Ain Shams University (#ASU/W/Sci-6M/23-3-55). Sixty adult *Sprague-Dawley* male rats weighing 230 ±5 g were obtained from the animal Breeding House of the National Research Centre (NRC), Giza, Egypt. Rats were allowed seven days for acclimation to adapt to the laboratory conditions. Rats were provided with a commercial pellet diet and water *ad libitum*. The experimental diet was obtained from the National Research Centre (NRC), Giza, Egypt. Afterward, they were housed in stainless steel cages at room temperature (22 ± 5°C), relative humidity (50 ± 10%), and 12 hours of light/dark cycles.

After acclimatization, the rats were randomly distributed into six groups (10 rats each) and treated as follows: Group I (healthy control); rats were given distilled water orally by gastric tube twice daily for 6 weeks. Group II (TiO_2-NPs intoxicated group); Rats were given 200 mg/kg b.wt./day of TiO_2-NPs suspended in distilled water for induction of neurotoxicity [22]. Group III (TiO_2-NPs +QSE-low dose); Rats were given TiO_2-NPs water suspension and then received quinoa seeds extract (QSE) (100 mg/kg b.wt./day dissolved in distilled water) [9]. Group IV (TiO_2-NPs +QSE-high dose); Rats were given a TiO_2-NPs water suspension and then received QSE (200 mg/kg b.wt./day dissolved in distilled water) [23]. Group V (TiO_2-NPs +MSE-low dose); Rats were given a TiO_2-NPs water suspension and then received moringa seeds extract (MSE) (100 mg/kg b.wt./day dissolved in distilled water) [2]. Group VI (TiO_2-NPs +MSE-high dose); Rats were given a TiO_2-NPs water suspension and then received MSE (200 mg/kg b.wt./day dissolved in distilled water) [24]. All treatments were given orally by gastric tube daily for six weeks. At the end of the study, the animals were sacrificed under sodium barbiturate anesthesia, and then blood and brain tissue samples were collected.

2.10. Behavioral assessment.

Behavioral tests were performed in the last week before animal sacrifice in the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. The Y-Maze Spontaneous Alternation (SAP) Test and open field test were done (between 8 am and 12 pm a day).

2.10.1. Y-maze spontaneous alternation (SAP) test.

Y-Maze Spontaneous Alternation reflects cognitive deficits in spatial working memory, a type of short-term memory. As described previously [25], it was a black wooden Y-maze with three arms marked A, B, or C and a symmetrical triangular central region. Rats were positioned at one arm's edge and given eight minutes to move freely throughout the maze. When the rat's rear paws were fully inside the arm, the rat’s entries were tallied. The following formula was used to determine SAP based on the total number of arm entries and alternations:

$$SAP\ (\%) = \frac{number\ of alternations}{total\ arm\ entries - 2} * 100 \qquad (1)$$

2.10.2. Open field test (OFT).

Each animal was carefully placed in the center of an 80 x 80 x 40 cm wooden box with red walls and a white, smooth, polished floor divided into 16 (4 x 4) identical squares by black lines. Five minutes of the animals' unrestricted exploration of the area were timed using a stopwatch, and their activities were recorded using a video camera placed in front of the test box [26].

2.11. Total oxidative stress and antioxidant biomarker levels in blood.

Total antioxidant capacity (TAC) and total oxidative stress (TOS) were determined by Enzyme-Linked Immunosorbent Assay (ELISA) kits (CAT.NO: MBS1600693 and CAT.NO: MBS1600508, respectively, from My BioSource, San Diego, California, USA). The oxidative stress index (OSI) was calculated according to the following formula [27]*:*

$$\text{OSI (arbitrary unit)} = \frac{\text{TOS (micromolar H2O2 equivalent/liter)}}{\text{TAC (micromolar Trolox equivalent/liter)}} \qquad (2)$$

2.12. Inflammatory biomarkers (IL-1β and TNF-α) in serum and (NF-κB and TLR-4) in brain tissues.

Measurement of interleukin-1 β (IL-1β) and tumor necrosis factor- α (TNF-α) in serum, as well as nuclear factor-kappa B (NF-κB) and toll-like receptor-4 (TLR4) concentrations in brain tissues was employed by using ELISA kits (CAT.NO: MBS825017, CAT.NO: CSB-E11987r, CAT.NO: MBS2505513 and CAT.NO: CSB-E15822r, respectively) according to the manufacturer’s instruction.

2.13. Measurement of melatonin, glutamate levels, and acetylcholinesterase activity in brain tissues.

Measurement of melatonin and glutamate levels and Acetylcholinesterase activity in brain tissues was employed by using the ELISA kits (CAT.NO: MBS764385 and CAT.NO: MBS756400 from My BioSource, San Diego, California, USA, and CAT NO: CSB-E11304r

from CUSABIO BIOTECH Co., China, respectively) according to the manufacturer's instructions.

2.14. Measurement of the gene expression of PERK, ATF, CHOP, Caspase 3, and Bax by real-time polymerase chain reaction (RT-PCR).

Based on the manufacturer's instructions, total messenger ribonucleic acid (mRNA) was extracted from brain tissues by using a spin or vacuum (SV) Total RNA Isolation system (Thermo Scientific, USA). A high-capacity cDNA reverse transcription kit (#K4374966 from Thermo Fisher Scientific, USA) was used to reverse transcribe mRNA into complementary DNA (cDNA). Using 2X SYBR Green PCR Master Mix (Applied Biosystem) and software version 3.1 (StepOneTM, USA), real-time quantitative PCR (RT-PCR) amplification and analysis were carried out.

The primer sequences (Table 1) for Protein Kinase RNA-Like ER kinase (PERK), Activating transcriptional 4 (ATF), C/EBP homologous protein (CHOP), caspase-3, BAX, and housekeeping β-actin genes were designed as described previously [28, 29]. Using the comparative threshold cycle approach, the relative expression of the genes under investigation was determined in relation to the reference β-actin gene [30].

2.15. Assessment of percentage DNA fragmentation in brain tissues.

DNA fragmentation was determined by the ELISA method using the My Biosource kit according to the manufacturer's instructions [31].

2.16. Isolation of cerebral mitochondria.

The isolation of mitochondria from cerebral brain tissue was done following the instructions of the Mitochondria Isolation Kit (CAT.NO: NBP2-29448).

2.17. Assessment of cerebral mitochondrial function.

Mitochondrial function was evaluated using the MTT reduction assay [32]. This test relies on the mitochondrial dehydrogenases' ability to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a formazan that can be measured spectrophotometrically at 550 nm.

2.18. Histological examination of brain tissue sections.

Rats in various groups had autopsy samples extracted from their brains, which were then preserved for 24 hours in 10% formol saline. Methyl, ethyl, and 100% ethyl alcohol serial dilutions were utilized after washing in tap water. After being cleaned with xylene, the specimens were embedded in paraffin for 24 hours at 56°C in an air oven. Using a rotating LEITZ microtome, tissue blocks made of paraffin wax were created for sectioning at a thickness of 4 microns. The resulting tissue slices were placed on glass slides, deparaffinized, stained with hematoxylin and eosin, and examined under a light microscope at 400x magnification [33].

2.19. Statistical analysis.

The mean ± standard deviation (SD) was used to represent all data in the tables and figures. One-way analysis of variance (ANOVA) was used for statistical analysis, and SPSS software, version 29 (IBM, Armonk, NY, USA), was used. Significant values were those with $p \leq 0.05$ [34].

3. Results and Discussion

3.1. Characterization of TiO_2 nanoparticles.

3.1.1. X-ray diffraction analysis.

The XRD pattern (Figure S1A) confirms the successful synthesis of crystalline anatase TiO_2 nanoparticles. Well-defined diffraction peaks at 2θ values of 25.3°, 38.5°, 48.1°, 53.9°, and 62.7° correspond to the (101), (004), (200), (105), and (204) crystallographic planes, respectively, matching the ICDD PDF card (No. 21-1272) for anatase TiO_2. The sharp, high-intensity peaks indicate well-crystallized nanoparticles with no additional rutile or brookite phases present, demonstrating phase purity. The average crystallite size calculated using the Scherrer equation is approximately 20 nm, suggesting isotropic particle growth. Calculated lattice parameters (a = b = 3.785 Å, c = 9.513 Å) closely match theoretical values for the anatase crystal structure.

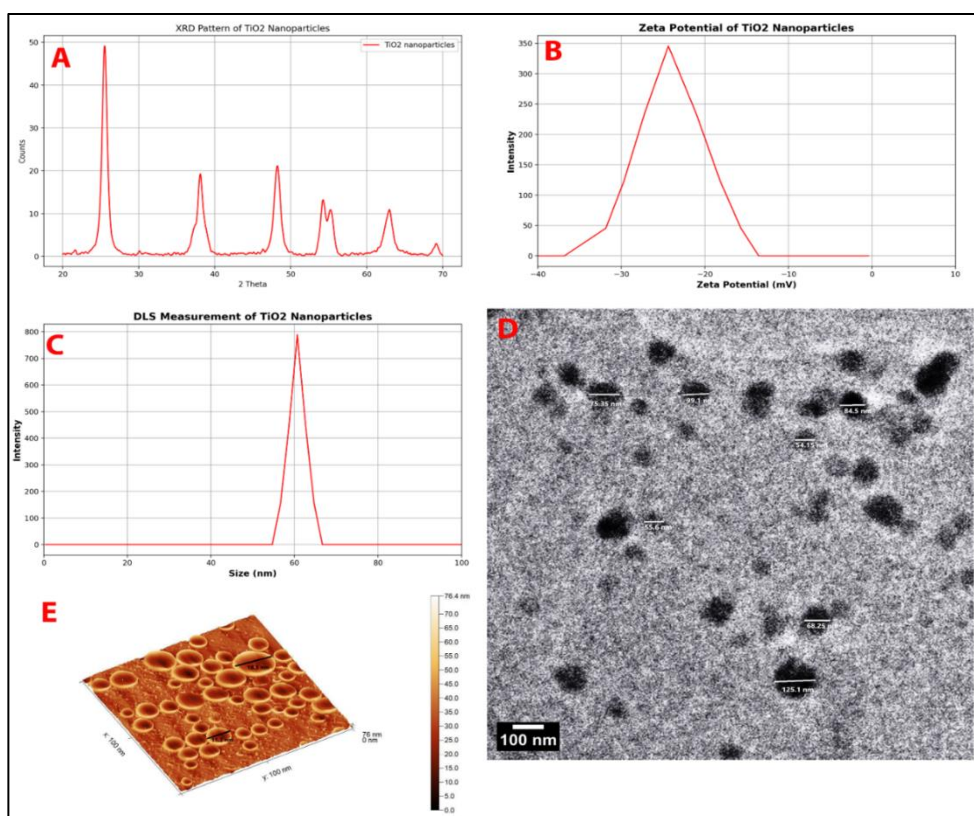

Figure S1. Comprehensive characterization of synthesized TiO_2 nanoparticles. **(A)** XRD pattern showing characteristic peaks of anatase phase with high crystallinity; **(B)** Zeta potential distribution indicating moderate negative surface charge (-25.4 mV) and colloidal stability; **(C)** DLS measurement revealing uniform size distribution with average hydrodynamic diameter of approximately 65 nm and low polydispersity; **(D)** TEM micrograph confirming spherical morphology with average particle size of 62 ± 5 nm and good interparticle spacing; **(E)** 3D AFM topography image showing surface morphology with textured features and moderate roughness across a 100 nm × 100 nm scanning area.

https://doi.org/10.33263/LIANBS151.043

3.1.2. Zeta potential analysis.

Zeta potential measurements (Figure S1B) show a distinct negative surface charge distribution with a peak value of -25.4 mV, indicating moderate electrostatic stabilization. The symmetrical distribution curve with a relatively narrow width (12.8 mV) demonstrates uniform surface charge characteristics across the nanoparticle population. The measured electrophoretic mobility (-1.98 μmcm/Vs) correlates well with the observed zeta potential. The negative surface charge can be attributed to the deprotonation of surface hydroxyl groups at neutral pH, contributing to electrostatic stabilization and preventing spontaneous aggregation.

3.1.3. Dynamic light scattering analysis.

DLS analysis (Figure S1C) reveals a monomodal size distribution with a sharp, symmetrical peak centered at approximately 65 nm, indicating uniform particle size distribution and excellent colloidal stability. The low polydispersity index (PDI) of 0.182 confirms the successful synthesis of well-dispersed nanoparticles with minimal aggregation. The observed hydrodynamic diameter (65 nm) is larger than the crystallite size from XRD analysis (~20 nm) due to the formation of the electrical double layer and hydration shell around the nanoparticles in aqueous suspension. The high peak intensity (785 kcps) and absence of secondary peaks indicate strong light scattering from suspended particles without significant agglomeration.

3.1.4. Transmission electron microscopy.

TEM analysis (Figure S1D) confirms the successful synthesis of discrete spherical nanoparticles with an average diameter of 62 ± 5 nm, correlating well with DLS measurements. The particles exhibit excellent size uniformity with approximately 92% falling within the 55-70 nm size range. The high-contrast image reveals distinct particle boundaries and adequate interparticle spacing (15-25 nm), indicating effective surface stabilization that prevents significant agglomeration. The uniform contrast across individual particles suggests homogeneous internal structure, while clear particle edges confirm well-crystallized TiO_2 nanostructures.

3.1.5. Atomic force microscopy.

AFM analysis (Figure S1E) provides detailed information about surface topography, revealing relatively uniform morphology with well-distributed spherical features. The root mean square (RMS) roughness value of 9.38 nm indicates moderate surface roughness, beneficial for applications requiring high surface area accessibility. The positive skewness value (0.836) suggests an asymmetric height distribution with prominent peaks, as indicated by the maximum peak height (42.78 nm) and the maximum valley depth (33.62 nm). The significant difference between the projected area (10,000 nm^2) and the actual surface area (229,679 nm^2) yields a surface area ratio of approximately 23, indicating the highly textured nature of the nanoparticle film. These properties are advantageous for catalytic and sensing applications requiring a high surface area.

3.2. Phytochemical analysis of the quinoa and moringa seeds' ethanolic extracts.

The total polyphenol content in quinoa and moringa seed extract was measured in mg as gallic acid equivalent (GAE%), and total flavonoids were measured in mg as catechin equivalent (CE%). Results showed that the total polyphenol content in quinoa and moringa seed extracts was 6.56 mg and 4.06 mg GAE, respectively. The total flavonoid content in quinoa and moringa seed extracts was 0.6 mg and 0.25 mg CE, respectively, as shown in Figure S2. Moreover, high-performance liquid chromatography (HPLC) was used to detect phenolic compounds in quinoa and moringa seed extracts quantitatively. The results indicated 12 main bioactive phenolic compounds in QSE, including protocatechuic acid, ferulic acid, sinapic acid, p-hydroxybenzoic acid, cinnamic acid, caffeic acid, p-coumaric acid, vanillic acid, syringic acid, gallic acid, and flavonoids (rutin and quercetin). Their amounts per gram of QSE are listed in Figure S3A. Moreover, MSE contained 13 main bioactive phenolic compounds, which included chlorogenic acid, p-hydroxybenzoic acid, gallic acid, vanillic acid, syringic acid, p-coumaric acid, cinnamic acid, ferulic acid, and flavonoids; daidzein, catechin, kaempferol, chrysin, and quercetin. Their amounts are indicated in Figure S3B.

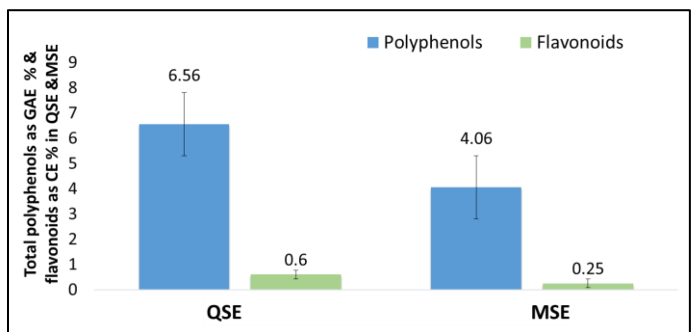

Figure S2. Total polyphenols and flavonoids content in QSE and MSE.

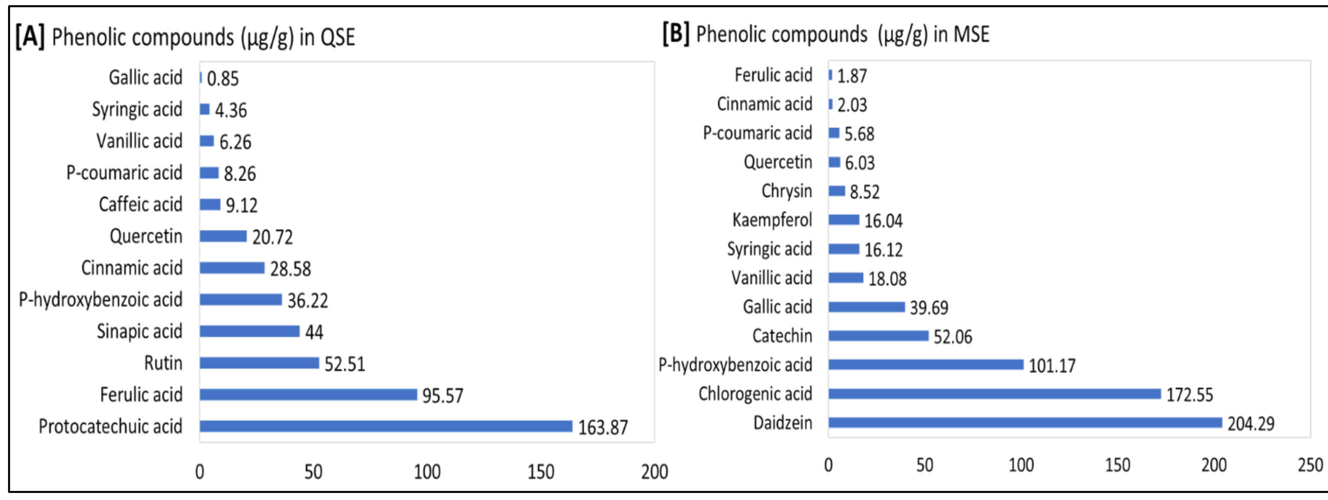

Figure S3. Different fractions of phenolic compounds in **(A)** QSE; **(B)** MSE were measured by HPLC.

3.3. Oral administration of QSE and MSE alleviated behavioral changes in intoxicated rats.

The effect of QS and MS extracts on behavioral tests such as the Y-maze spontaneous alternation (SAP) test, and the Open field test (ambulation frequency, OFT rearing frequency, and OFT Grooming), is illustrated in Figure S4. Y-maze test spontaneous alternation, ambulation frequency, OFT rearing frequency, and OFT grooming significantly ($P \leq 0.05$) decreased in the TiO_2-NPs control group compared to the normal control group, reflecting cognitive deficits and decreased exploratory and locomotor activity. However, treatment with

QS and MS extracts at 100 and 200 mg/kg b.wt. significantly ($P \leq 0.05$) enhanced the results of these tests compared to the TiO_2-NPs group ($P \leq 0.05$). Moreover, the administration of TiO_2-NPs significantly increased the latency time compared to the control group ($P \leq 0.05$), indicating disrupted motor coordination caused by TiO_2-NP neurotoxicity. However, the oral administration of QS and MS extracts at 100 and 200 mg/kg body weight significantly ($P \leq 0.05$) preserved nerve function and motor coordination compared to the TiO_2-NPs group. No significant difference was observed between low and high doses of QSE and MSE in all behavioral tests except the Y-maze test, which shows no significant difference between low doses of QSE and MSE only.

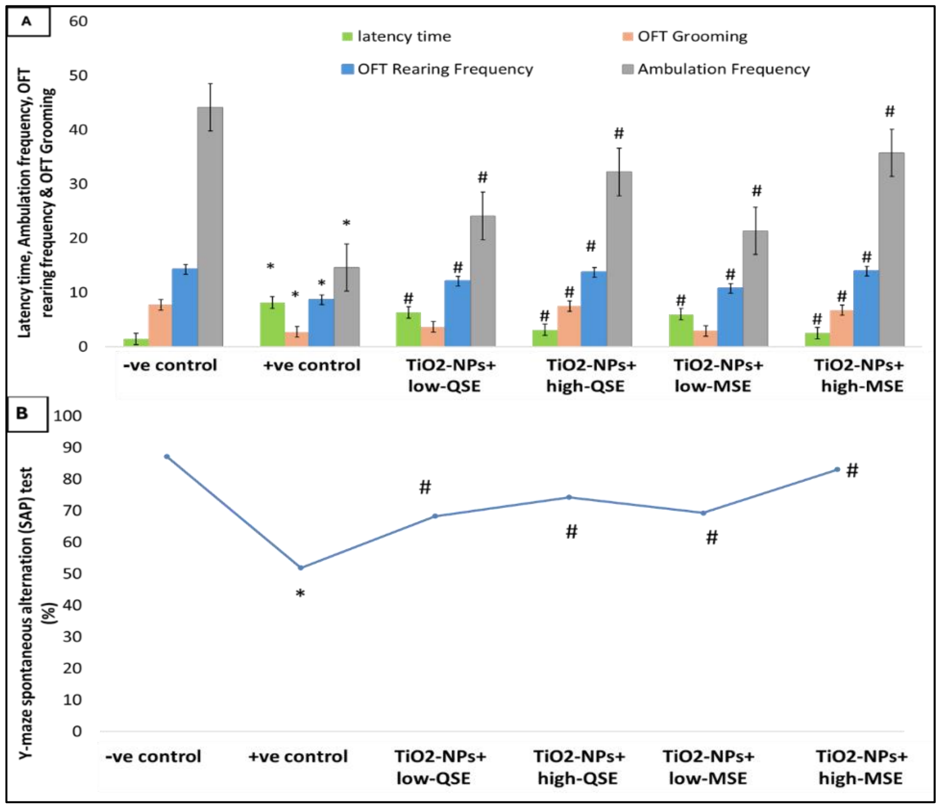

Figure S4. Behavioral tests of experimental groups. **(A)** Latency time, Ambulation frequency, OFT rearing frequency, and OFT Grooming. **(B)** Y-maze test (SAP). Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group;(#) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.4. Quinoa seeds and moringa seeds extracts inhibited TiO_2-NPs-induced oxidative stress.

Results presented in Figure S5 showed that TiO_2-NPs caused a significant decrease in serum TAC compared to the negative control group ($p \leq 0.05$). While QS and MS extracts, low and high doses administration caused a significant increase in the antioxidant capacity when compared to the TiO_2-NPs group. Moreover, TOS and OSI were significantly increased by TiO_2-NPs administration compared to the negative control group and significantly ($p \leq 0.05$) reduced by QS and MS extracts administration compared to the TiO_2-NPs control group. No significant difference was observed between the MSE high dose, QSE high dose, and the negative control group. Also, no significant difference was observed between the MSE low dose and the QSE low dose. Therefore, QS and MS extracts similarly inhibited oxidative damage caused by TiO_2-NPs.

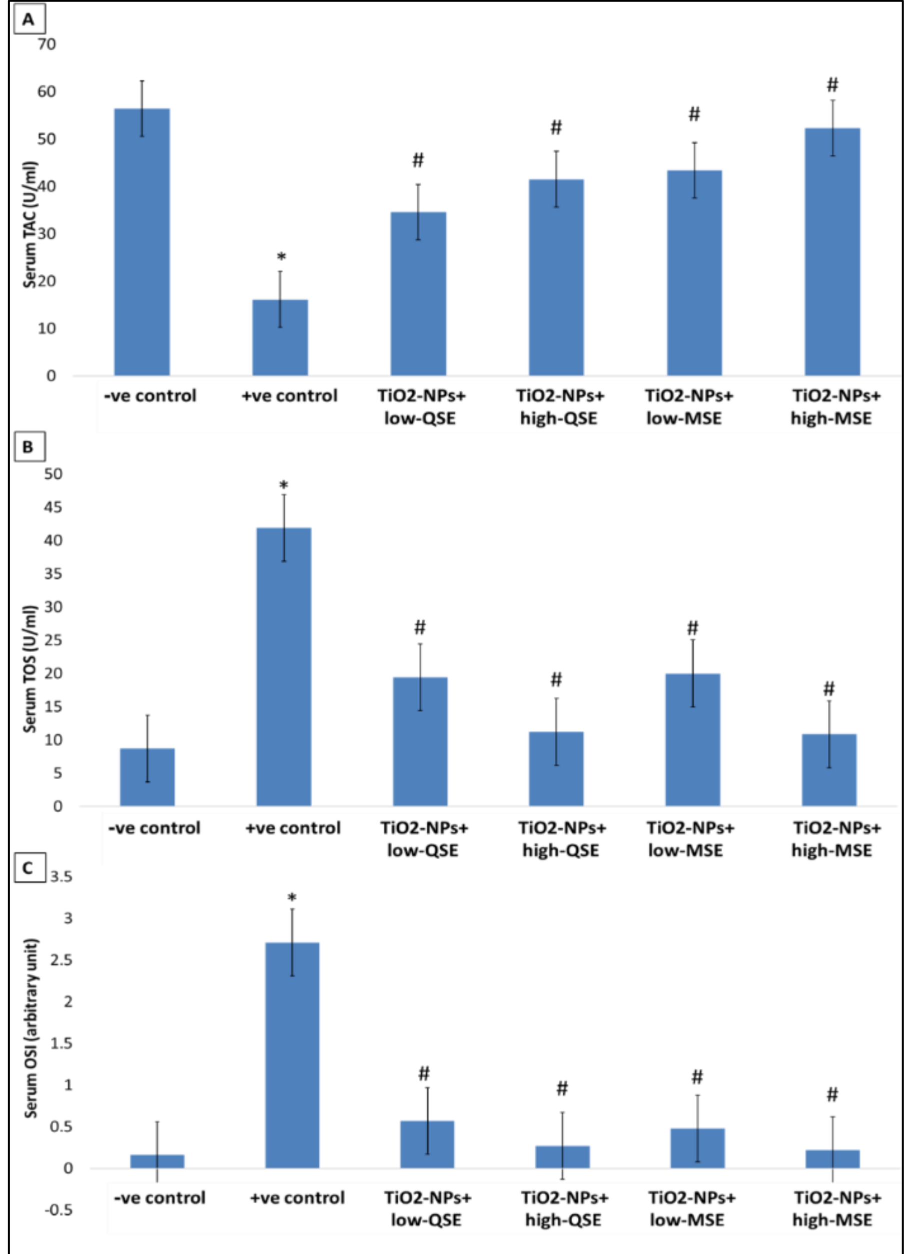

Figure S5. Serum TAC, TOS, and OSI of experimental groups. **(A)** TAC; **(B)** TOS; **(C)** OSI. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; ($^{\#}$) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.5. Downregulation of neuroinflammation by oral QSE and MSE in TiO_2-NPs intoxicated rats.

Oxidative stress caused by TiO_2-NPs was also associated with neuroinflammation. Results in Figure S6 confirmed the neuroprotective effect of QS and MS extracts. Serum levels of IL-1β, TNF-α, and brain tissue levels of TLR-4 and NF-κB were significantly ($p \leq 0.05$) increased in the TiO_2-NPs group compared to the negative control group. Treatment with QS AND MS extracts at the doses of 100 and 200 mg/kg b.wt. significantly reversed the elevated brain inflammatory biomarker levels compared to the TiO_2-NPs group. Thus, both QSE AND MSE possess an anti-inflammatory effect and a neuroprotective effect. Moreover, no significant difference was observed between the low dose of MSE and QSE in IL-1β. Also, no significant difference was observed between the high dose of MSE and QSE in TNF-α. So the high dose of MSE showed the most significant reduction in all inflammatory biomarkers.

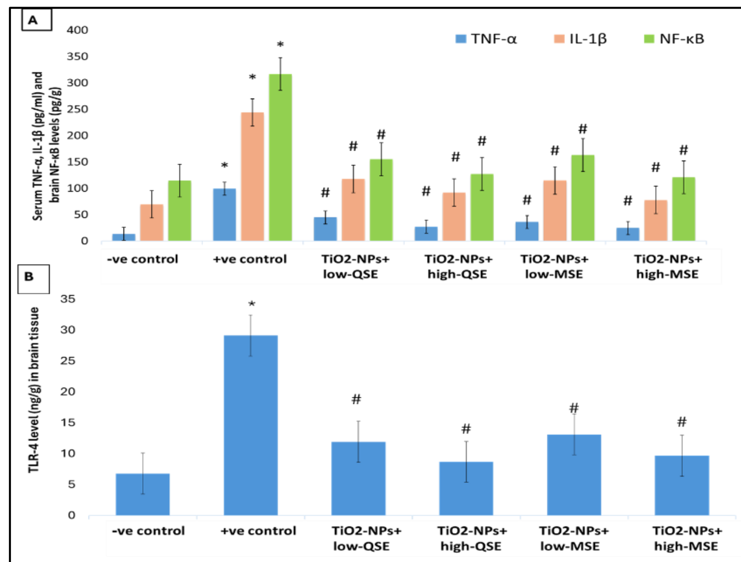

Figure S6. Some inflammatory biomarkers in experimental groups. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; (#) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.6. Oral administration of QSE AND MSE alleviated ERS and apoptotic gene expression in TiO_2-NPs-intoxicated rats' brains.

Administration of TiO_2-NPs induced endoplasmic reticulum stress (ERS) and apoptosis, as presented in Figure S7. Our results showed that TiO_2-NPs significantly ($p \leq 0.05$) upregulated the mRNA expression level of ERS (PERK, ATF, and CHOP) and pro-apoptotic genes (Caspase 3 and Bax) compared to the control rats. Coadministration with either low or high doses of QS AND MS extracts caused a significant ($p \leq 0.05$) reduction in the gene expression levels of PERK, ATF, CHOP, Caspase-3, and Bax compared to the control TiO_2-NPs intoxicated group. Notably, QS AND MS extracts at different doses efficiently restored the apoptotic machinery and reversed endoplasmic reticulum stress; the high dose showed the most significant ameliorative effect.

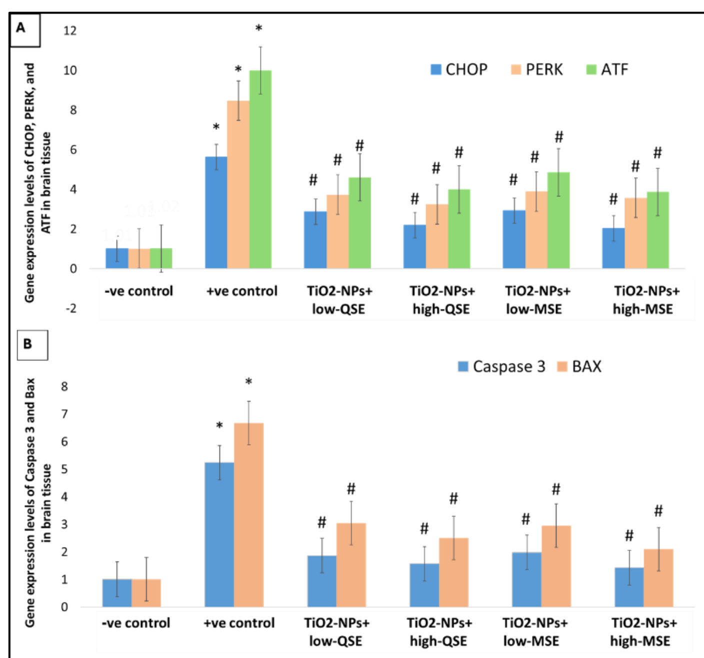

Figure S7. The mRNA expression of **(A)** ERS genes; **(B)** proapoptotic genes in the brain tissue of experimental groups. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; (#) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.7. Effect of oral administration of QSE AND MSE on cerebral mitochondrial function and DNA fragmentation % of experimental groups.

The present study showed that TiO_2-NPs markedly decreased cerebral mitochondrial function and increased the percentage of DNA fragmentation in brain tissues compared to the normal control group in Figures S8 and S9. Whereas low and high doses of QS and MS extracts significantly ($p \leq 0.05$) decreased DNA fragmentation % and increased cerebral mitochondrial function, which correlates with the reduction in oxidative stress as previously reported.

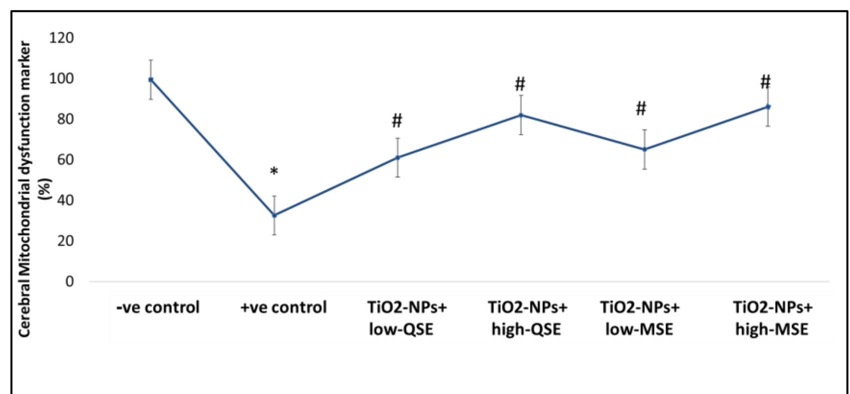

Figure S8. Cerebral mitochondrial function in brain tissues of experimental groups. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; ($^{\#}$) indicates significance at ($P \leq 0.05$), compared with the positive control group.

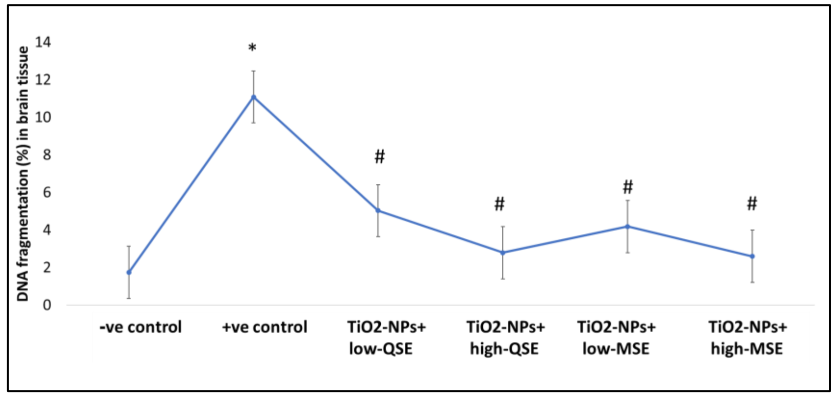

Figure S9. DNA fragmentation % in the brain tissues of experimental groups. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; ($^{\#}$) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.8. Regulation of some neuronal markers by QSE AND MSE in TiO_2-NPs intoxicated rats.

It has been reported that neuronal intoxication can be associated with altered neurotransmitter levels; our results in Figure S10 revealed that TiO_2-NPs administration caused a significant elevation in glutamate levels and AchE activity, with a reduction in melatonin levels compared to the healthy control group. Meanwhile, QS and MS extracts administration caused a significant ($p \leq 0.05$) reduction in glutamate levels and AchE activity with an elevation in melatonin levels compared to the TiO_2-NPs control group. Furthermore, there was a significant difference in melatonin and glutamate levels between MSE and QSE low and high doses; no significant difference was observed between quinoa and moringa groups in ACHE activity.

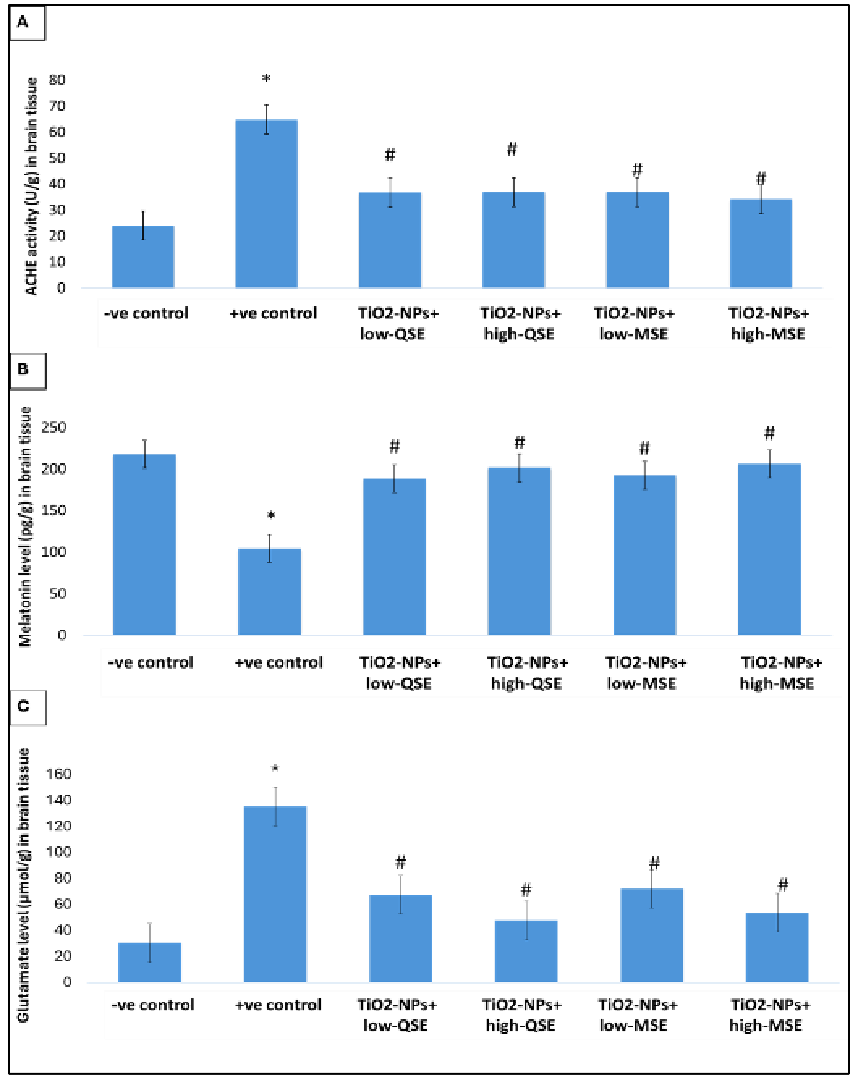

Figure S10. Melatonin, Glutamate levels, and ACHE activity in serum of experimental groups. Data are expressed as mean ±SD. (*) indicates significance at ($P \leq 0.05$), compared with the control group; (#) indicates significance at ($P \leq 0.05$), compared with the positive control group.

3.9. Histological examination or brain tissue sections.

Photomicrographs depicted in Figure S11 show the brain tissue of the cerebral cortex in the experimental groups. It reveals that the negative control group shows normal histological structures (Lesion Score: 0) with most neurons intact in a cerebral cortex section, which were severely disrupted in the intoxication group. TiO_2-NPs caused severe neurotoxicity, indicated by severe neuronal cell loss due to apoptosis (Lesion Score: +++), and diffuse cytotoxic edema (Lesion Score: +++). The coadministration of QS AND MS extracts at low and high doses ameliorated the neurotoxic effect of TiO_2-NPs. Cerebral cortex sections from rats administered low-dose QSE showed intracellular vacuolation and edema associated with the proliferation of glial cells (Lesion Score: ++). Cerebral cortex sections of rats in the TiO_2-NPs+ high dose-QSE group showed a moderate neuronal cell loss (Lesion Score: ++). Cerebral cortex sections of rats administered a low dose of MSE showed a congested blood vessel, perivascular and pericellular edema, and pyknosis (Lesion Score: +). Cerebral cortex sections of rats in the TiO_2-NPs+ high dose-MSE group showed a normal histological structure, and most neurons are intact (Lesion Score: 0). Moreover, the high dose MSE showed the most ameliorative effect and better preservation for the brain and neurons' normal structure. Histopathological results are in line with biochemical results.

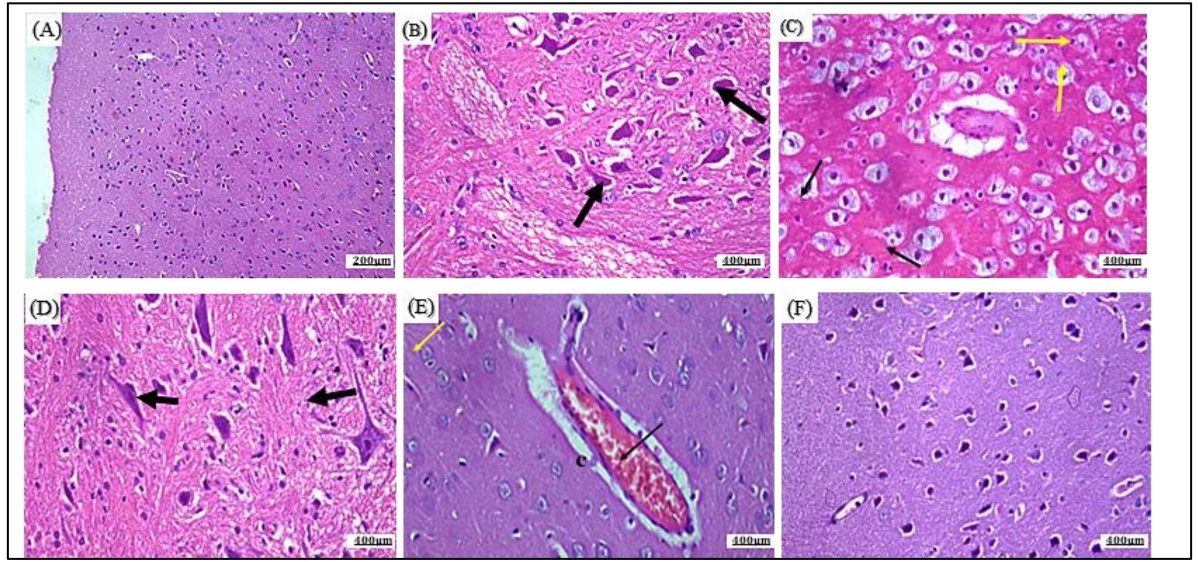

Figure S11. Photomicrographs of the cerebral cortex in different groups. **(A)** negative control group; showing a normal histological structure, and most neurons are intact, (Lesion Score: 0); **(B)** TiO_2-NPs group, showing severe neuronal cell loss (black arrows) due to apoptosis (Lesion Score: +++); **(C)** TiO_2-NPs group +QSE low dose; showing intracellular vacuolation and edema (black arrows) associated with proliferation of glia cells (yellow arrows), (Lesion Score: ++); **(D)** TiO_2-NPs group +QSE high dose; showing moderate neuronal cell loss (black arrows), (Lesion Score: ++); **(E)**TiO_2-NPs group +MSE low dose; showing congested blood vessel, Perivascular and pericellular edema (black arrow), and pyknosis (yellow arrow), (Lesion Score: +); **(F)** TiO_2-NPs group +MSE high dose; showing normal histological structure, and most neurons are intact, (Lesion Score: 0).

3.10. Discussion.

The brain is an essential organ since damage to it is difficult to heal. Consequently, NPs' neurotoxicity should be of great concern. Titanium dioxide nanoparticles can migrate toward the nervous system, where they may induce oxidative stress, neuroinflammation, tissue necrosis, and neuronal cell apoptosis, leading to cytotoxic effects and brain damage [35]. For this reason, we discussed how TiO_2-NPs can damage the brain and how natural extracts can reduce their neurotoxic risk.

The neurological alteration caused by TiO_2-NPs might affect the behavioral activities of animals; thus, the open field and Y-maze tests were applied in our study, which indicated significant alteration in the behavioral functions of TiO_2-NP-intoxicated rats. TiO_2-NPs could have inhibitory effects on locomotor activity, central square duration, and the distance traveled in the open field test's center, which examines mice's exploratory behavior and general activity [36]. It could also considerably lower the number of entries made into the Y-maze's new arms [37]. Our results matched those of Naima *et al*. [38], who found that the cognitive capacity and emotional reactivity were significantly disrupted in TiO_2-NPs-administered rats. This might be due to oxidative damage, altered neurotransmitter concentrations, neuronal death, reduced neurogenesis, and poor synaptic plasticity induced by TiO_2-NPs [39].

Oxidative stress is thought to be a major reason for the detrimental neurological effects of TiO_2-NPs, which produce excessive ROS in the brain and have cytotoxic effects. In particular, the brain's high energy requirements, low endogenous antioxidant levels, and high neuronal concentrations of polyunsaturated fatty acids make it extremely vulnerable to ROS-induced damage [35]. In our study, TiO_2-NPs induced a depletion of total antioxidant capacity, along with elevated total oxidative stress and oxidative stress index. It can cause oxidative

stress by producing multiple hydroxyl radicals, exacerbating DNA damage [40], and leading to a substantial drop in TAC levels, an increase in OSI, and increases in malondialdehyde and TOS, resulting in increased oxidative damage and neurological dysfunction [2].

Oxidative stress and inflammation are interrelated processes in a vicious cycle. Pro-inflammatory mediators can be secreted as a result of the redox imbalance and inflammatory pathway activation induced by TiO_2-NPs [41]. These agree with our results, in which the oral doses of TiO_2-NPs upregulated NF-κB, IL-1β, and TNF-α along with the activation of TLR4. Xiong *et al*. [3] reported that, regardless of size or surface coating, exposure to TiO_2-NPs could markedly increase ROS, NOS, IL-6, and TNF-α levels. By inducing Ca^{+2}/PKC/p38/MAPK signaling and upregulating NF-κB, TiO_2-NPs induced the overproduction of inflammatory cytokines [42].

Additionally, these NPs induce oxidative stress, which can impair the function of vital cell organelles, including the endoplasmic reticulum (ER) and mitochondria. The ER plays a crucial role in controlling the synthesis, folding, and transport of proteins as well as the storage of Ca^{+2}. High Ca^{+2} levels and a strongly oxidizing environment are necessary for the ER to support protein folding and allow disulfide bond formation [43]. Oxidative stress can thus result in ER stress and in the buildup of misfolded proteins [44], which leads to neurodegenerative and neuroinflammatory disorders. TiO_2-NPs can lengthen ER-mitochondrial contact sites and induce Ca^{+2} shifting to mitochondria, thus altering mitochondrial homeostasis, causing apoptosis, and ERS [45]. Specifically, TiO_2-NPs could increase the PERK, ATF, CHOP, and Bax gene expression in our study, indicating an enhanced neuronal ERS condition. One of the ERS-dependent feedback loops is the PERK/ATF/CHOP pathway [46]. It has been reported that TiO_2-NPs can induce ERS by sharply upregulating CHOP expression, which controls the ratio of pro-apoptotic (Bax) to anti-apoptotic B-cell lymphoma-2 (Bcl-2) family proteins, thereby initiating apoptosis [47,48]. Through nuclear pyknosis and caspase 3 activation, TiO_2-NPs can also result in damage to hippocampus neurons and decreased neuronal cell viability [49].

The synthesis of ATP, the synthesis and degradation of many metabolites, and the generation and elimination of ROS are all processes that mitochondria can regulate [50]. Our results indicated a significant reduction in mitochondrial activity and ATP production after oral administration of TiO_2-NPs. These nanoparticles can enter mitochondria, enlarge the tubules, and damage mitochondrial structure, thereby interfering with their function [51]. TiO_2-NPs can demolish the adenine and phosphate moieties of ATP and disrupt mitochondrial respiration [5]. Early-life neurodegeneration was linked to mitochondrial damage, protein misfolding, and other subcellular oxidative damage caused by inhaled TiO_2 nanorods. ROS can directly damage DNA and the mitochondrial membrane, leading to mitochondrial dysfunction, increased permeability of the mitochondrial transition pores, and apoptotic cell death [35]. This could be confirmed in our study by histological examination of brain tissues. The cerebral section of rats treated with TiO_2-NPs showed severe neuronal cell loss due to apoptosis and diffuse cytotoxic edema.

Furthermore, oral administration of TiO_2-NPs leads to an elevation in DNA fragmentation in rats' brain tissue, as shown in our results. TiO_2-NPs can induce DNA damage through ROS generation, interference with DNA repair proteins, and epigenetic modifications [52]. Our results matched those of Mohamed *et al*. [53], who reported that TiO_2-NPs produced more ROS, which resulted in severe DNA damage. It can directly disrupt the structure and functionality of genomic DNA, leading to chromosomal damage, genotoxicity, and DNA

double-strand breaks. Ali and his colleagues [54] reported that when mice were given varying dosages of TiO_2-NPs (50, 250, and 500 mg kg-1 body weight, NPs sized at 21 and 80 nm) for five consecutive days, chromosomal abnormalities occurred at a dose-dependent pace, with the smaller particles exhibiting increased genotoxicity. This led to changes in brain processes and the death of neuronal cells. For that, we measured the concentrations of a few neurotransmitters in the brain tissue of rats.

Our study showed significant alterations in neurotransmitters, a marked reduction in melatonin, and an elevation in glutamate levels and acetylcholinesterase activity after administration of TiO_2-NPs. In line with our results, Naima *et al.* [38] found that rats given TiO_2-NPs exhibited notable alterations in the neurotransmitter composition of their brains as a result of oxidative brain injury. Among these, TiO_2-NPs were found to dramatically reduce melatonin, which has a potent antioxidant effect and protects against oxidative stress [55]. Through several pathways, such as oxidative stress, apoptosis, neuroinflammation, and compromised synaptic plasticity, it can also cause neurotoxicity, resulting in compromised metabolism of neurotransmitters, particularly glutamate and dopamine [56]. Hu and his colleagues [57] also demonstrated that adrenergic, cholinergic, dopaminergic, and serotonergic neurotransmitter systems were significantly altered in mice that received intragastric gavage of TiO_2-NPs, as evidenced by a marked increase in glutamate and NO levels, as well as AChE activity. AChE is a crucial enzyme for the hydrolysis of acetylcholine (ACh), a cholinergic neurotransmitter essential for peripheral and central nervous system function, and may cause a subsequent disturbance in neuronal excitability [58].

Functional foods contain specific components that benefit our health and wellness beyond their nutritive value. Moringa and quinoa seeds are functional foods, possessing antioxidant activities due to their bioactive components [59]. Our results concerning the quantitative analysis of phytochemicals by HPLC in MSE and QSE indicated the presence of higher contents of total polyphenols and flavonoids in the red QSE. The QSE analysis showed a higher percentage of protocatechuic acid and ferulic acid, while the MSE showed a higher percentage of daidzein and chlorogenic acid. A study by Liu and his colleagues [60] found that Protocatechuic acid, ferulic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, P-coumaric acid, sinapic acid, and isoferulic acid are among the phenolic acids found in quinoa seeds. It should be noted that the antioxidant activity and phenolic profiles of quinoa vary depending on the color of the seeds. Compared to white seeds, the darker quinoa seeds—such as red and black quinoa—have more phenolics, flavonoids, and antioxidant activity [61]. Similarly, the abundance of phenolic content in moringa seeds indicates their high antioxidant activity [62]. Similar to our findings, Singh and his colleagues [63] found that the phenolic compounds present in moringa seed extract could include gallic acid, catechin, coumaric acid, sinapic acid, chlorogenic acid, caffeic acid, and salicylic acid, while the flavonoids included kaempferol and quercetin.

Notably, our research showed that rats' behavioral activity in the Y-maze and open-field tests was significantly enhanced when QSE or MSE was coadministered with TiO_2-NPs. The study by Abdelghany and colleagues [64] showed that after 70 days of oral gavage with an ethanolic extract of Moringa oleifera at a dose of 200 mg/kg body weight, the spontaneous alternation behavior percentage (SAP) in the Y-maze test increased. In a separate investigation, motor dysfunction in the open field test significantly improved in mice given 200 mg/kg of MSE orally for 28 days. They were also protected from locomotor deficiencies by their antioxidant properties. This antioxidant action is associated with their high phenolic content

[24]. The ethanolic extract of M. oleifera seeds has shown a neuroprotective potential when mice were administered scopolamine [65]. In fact, MSE contains several bioactive compounds with antioxidant properties that may protect Substantia Nigra (SN) neurons and mitigate the severity of motor impairment in rats [66]. Similarly, Terreros and associates [67] demonstrated that locomotor activity was not affected by stress treatment during the quinoa seed-based diet's. It significantly decreased the quantity of entries made into the Y-maze's new arms. Consuming quinoa effectively regulated spatial memory, preventing the stress-induced memory deficit and modulating cognitive characteristics. Quinoa seeds' neuroprotective properties are related to the vast range of phenolic compounds with potent antioxidant properties [68].

Coadministration of quinoa and moringa seed extracts with TiO_2-NPs markedly restored the antioxidant capacity of brain tissue in our study. Following our results, Souza and his colleagues [9] showed that because of the high content of total phenolic compounds and total flavonoids, oral red quinoa seed extract (100 mg/kg for 7 days) completely restored the antioxidant indicators in the cerebral cortex and hippocampal regions. Effective antioxidant activity has been demonstrated by quinoa ethanolic extract in 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests [69]. Aniess and his colleagues [70] indicated that rats fed a meal supplemented with 5g and 10g of red quinoa (RQ) seeds showed a considerable increase in antioxidant enzymes. The various phenolic compounds and flavonoids found in QSE confirm its antioxidant activity. According to recent research, those phenolic compounds possess a variety of pharmacological activities, particularly anti-inflammatory, anti-apoptotic, and antioxidant properties, as well as neuroprotective functions [71]. They can suppress ROS production, scavenge free radicals, boost Nrf2 expression, and alter multiple signaling pathways that combat oxidative stress [72]. Moreover, Alanazi *et al.* [73] demonstrated that mouse brain tissues treated with 50 and 100 mg/kg b.wt. MSE markedly raised antioxidant markers and lowered oxidative stress markers. Furthermore, MSE has anti-inflammatory, antioxidant, and neuroprotective properties [74]. Also, Al-Obaidi and his colleagues [75] reported that TAC was significantly increased after 45 days of administration of 160 mg/kg b.wt./day of M. oliefera seed extract, whereas TOS and OSI were dramatically reduced. Therefore, by triggering the antioxidant signaling response and increasing cellular antioxidant content, moringa seeds demonstrated a notable suppression of oxidative stress biomarkers. Their bioactive components, flavonoids and phenolics, exhibit numerous therapeutic benefits, including tackling oxidative stress, scavenging free radicals, and enhancing neuroprotection [76].

Notably, rats administered the ethanolic extracts of quinoa and Moringa seeds showed a marked improvement in the neuroinflammatory condition associated with TiO_2-NPs in the present study. The quinoa's anti-inflammatory properties can be confirmed by Ng and Wang [7], who suggested that quinoa supplementation reduces the overproduction of inflammatory mediators, as seen by a notable drop in the percentage of DNA fragmentation and serum levels of TNFα, IL-1β, and IL-6. Quinoa's phytochemical composition may contribute to its anti-inflammatory properties. Flavonoids and phenolic acids found in quinoa seeds have several physiological benefits, including antioxidant, anti-inflammatory, antibacterial, cardioprotective, and anticarcinogenic effects [77]. Repressing NF-κB, a crucial modulator of the proinflammatory cytokine signaling pathway, can reduce the production of proinflammatory molecules [78]. Similarly, by enhancing Nrf_2 signaling, increasing antioxidant activity, and lowering proinflammatory cytokine levels like IL-1β and TNF-α, the ethanolic extract of moringa seeds demonstrated neuroprotective potential [79]. In addition,

Mthiyane and his colleagues [74] found that by blocking NF-κB translocation or boosting Nrf2's antioxidant response, MSE reduces oxidative stress and undesirable pro-inflammatory response. The phenolic compounds in MSE potentially protect the nervous system from oxidative stress and neuroinflammation via their antioxidant and anti-inflammatory properties [80]. Phenolic substances may have neuroprotective benefits by modulating metabolic and anti-inflammatory/oxidative pathways to counteract inflammatory mediators and inflammation-related tissue injury [81].

Meanwhile, the antioxidant and anti-inflammatory properties of QSE and MSE are reflected in protecting against the ERS and apoptotic events associated with TiO_2-NPs via the marked reduction in PERK, ATF, CHOP, caspase 3, and Bax gene expression in our results. The presence of antioxidant components, such as bioactive phenolic compounds, is responsible for the strong antioxidant activity of the seeds [82]. Interestingly, ferulic acid, one of the phenolic acids in QSE, significantly elevated Bcl-2 levels while suppressing caspase-3 and Bax levels in rat brain tissues. This is attributed to ROS and cellular redox state modulation [83]. The phenolic compounds in quinoa seeds may alleviate oxidative stress and suppress the expression of ERS markers CHOP, GRP78, and eIF2α [84]. Moreover, Ekici and his colleagues [85] reported that sinapic acid, present in QSE, possesses potent anti-inflammatory and antioxidant activities in the cortex and hippocampus of rats. Additionally, it decreased the levels of hippocampus caspase 3 and caspase 9. Similarly, MSE suppresses oxidative stress by empowering the Nrf_2 cellular antioxidant signaling pathway, which has a feedback response, preventing ERS and its mediators [86]. In agreement with our results, Kandeil and his colleagues [2] showed that rats received 100 mg/kg b.wt. of MSE for 30 days showed an anti-apoptotic effect through Nrf2 activation that reduced caspase-3 concentration in their brain tissues. Through the downregulation of apoptosis executors, Nrf2 shields the cell from cellular apoptosis [87]. It has been speculated that moringa seed extracts inhibit caspase activation, prevent cytochrome c release from mitochondria, and downregulate the expression of proapoptotic markers while upregulating Bcl-2 expression [88].

Notably, the coadministration of QSE and MSE with TiO_2-NPs could significantly enhance mitochondrial activity. An effect that might be related to their potent antioxidant properties. In line with our results, Hu and his colleagues [89] showed that Quinoa seeds (100, 400, and 800 mg/kg b.wt.) given orally for eight weeks reduced oxidative stress in the brain tissues of mice; this effect may have been caused by a decrease in lipid peroxidation and an increase in antioxidant enzymes. This could hinder mitochondrial malfunction by preserving DNA stability and mitochondrial membrane integrity. Quercetin, a naturally occurring flavonoid in quinoa seeds, protects neuronal cells from mitochondrial damage caused by neuroinflammation [90]. Cinnamic acid and ferulic acid (FA) are strong antioxidants. In rat neurons, they can reduce oxidative stress, restore mitochondrial dynamics, and stop mitochondrial malfunction, all of which may boost the production of ATP in brain tissue [91, 92]. In agreement with our results, Kandeil and his colleagues [2] found that rats given 100 mg/kg b.wt./day of MSE for 30 days showed increased mitochondrial viability in their brains. Increased Nrf2 expression suppresses pro-inflammatory and apoptotic mediators and restores antioxidant activity. The availability of NADH and FADH2/succinate substrates, which are necessary for mitochondrial function and cellular respiration, can be enhanced by the antioxidant activity of MSE flavonoids and phenolics [86]. Catechin, one of the bioactive substances in MSE, can improve ATP levels by 50–85% in mitochondria isolated from the rat hippocampus, cortex, and striatum, decrease ROS production, and restore mitochondrial

respiratory rates and mitochondrial membrane potential [93]. These results can be confirmed by the histological features observed in our results, a significant amelioration of brain tissue damage and preservation of intact neurons after administration with QSE and MSE.

In the current investigation, oral administration of either QSE or MSE significantly decreased the proportion of DNA fragmentation caused by oral doses of TiO_2-NPs. Hu and his colleagues [89] demonstrated that quinoa seeds (administered orally at up to 800 mg/kg b.wt.) for 8 weeks reduced oxidative stress and lipid peroxidation in mouse brain tissue, consistent with our findings. This can protect neuronal DNA from oxidative damage and fortify the machinery that repairs nuclear DNA. Our findings indicate that the bioactive phenolic compounds in QSE have a strong potential to reduce oxidative brain damage and prevent DNA damage. In addition to reducing oxidative stress and cytokine toxicity, quercetin may lower the percentage of DNA fragmentation in rat brain cells [94]. In line with our results, Kandeil and his associates [2] found that moringa seed extract dramatically reduced DNA fragmentation, lowered TiO2-NP-induced oxidative damage, and boosted antioxidant activity in the brain. Furthermore, it was found that MSE at a dose of 100 mg/kg prevented oxidative damage and suppressed the pro-inflammatory signaling cascade, thereby protecting neuronal DNA from oxidative damage [73]. This could be because of the dramatic increase in Nrf2 expression, which strengthens the cytoprotective response of brain cells by inhibiting pro-inflammatory and apoptotic mediators [2].

In the brain tissue of rats given oral doses of TiO_2-NPs, the neuronal cytoprotective action of QSE and MSE also manifested as a notable regulation in neurotransmitters. Souza and his colleagues' findings [9] corroborate our findings, which reported that rats administered red quinoa seed extract (100 mg/kg b.w.) for seven days show low AChE activity in their hippocampal and cerebral cortex. This is due to the high concentrations of flavonoids and phenolics, which have antioxidant and AChE-inhibiting properties, thereby preventing neurodegeneration. The polyphenolic compounds in quinoa seed extracts, both in aqueous and organic solvents, exhibit AChE-inhibitory activity and enhance memory function simultaneously. PCA, a component of QSE, can dramatically elevate GABA levels, lower AChE activity and glutamate levels, and prevent excessive ROS production, neuroinflammation, and β-amyloid plaque accumulation in the central nervous system, thereby preventing several neurodegenerative diseases and protecting against behavioral and cognitive deterioration [95]. Rutin can also decrease the quantity of excitatory amino acids such as glutamate and glutamatergic transmission in the extracellular space in the cortical and subcortical regions of rats [96]. Similarly, Zhou and his colleagues [65] found that oral MSE treatment at 200–400 mg/kg BW for 7 or 21 days effectively blocked the hyperactivation of AChE and dramatically reversed the decline in ACh in the brain tissues of mice. MSE exhibits neuroprotective effects via empowering cholinergic activity, synaptic plasticity, and hippocampus neurogenesis. Furthermore, several phenolic compounds found in moringa seeds, including gallic acid, catechin, quercetin, rutin, and quercitrin, are strong antioxidants and modulators of brain neurotransmitter levels [97]. Several polyphenolic bioactive compounds protect the cerebral cortex from glutamate-induced neuronal cell death and prevent neurodegenerative disorders by regulating Ca^{+2} entry into neurons [98].

Lastly, all biochemical results were confirmed by the cerebral histopathological features. The oral administration of MS and QS extracts at low and high doses mitigated the neurotoxic effect of TiO_2-NPs, showing better preservation of brain cells and less damaging effects of TiO_2-NPs. Moringa seed at low and high doses offered better protection against TiO_2-

NPs. The moringa high dose showed the most significant neuroprotective effect, as indicated by normal histological structure, as indicated by lesion scores and photomicrographs, with most neurons intact.

4. Conclusions

Quinoa and moringa seeds are promising functional foods with neuroprotective activity against TiO_2-NPs intoxication. The widespread use of TiO_2-NPs in the food industry as a food colorant has led to severe neurotoxic effects, including neurobehavioral changes, oxidative damage, inflammation, endoplasmic reticulum stress, neuroapoptosis, mitochondrial damage, and DNA damage. Herein, the neuroprotective effect of QS and MS ethanolic extracts was mechanistically mediated through modulation of oxidative/antioxidant biomarkers, the pro-inflammatory cascade, ERS, and pro-apoptotic cascades, and protection against mitochondrial and DNA damage. This was reflected in enhanced locomotor activities and anxiety behavior, as well as the neurotransmitter levels in the brain. Thereby, Quinoa and moringa seeds are promising candidates for their effectiveness against the neurotoxicity of TiO_2-NPs in rats.

Author Contributions

Conceptualization, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; methodology, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; software, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; validation, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; formal analysis, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; investigation, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; resources, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; data curation, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; writing—original draft preparation, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; writing—review and editing, H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E.; visualization, A.A.M.E.-R., H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E; supervision, H.M.A.E.-F., N.G.E.-R. and H.M.I.A.-E. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study experimental measures were performed according to the recommendations for the care and use of laboratory animals and following the local animal ethics committee of Ain Shams University (#ASU/W/Sci-6M/23-3-55).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Acknowledgments

Not applicable.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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