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Hesperidin Attenuates Titanium Dioxide Nanoparticle-Induced Neurotoxicity in Rats by Regulating Nrf-2/TNF-α Signaling Pathway, the Suppression of Oxidative Stress, and Inflammation

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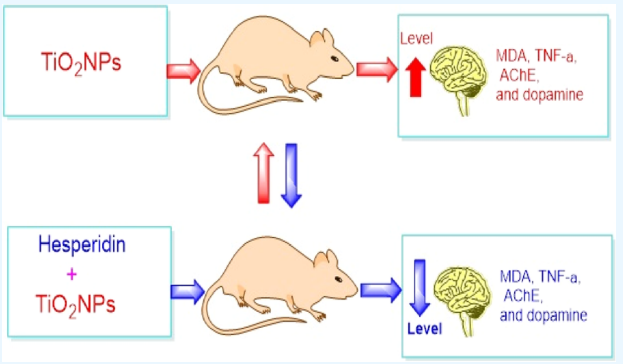
ABSTRACT: Background: Titanium dioxide nanoparticles (TiO_2NPs) are widely utilized and consumed mainly as food additives. Oxidative stress is considered to be the basic effect of TiO_2NPs through biological interactions. Hesperidin (HSP) is a bioflavonoid (flavanone glycoside) with lipid-lowering, inflammation, oxidative stress suppression, antihypertensive, cancer-fighting, and antiedema effects. **Objective**: This study was to investigate the possible protective influences of HSP of subchronic oral TiO_2NP exposure on the brains of rats, including neurotransmitters, oxidative stress/antioxidant parameters, inflammatory markers, and histological changes in the brains of adult male albino rats. **Methodology:** The experiment was executed on 80 albino rats. The animals were randomly divided into 4 equal groups. The first group served as a control; the second group was treated with oral doses of HSP (100 mg/kg Bw daily); the third group received TiO_2NPs (200 mg/kg Bw orally daily); and the fourth group was treated with TiO_2NPs and an oral dose of HSP daily for 8 weeks. Blood samples were obtained for biochemical analysis. Neurotransmitters, oxidative stress biomarker levels, and inflammatory markers were measured in brain homogenates. Histological examination of the brain was performed through H&E staining. **Results**: Coadministration of hesperidin with TiO_2NPs orally for 8 weeks decreased the levels of MDA, TNF-α, AChE, and dopamine in brain homogenates, which were increased in the TiO_2NP group. It increased the other oxidative biomarkers (SOD, CAT, and GPx) and Nrf-2 expression levels. Brain histological sections of the TiO_2NP-treated group show degeneration, necrosis, congestion, and inflammatory cell infiltration that decreased markedly in the coadministration of hesperidin with the TiO_2NP group. **Conclusion:** Hesperidin cotreatment offers significant protection against TiO_2NP-induced oxidative stress and biochemical and histological alteration in the brain.



1. BACKGROUND

Because of its intriguing features and uses, nanotechnology has garnered a lot of interest throughout the years.[1] Nanoparticles are particles that range in size from one to one hundred nanometers and are formed of carbon, metal, metal oxides, or organic substances.[2] Titanium dioxide nanoparticles (TiO_2NPs) are a type of nanomaterial that has become widely employed in several applications in recent years, including food, pharmaceutical additives, paints, paper, cosmetics, sunscreens, electronics, and other industrial items.[3]

Numerous researchers have been undertaken to investigate the harmful effects of TiO_2NPs and have demonstrated their capacity to pack in various tissues and organs in rodents and aquatic creatures.[4] TiO_2NPs were absorbed by the GIT after oral dosing, with extremely limited bioavailability and delayed tissue clearance.[5] TiO_2NP accumulation in hepatic, cardiac, and neurological tissues has previously been documented.[4]

Hesperidin [(HSP), 3,5,7-trihydroxy flavanone-7-rhamnoglucoside], is a bioflavonoid abundant in all citrus fruits, such as oranges, lemons, and grapefruits that have active pharmacological activities.[6] HSP has been shown to have lipid-lowering, inflammation and oxidative stress suppression, antihypertensive, cancer-fighting, and antiedema effects.[7]

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In the current research, we examined the drawbacks of subchronic oral TiO_2NP exposure on the brain of rats to comprehend its neurotoxicity as well as the potential protective impact of hesperidin on diverse neuronal biomarkers, such as neurotransmitters, oxidative stress/antioxidant variables, inflammatory markers, and histological alterations in the brain of adult male albino rats.

2. MATERIALS AND METHODS

The current research was performed following the recommendations for the care and use of laboratory animals confirmed by the Research Ethical Committee of Beni-Suef University, Egypt.

2.1. Chemicals. Titanium dioxide (TiO_2) was obtained from Sigma-Aldrich Chemical Co. in the United States. Titanium dioxide nanoparticles (TiO_2NPs) were adjusted at Beni-Suef University's Nanotechnology Unit, which is part of the School of Postgraduate Studies in Advanced Sciences. X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to characterize the TiO_2NP fine powder that was produced. Just before oral delivery, solutions of dispersed TiO_2NPs were produced by ultrasonication for 15 min. Hesperidin is available as a light brown powder from Sigma-Aldrich Chemical Co. in the United States. Biodiagnostic Co. (Giza, Egypt) and Sigma Chemical Co. (St. Louis) supplied all of the residual chemicals and diagnostic kits.

2.2. Animals and Treatment. The experiment has been conducted on 80 mature male albino rats weighing between 100 and 150 g. All animals were kept in cages made of plastic in a room that was kept at a constant, suitable atmospheric temperature (22 °C) and humidity level of 50%. The experimental rats experienced a 12 h cycle of light and dark. Before beginning the experiment, the animals were given unrestricted access to food and water in well-ventilated rooms for 2 weeks. Four groups of 20 rats each were allocated. The first group was given distilled water and served as the control group. According to Menze et al.,[8] the second group of rats received hesperidin at a dosage of 100 mg/kg (BW) daily. According to Chen et al., the third group was prepared for the daily delivery of TiO_2NPs at a dosage of 200 mg/kg (BW). In the fourth group,[9] rats were given TiO_2NPs as well as hesperidin (TiO_2NPs + HSP; 200 and 100 mg/kg BW/day, respectively). Both TiO_2NPs and hesperidin were newly produced soon before administration according to the manufacturer's directives. For 8 weeks, all therapies were administered orally.

Lastly, blood sampling was from the retro-orbital venous plexus. Centrifugation at 3000 rpm for 10 min was followed. All rats were sacrificed through decapitation under light anesthesia by intraperitoneal injection of 50 mg/kg ketamine, and the following examinations were performed.

2.3. Brain Tissue Homogenates: Preparation and Estimation of Neurotransmitters. Each animal's brain was excised whole and cleaned, and the remaining blood was washed away with 0.01 M, pH = 7.4, precooled phosphate-buffered saline (PBS). After weighing the tissue, it was cut up and homogenized in PBS using a glass homogenizer on ice. The homogenates are then centrifuged at 5000*g* for 5 min to get the supernatant. Following that, the brain supernatant was collected and processed according to neurotransmitter guidelines.[10] Dopamine was determined using the Rat Dopamine (DA) ELISA Kit provided by CUSAPIO using the technique described by Jia et al.[11] Acetylcholine esterase (AChE) was measured using the Rat Acetylcholine Esterase (AChE) ELISA Kit provided by CUSAPIO and the technique outlined by Ellman et al.[12] Glutamate was determined using BioAssay Systems' EnzyChromTM Glutamate Assay Kit (EGLT-100) using the method given by Matsumura and Miyachi.[13]

2.4. Measurement of Oxidative Stress Parameters. SOD was measured photometrically using BIODIAGNOSTIC SOD test kits and the technique reported by Nishikimi et al.[14] CAT was calculated photometrically using CAT test kits provided by BIODIAGNOSTIC and the Aebi technique.[15] MDA was measured photometrically with MDA test kits provided by BIODIAGNOSTIC and the technique described by Ohkawa et al.[16] GPx was measured photometrically using BioVision's GPx Colorimetric Assay kits and the technique published by Rotruck et al.[17]

2.5. Gene Expression. The RNeasy Mini Kit (Qiagen Cat No./ID: 74104) was used to isolate total RNA from the brain.[18] First-strand cDNA was synthesized according to the manufacturer's instructions using SuperScript Reverse Transcriptases (Thermo Scientific).[19] SYBRTM Green PCR Master Mix (Thermo Scientific Cat number: 4309155) was used for quantitative polymerase chain reaction (PCR). The ABI Prism Step One Plus Real-Time PCR System (Applied Biosystems) was used, as directed by the manufacturer.[20] The expression of target mRNAs was standardized to that of ACTB (Table 1).

Table 1. Primer Sets of the Assessed Genes

gene	GenBank accession number	gene sequence
TNF-α	NM_012675.3	F:ACACACGAGACGCTGAAGTA
		R:GGAACAGTCTGGGAAGCTCT
Nrf-2	NM_031789.2	F:TGTAGATGACCATGAGTCGC
		R:TCCTGCCAAACTTGCTCCAT
ACTB	NM_031144.3	F:CCGCGAGTACAACCTTCTTG
		R:CAGTTGGTGACAATGCCGTG

2.6. Histopathological Examination. The rats in the 4 experimental groups were autopsied. The brain samples were then treated with a 4% paraformaldehyde solution for a whole day. All samples were rinsed in tap water before being serially diluted with 100% ethyl alcohol to dehydrate them. Specimens were treated in xylene and immersed in paraffin for 24 h in a 56 °C adjusted air oven. Sledge microtome slices were cut from blocks of paraffin beeswax tissue at four microns in thickness. The slates were mounted on glass slides, deparaffinized, and stained with H&E stain for histological inspection using a light microscope.

2.7. Statistical Analysis. SPSS (SPSS Inc., Chicago, Illinois) was utilized for statistical analysis. The analysis of variance (ANOVA) test was used to determine the level of significance among various treatment groups. The data that were not normally distributed, as determined by the Shapiro–Wilk Normality Test, were analyzed using the Kruskal–Wallis test (a nonparametric ANOVA test), followed by Bonferroni posthoc analysis for pairwise comparison to determine the significance among the examined groups. At *p*-values of 0.05, differences in means were deemed statistically significant. The gene expression calculation method: The results of the qRT-PCR were evaluated by harnessing CT, ΔCT, ΔΔCT, and $2^{-\Delta\Delta CT}$.[21]

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3. RESULTS

3.1. Characterization of TiO_2 NPs. XRD patterns of activated TiO_2NPs show that they are made in a semicrystalline anatase juncture with an average crystallite size of 27 nm. SEM photomicrographs show that the TiO_2 spherical nanoparticles are homogeneous in size and shape (Figure 1a,b).

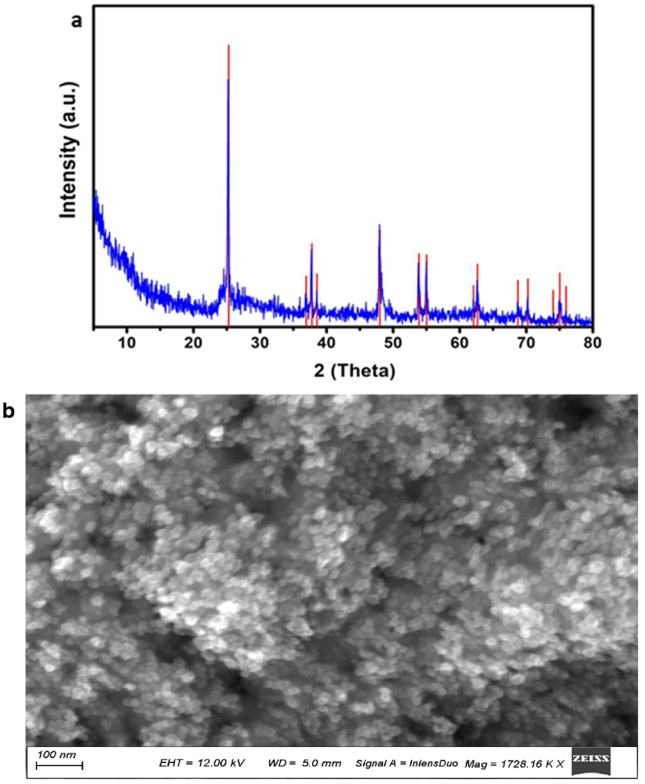

Figure 1. (a) X-ray diffraction patterns of TiO_2NPs. (b) SEM image showing the spherical shape of TiO_2NPs.

3.2. Acetylcholine Esterase Activity and Brain Neurotransmitters. As demonstrated in Table 2, the activity of AChE plus dopamine was considerably enhanced in TiO_2NP-intoxicated rats, but glutamate levels were significantly lowered ($p \geq 0.05$). Hesperidin substantially decreased AChE enzymatic activity and dopamine levels in the TiO_2NPs + HSP rats compared to the TiO_2NP group ($p \geq 0.05$). Hesperidin treatment did not affect AChE enzyme, dopamine, or glutamate levels when compared to the control rats ($p \geq 0.05$).

3.3. Oxidative/Antioxidant Parameters. Table 3 shows that, compared to control rats, oral delivery of TiO_2NPs to male rats raised MDA levels ($p \geq 0.05$), while hesperidin therapy resulted in a nonsignificant drop in MDA levels. However, in the TiO_2NPs + HSP group, coadministration of hesperidin substantially decreased the rise in MDA levels caused by TiO_2NPs alone ($p \geq 0.05$). In terms of antioxidant parameters, the vigor of SOD, CAT, and GPx in the TiO_2NP rat group was considerably lower than that in the control group. When compared to the TiO_2NP-treated group, hesperidin considerably enhanced its activity ($p \geq 0.05$).

3.4. Oxidative/Antioxidant Parameters. *3.4.1. Gene Expression.* TiO_2NP intake upregulated the expression levels of TNF-α but downregulated the expression levels of Nrf-2 significantly compared with that of control rats. Treatment of TiO_2NPs with hesperidin reversed the previous expressions (Figure 2).

3.4.2. Histopathological Examination. As shown in Figure 3, brain cut sections of the control rats showed the average histological structure of both the hippocampus and cerebral cortex. The hesperidin-treated group showed minimal necrosis of neurons in the hippocampus and mild congestion in the cerebral cortex. Sections in the TiO_2NP-treated group were markedly affected, showing intensive degenerative changes and necrosis of neurons in addition to vacuolation in the hippocampus and also intensive multifocal degenerative and necrotic changes in the cerebral cortex layers when matched

Table 2. Brain Neurotransmitters in the Studied Different Groups

groups		control	HSP	TiO_2NPs	TiO_2NPs + HSP	*p*-values	
DA (ng/mL)	mean ± SD	0.27 ± 0.07	0.22 ± 0.06	1.40 ± 0.30	1.00 ± 0.20	<0.001*	0.752[a]
							<0.001*[b]
							<0.001*[c]
	range	0.21−0.34	0.17−0.28	1.12−1.72	0.82−1.22		**<0.001***[d]
							<0.001*[e]
							0.032*[f]
AChE (pg/mL)	mean ± SD	10.21 ± 1.04	9.23 ± 0.96	29.67 ± 3.47	19.78 ± 2.31	<0.001*	0.601[a]
							<0.001*[b]
							<0.001*[c]
	range	9.06−11.09	8.16−10.02	26.37−33.29	17.58−22.19	<0.001*	**<0.001***[d]
							<0.001*[e]
							<0.001*[f]
GLUTAMATE (mM)	mean ± SD	0.99 ± 0.03	1.08 ± 0.04	0.43 ± 0.13	0.56 ± 0.17	<0.001*	0.305[a]
							<0.001*[b]
							<0.001*[c]
	range	0.96−1.02	1.05−1.13	0.32−0.57	0.41−0.74	<0.001*	**<0.001***[d]
							<0.001*[e]
							0.189[f]

*p-value ≤0.05 is statistically significant. Statistical analysis was carried out using Kruskal−Wallis test, followed by Bonferroni posthoc. [a]Difference between control and HSP. [b]Difference between control and TiO_2NPs. [c]Difference between control and TiO_2NPs + HSP. [d]Difference between HSP and TiO_2NPs. [e]Difference between HSP and TiO_2NPs + HSP. [f]Difference between TiO_2NPs and TiO_2NPs + HSP.

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Table 3. Comparison of Rat Brain Tissue Antioxidants among 4 Studied Groups

groups		control	HSP	TiO_2NPs	TiO_2NPs + HSP	*p*-values	
SOD (U/g)	mean ± SD	2.00 ± 0.07	2.20 ± 0.07	0.78 ± 0.06	1.17 ± 0.08	<0.001*	**0.010**^{*a}
							<0.001^{*b}
							<0.001^{*c}
							<0.001^{*d}
							<0.001^{*e}
	range	1.95−2.08	2.14−2.28	0.72−0.83	1.08−1.24		**<0.001**^{*f}
CAT (U/g)	mean ± SD	2.64 ± 0.18	2.90 ± 0.20	1.14 ± 0.08	1.72 ± 0.12	<0.001*	0.071^{a}
							<0.001^{*b}
							<0.001^{*c}
							<0.001^{*d}
							<0.001^{*e}
	range	2.45−2.81	2.69−3.09	1.06−1.21	1.59−1.83		**0.002**^{*f}
MDA (nmol/g)	mean ± SD	1.12 ± 0.08	1.01 ± 0.06	2.21 ± 0.27	1.47 ± 0.18	<0.001*	0.435^{a}
							<0.001^{*b}
							0.035^{*c}
							<0.001^{*d}
							0.010^{*e}
	range	1.04−1.19	0.94−1.06	1.99−2.51	1.32−1.67		**<0.001**^{*f}
GPx (U/g)	mean ± SD	2.18 ± 0.04	2.39 ± 0.06	0.88 ± 0.04	1.23 ± 0.06	<0.001*	**0.001**^{*a}
							<0.001^{*b}
							<0.001^{*c}
							<0.001^{*d}
							<0.001^{*e}
	range	2.14−2.22	2.34−2.45	0.84−0.92	1.17−1.28		**<0.001**^{*f}

*p-value ≤0.05 is statistically significant. Statistical analysis was carried out using Kruskal−Wallis test, followed by Bonferroni posthoc. ^{a}Difference between control and HSP; ^{b}Difference between control and TiO_2NPs. ^{c}Difference between control and TiO_2NPs + HSP. ^{d}Difference between HSP and TiO_2NPs. ^{e}Difference between HSP and TiO_2NPs + HSP. ^{f}Difference between TiO_2NPs and TiO_2NPs + HSP.

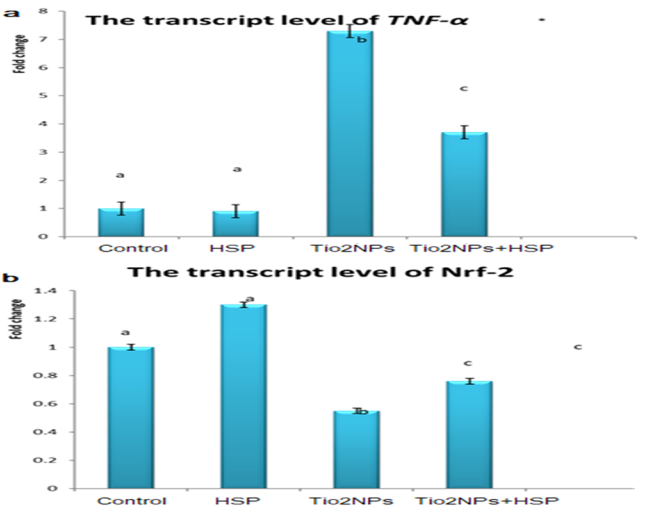

Figure 2. (a) Chart showing the brain transcript level of TNF-α. (b) Chart showing the brain transcript level of Nrf-2. Values are presented as mean ± standard error of the mean (n = 20 rats/group). Different superscript letters indicate statistically significant differences at $p \leq 0.05$.

with the control group. Rats fed on TiO_2NPs and HES were mildly affected and showed mild degenerative changes of neurons in the hippocampus and mild degenerative alterations and necrosis in the cerebral cortex.

4. DISCUSSION

TiO_2NPs are vastly employed in a variety of implementations, such as paints, transparent plastics, papers, cosmetics, the food industry, medicines, water purgation, antimicrobial vibes in decontamination, and photoactive materials in solar cells.[22] As a result, human and animal exposure to TiO_2NPs is unavoidable.[23]

HSP is a pharmacologically active flavonoid glycoside and flavonoid subdivision that is abundant in all citrus species (blood orange, orange, lemon, and lime).[24] This flavanone has been shown to have several pharmacological effects, implicating antioxidant, inflammatory fighter, analgesic, anticarcinogenic, antiviral,[25] anticoagulants, hypolipidemic, and hypoglycemic activities.[26] So, in this study, we use a number of measures to look into the neurotoxic effect of oral TiO_2NPs and the possible protective effect of hesperidin in adult rats.

Neurotransmitters are tiny chemicals that convey messages between neurons and other cells via synapses, causing a remarkable action to be triggered.[27] The human system recycles or breaks down neurotransmitters once they deliver signals. The most well-known neurotransmitters that control several vital processes include acetylcholine, serotonin, dopamine, and norepinephrine.

We found that in the TiO_2NP-treated group, there was a significant rise in AChE enzymatic activity with dopamine and a significant decrease in glutamate when matched to control groups. These notifications were contrary to Halawa et al.[28] and Grissa et al.[29] In our opinion, the alterations in neurotransmitter levels in brain tissues are associated with the physicochemical properties of nanoparticles, which were changed by many factors during the synthesis of TiO_2NPs.

The group treated with TiO_2NPs in combination with hesperidin showed a significant decrease in the level of AChE enzyme activity and dopamine when compared to that of the TiO_2NP-treated group. These results were in agreement with the study done by Thenmozhi et al.[30] and Jaiswal et al.[31]

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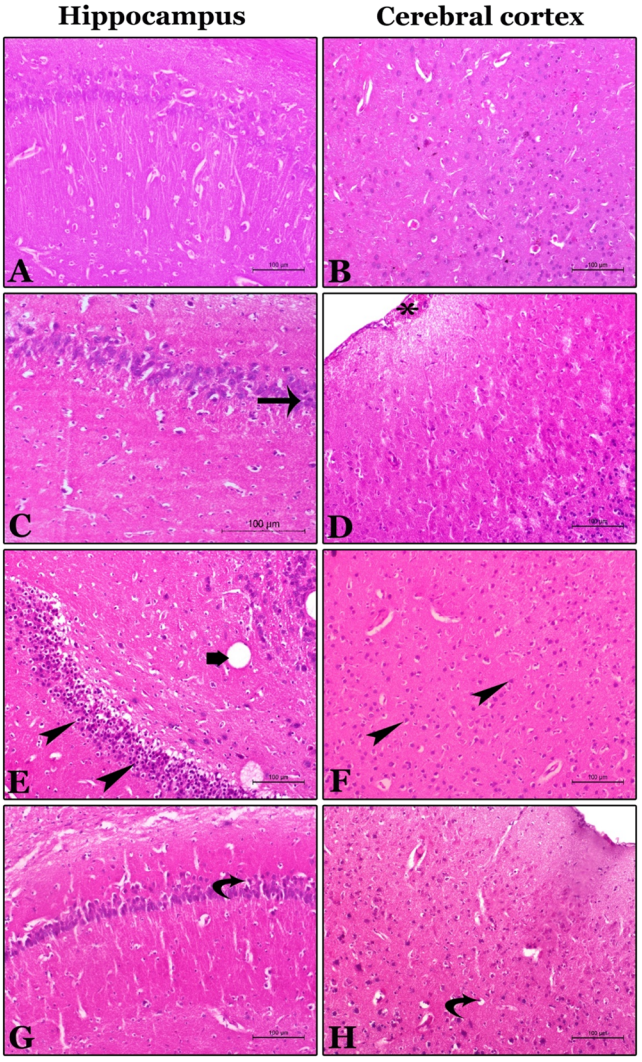

Figure 3. Histopathological changes in rat brain tissues stained with H&E ×100. The A and B sections of the control group showed normal histological structure. The C and D sections of the hesperidin-treated group showed minimal necrosis of neurons of the hippocampus (arrow) and mild congestion (*) in the cerebral cortex. The E and F sections of the TiO_2NP-treated group showed marked neuronal degenerative and necrotic changes (arrowheads) and vacuolation (thick arrows) in the hippocampus and severe neuronal degeneration and necrosis in the cerebral cortex (arrowheads). The G and H sections of TiO_2NPs and HES showed mild degenerative changes and necrosis of neurons in the hippocampus and cerebral cortex (curved arrows).

These findings contradicted the findings of Antunes et al.;[32] they found that hesperidin therapy protects against cognitive decline caused by 6-OHDA and keeps spatial memory by acting as an antioxidant and increasing dopamine. Hesperidin has also been shown to diminish AChE inhibition in the brains of rats exposed to Cd.[33]

Aerobic metabolism produces ROS, such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide. They are formed as active oxygen derivatives by oxidation−reduction reactions.[34] The three basic forms of intracellular antioxidant enzymes are SOD, CAT, and peroxidase, with GPx being the most well-known. SODs degrade superoxide radicals into hydrogen peroxide and molecular oxygen (O_2), whereas catalase and peroxidases degrade H_2O.[35]

Our study showed that the oxidative stress biomarkers in brain tissue in the TiO_2NP-treated group had a significant fall-off in SOD, CAT, and GPx, but there was a significant increase in the MDA level when matched with control groups. These findings concurred with those of Jia et al.[4] Similarly, after nasal or intra-abdominal exposure to TiO_2NPs, ROS were produced in a dose-dependent manner in the brains of mice.[36] GSH activity was also significantly reduced in rats given intraperitoneal TiO_2NPs (30−50 nm) at dosages of 120 or 160 mg/kg on alternate days for 28 days.[37] As a result, the dose, the size of the nanotitanium, and the time of exposition all have an impact on the results.

Compared to rats that were only given TiO_2NPs, rats that were also given hesperidin and TiO_2NPs showed a big drop in MDA and a big increase in SOD, CAT, and GPx. Our findings are consistent with previous research in which hesperidin cotreatment significantly corrected the oxidative stress state in different brain parts.[38]

Because enzymes responsible for eliminating free radicals, including catalase, superoxide dismutase, and glutathione peroxidase, have decreased bounce in the brain, the brain is more vulnerable to oxidative stress. Furthermore, because iron has the propensity to produce ROS, excess iron in the brain can be more hazardous than that in other cells. Furthermore, higher iron levels in the brain decrease the level of occludin expression. Because the protein participates in the BBB, decreased expression of occluding, a tight junction protein, might disrupt BBB function and injure the brain.[39]

The majority of macrophages and monocytes produce TNF-α, a multifunctional inflammatory cytokine. By stimulating neutrophils and lymphocytes, it can promote the generation and release of other cytokines.[40]

The transcription factor, nuclear factor-erythroid-2-related factor 2 (Nrf-2), adjusts the expression of genes encompassed in cellular safeguard against oxidants, electrophiles, and inflammatory laborers, as well as the preservation of mitochondria, cellular redox, and protein homeostasis.[41]

In comparison to the control rats, TiO_2NPs dramatically increased the expression levels of TNF-α and decreased the expression levels of Nrf-2 in brain tissue. This conclusion was consistent with Kandeil et al.,[42] who discovered a substantial drop in Nrf-2 levels and an increase in TNF-α and IL-1 in TiO_2NP-treated animals compared to control rats. This discovery concurred with McCoy and Cookson,[43] who observed excessive ROS generation following a decrease in the expression of Nrf-2.

As shown by a large drop in Nrf-2 levels, our results suggest that Nrf-2 plays a key role in the reduction of antioxidant vindication in neuronal cells caused by TiO_2NP toxicity. ROS production is linked to proinflammatory arbiters,[44] which goes against our findings that oxidative stress caused by TiO_2NP exposure led to more proinflammatory cytokines in neurons like TNF-α. These findings might indicate that an inflammatory response led to TiO_2NP neurotoxicity.

The rats in the TiO_2NPs + hesperidin group had significantly less TNF-α and more Nrf-2 than the rats in the TiO_2NP rat group. These outlines concurred with the findings of Justin-Thenmozhi et al.,[45] who discovered that hesperidin administration decreased the production of proinflammatory intermediates such as TNF-α in animal models of $AlCl_3$-induced neuroinflammation in the hippocampus. This conclusion was also consistent with the results of Zhu et al.,[46] who discovered that hesperidin invigorates Nrf-2, one of

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the cellular protection mechanisms against oxidative stress. Hesperidin's neuroprotective benefits are mostly based on its oxidation and inflammation-defending properties.[45]

5. CONCLUSIONS

Our study showed that administration of hesperidin with TiO_2NPs orally for 8 weeks decreased the levels of MDA and TNF-α in brain homogenates that were increased in the TiO_2NP group. It increases the other oxidative biomarkers (SOD, CAT, and GPx) and the Nrf-2 expression levels. It decreases the levels of AChE and dopamine in brain tissue homogenate, which were increased in the TiO_2NP-treated group. Brain histological sections of the TiO_2NP-treated group showed degeneration, necrosis, congestion, and inflammatory cell infiltrations. Hesperidin cotreatment offers significant protection against TiO_2NP-induced oxidative stress and biochemical and histological alteration in the brain.

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A.E.k. and S.S.G.: Writing—original draft, writing—review and editing, and supervision; A.Z.: Supervision, writing—review and editing; R.H.A.-S.: Conceptualization, methodology, supervision, writing—review and editing; M.I. and R.M.H.: Investigation, writing—original draft, writing—review and editing; A.A.F.: Investigation, writing—original draft; M.A.A. and L.E.A.: Project administration, investigation, writing—original draft, writing—review and editing, funding acquisition, resources; M.A., M.M.G.: writing—review, editing, and project administration.

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Notes

The authors declare no competing financial interest.

Ethical Statement This study was approved by Beni-Suef University's Institutional Animal Care and Use Committee (BSU-IACUC) established ethical standards for this research (approval no.: 022-421).

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REFERENCES

(1) Abd Elkodous, M.; El-Sayyad, G. S.; Mohamed, A. E.; Pal, K.; Asthana, N.; de Souza Junior, F. G.; El-Batal, A. I. Layer-by-layer preparation and characterization of recyclable nanocomposite (CoxNi1−xFe2O4; X = 0.9/SiO2/TiO2). *J. Mater. Sci.: Mater. Electron.* **2019**, *30* (9), 8312−8328.

(2) Attia, M. S.; El-Sayyad, G. S.; Saleh, S. S.; Balabel, N. M.; El-Batal, A. I. Spirulina platensis-polysaccharides promoted green silver nanoparticles production using gamma radiation to suppress the expansion of pear fire blight-producing Erwinia amylovora. *J. Cluster Sci.* **2019**, *30* (4), 919−935.

(3) Baranowska-Wójcik, E.; Szwajgier, D.; Oleszczuk, P.; Winiarska-Mieczan, A. Effects of titanium dioxide nanoparticles exposure on human health—A review. *Biol. Trace Elem. Res.* **2020**, *193* (1), 118−129.

(4) Jia, X.; Wang, S.; Zhou, L.; Sun, L. The potential liver, brain, and embryo toxicity of titanium dioxide nanoparticles on mice. *Nanoscale Res. Lett.* **2017**, *12* (1), No. 478.

(5) Geraets, L.; Oomen, A. G.; Krystek, P.; Jacobsen, N. R.; Wallin, H.; Laurentie, M.; de Jong, W. H.; et al. Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. *Part. Fibre Toxicol.* **2014**, *11* (1), 1−21.

(6) Taslimi, P.; Caglayan, C.; Gulcin, İ. The impact of some natural phenolic compounds on carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes: An antidiabetic, anticholinergic, and antiepileptic study. *J. Biochem. Mol. Toxicol.* **2017**, *31* (12), No. e21995.

(7) Hamdy, S. M.; Shabaan, A. M.; Latif, A. K. M. A.; Abdel-Aziz, A. M.; Amin, A. M. Protective effect of Hesperidin and Tiger nut against Acrylamide toxicity in female rats. *Exp. Toxicol. Pathol.* **2017**, *69* (8), 580−588.

(8) Menze, E. T.; Tadros, M. G.; Abdel-Tawab, A. M.; Khalifa, A. E. Potential neuroprotective effects of hesperidin on 3-nitropropionic acid-induced neurotoxicity in rats. *Neurotoxicology* **2012**, *33* (5), 1265−1275.
(9) Chen, Z.; Wang, Y.; Ba, T.; Li, Y.; Pu, J.; Chen, T.; Jia, G.; et al. Genotoxic evaluation of titanium dioxide nanoparticles in vivo and in vitro. *Toxicol. Lett.* **2014**, *226* (3), 314−319.
(10) Owolabi, J. O.; Olatunji, S. Y.; Olanrewaju, A. J. Caffeine and cannabis effects on vital neurotransmitters and enzymes in the brain tissue of juvenile experimental rats. *Ann. Neurosci.* **2017**, *24* (2), 65−73.
(11) Jia, D.; Dou, Y.; He, Y.; Zhou, X.; Gao, Y.; Ma, M.; Wu, Z.; Li, W. Saponin extract of Baihe-Zhimu Tang ameliorates depression in chronic mild stress rats. *J. Funct. Foods* **2020**, *68*, No. 103905.
(12) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr; Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88−95.
(13) Matsumura, H.; Miyachi, S. [43] Cycling assay for nicotinamide adenine dinucleotides. In *Methods in Enzymology*; Academic Press, 1980; Vol. *69*, pp 465−470.
(14) Nishikimi, M.; Rao, N. A.; Yogi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Bioph. Res. Common.* **1972**, *46* (2), 849−854.
(15) Aebi, H. [13] Catalase in vitro. In *Methods in Enzymology*; Academic Press, 1984; Vol. *105*, pp 121−126.
(16) Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95* (2), 351−358.
(17) Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. Selenium: biochemical role as a component of glutathione peroxidase. *Science* **1973**, *179* (4073), 588−590.
(18) Ahmed, W. M. S.; Abdel-Azeem, N. M.; Ibrahim, M. A.; Helmy, N. A.; Radi, A. M. Neuromodulatory effect of cinnamon oil on behavioural disturbance, CYP1A1, iNOStranscripts and neurochemical alterations induced by deltamethrin in rat brain. *Ecotoxicol. Environ. Saf.* **2021**, *209*, No. 111820.
(19) Hassanen, E. I.; Ibrahim, M. A.; Hassan, A. M.; Mehanna, S.; Aljuaydi, S. H.; Issa, M. Y. Neuropathological and Cognitive Effects Induced by CuO-NPs in Rats and Trials for Prevention Using Pomegranate Juice. *Neurochem. Res.* **2021**, *46* (5), 1264−1279.
(20) Morgan, A. M.; Hassanen, E. I.; Ogaly, H. A.; Al Dulmani, S. A.; Al-Zahrani, F. A. M.; Galal, M. K.; Kamel, S.; Rashad, M. M.; Ibrahim, M. A.; Hussien, A. M. The ameliorative effect of N-acetylcysteine against penconazole induced neurodegenerative and neuroinflammatory disorders in rats. *J. Biochem. Mol. Toxicol.* **2021**, *35* (10), No. e22884.
(21) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25* (4), 402−408.
(22) Czajka, M.; Sawicki, K.; Sikorska, K.; Popek, S.; Kruszewski, M.; Kapka-Skrzypczak, L. Toxicity of titanium dioxide nanoparticles in central nervous system. *Toxicol. In Vitro* **2015**, *29* (5), 1042−1052.
(23) Miura, N.; Ohtani, K.; Hasegawa, T.; Yoshioka, H.; Hwang, G. W. Biphasic adverse effect of titanium nanoparticles on testicular function in mice. *Sci. Rep.* **2019**, *9* (1), No. 14373.
(24) Hemanth Kumar, B.; Dinesh Kumar, B.; Diwan, P. V. Hesperidin, a citrus flavonoid, protects against l-methionine-induced hyperhomocysteinemia by abrogation of oxidative stress, endothelial dysfunction and neurotoxicity in Wistar rats. *Pharm. Biol.* **2017**, *55* (1), 146−155.
(25) Ciftci, O.; Ozcan, C.; Kamisli, O.; Cetin, A.; Basak, N.; Aytac, B. Hesperidin, a citrus flavonoid, has the ameliorative effects against experimental autoimmune encephalomyelitis (EAE) in a C57BL/J6 mouse model. *Neurochem. Res.* **2015**, *40* (6), 1111−1120.
(26) Roohbakhsh, A.; Parhiz, H.; Soltani, F.; Rezaee, R.; Iranshahi, M. Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. *Life Sci.* **2015**, *124*, 64−74.
(27) Bai, R.; Zhang, L.; Liu, Y.; Li, B.; Wang, L.; Wang, P.; Chen, C.; et al. Integrated analytical techniques with high sensitivity for studying brain translocation and potential impairment induced by intranasally instilled copper nanoparticles. *Toxicol. Lett.* **2014**, *226* (1), 70−80.
(28) Halawa, A.; Elshopakey, G.; El-Adl, M.; Lashen, S.; Shalaby, N.; Eldomany, E.; Farghali, A.; Rezk, S. Chitosan attenuated the neurotoxicity-induced titanium dioxide nanoparticles in brain of adult rats. *Environ. Toxicol.* **2021**, *37* (3), 612−626, DOI: 10.1002/tox.23429.
(29) Grissa, I.; Guezguez, S.; Ezzi, L.; Chakroun, S.; Sallem, A.; Kerkeni, E.; Haouas, Z.; et al. The effect of titanium dioxide nanoparticles on neuroinflammation response in rat brain. *Environ. Sci. Pollut. Res.* **2016**, *23* (20), 20205−20213.
(30) Thenmozhi, A. J.; Raja, T. R. W.; Janakiraman, U.; Manivasagam, T. Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. *Neurochem. Res.* **2015**, *40* (4), 767−776.
(31) Jaiswal, P.; Mandal, M.; Mishra, A. Effect of hesperidin on fluoride-induced neurobehavioral and biochemical changes in rats. *J. Biochem. Mol. Toxicol.* **2020**, *34* (11), No. e22575.
(32) Antunes, M. S.; Goes, A. T.; Boeira, S. P.; Prigol, M.; Jesse, C. R. Protective effect of hesperidin in a model of Parkinson's disease induced by 6-hydroxydopamine in aged mice. *Nutrition* **2014**, *30* (11−12), 1415−1422.
(33) Khan, M. H. A.; Parvez, S. Hesperidin ameliorates heavy metal induced toxicity mediated by oxidative stress in brain of Wistar rats. *J. Trace Elem. Med. Biol.* **2015**, *31*, 53−60.
(34) Singh, N. A. Nanotechnology innovations, industrial applications and patents. *Environ. Chem. Lett.* **2017**, *15* (2), 185−191.
(35) Weydert, C. J.; Cullen, J. J. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat. Protoc.* **2010**, *5* (1), 51−66.
(36) Ze, Y.; Hu, R.; Wang, X.; Sang, X.; Ze, X.; Li, B.; Hong, F.; et al. Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles. *J. Biomed. Mater. Res., Part A* **2014**, *102* (2), 470−478.
(37) Latif, M. A.; Jabeen, F.; Ali, M.; Rasul, A.; Naz, S.; Akram, M. Neurotoxic effects of titanium dioxide nanoparticles on the brain of male sprague dawley rats. *Pak. J. Pharm. Sci.* **2019**, *32* (5), 2311−2316.
(38) Justin Thenmozhi, A.; William Raja, T. R.; Manivasagam, T.; Janakiraman, U.; Essa, M. M. Hesperidin ameliorates cognitive dysfunction, oxidative stress and apoptosis against aluminium chloride induced rat model of Alzheimer's disease. *Nutr. Neurosci.* **2017**, *20* (6), 360−368.
(39) Sripetchwandee, J.; Wongjaikam, S.; Krintratun, W.; Chattipakorn, N.; Chattipakorn, S. C. A combination of an iron chelator with an antioxidant effectively diminishes the dendritic loss, tau-hyperphosphorylation, amyloids-β accumulation and brain mitochondrial dynamic disruption in rats with chronic iron-overload. *Neuroscience* **2016**, *332*, 191−202.
(40) Pisetsky, D. S.; Ward, M. M. Advances in the treatment of inflammatory arthritis. *Best Pract. Res. Clin. Rheumatol.* **2012**, *26* (2), 251−261.
(41) Dinkova-Kostova, A. T.; Kostov, R. V.; Kazantsev, A. G. The role of Nrf-2 signaling in counteracting neurodegenerative diseases. *FEBS J.* **2018**, *285* (19), 3576−3590.
(42) Kandeil, M. A.; Mohammed, E. T.; Hashem, K. S.; Aleya, L.; Abdel-Daim, M. M. Moringa seed extract alleviates titanium oxide nanoparticles (TiO2-NPs)-induced cerebral oxidative damage and increases cerebral mitochondrial viability. *Environ. Sci. Pollut. Res.* **2020**, *27* (16), 19169−19184.
(43) McCoy, M. K.; Cookson, M. R. DJ-1 regulation of mitochondrial function and autophagy through oxidative stress. *Autophagy* **2011**, *7* (5), 531−532.
(44) Ansar, S.; Abudawood, M.; Hamed, S. S.; Aleem, M. M. Exposure to zinc oxide nanoparticles induces neurotoxicity and

https://doi.org/10.1021/acsomega.3c06198
ACS Omega 2023, 8, 37584−37591

proinflammatory response: amelioration by hesperidin. *Biol. Trace Elem. Res.* **2017**, *175* (2), 360−366.
(45) Justin-Thenmozhi, A.; Dhivya Bharathi, M.; Kiruthika, R.; Manivasagam, T.; Borah, A.; Essa, M. M. Attenuation of aluminum chloride-induced neuroinflammation and caspase activation through the AKT/GSK-3β pathway by hesperidin in Wistar rats. *Neurotoxic. Res.* **2018**, *34* (3), 463−476.
(46) Zhu, C.; Dong, Y.; Liu, H.; Ren, H.; Cui, Z. Hesperetin protects against H2O2-triggered oxidative damage via upregulation of the Keap1-Nrf2/HO-1 signal pathway in ARPE-19 cells. *Biomed. Pharmacother.* **2017**, *88*, 124−133.

https://doi.org/10.1021/acsomega.3c06198
ACS Omega 2023, 8, 37584−37591