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Structural Characterization of Polypropylene Nanoparticles and the Protective Role of Phycocyanin Against Neurotoxicity and Oxidative Stress in Brain Tissue of Juvenile Rats

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Abstract

Introduction: Recently, the most common environmental issue is form from nanoplastics. The aim of this research is the determination of the important physiological changes and sever neurotoxicity that cause by exposure to polypropylene nanoparticles (PP NPs) in the juvenile white rats. Also, protective role of this phycocyanin compound.

Methods: The experiment was conducted on the 45 albino rats for the 30 days, which were divided into groups that included exposure to the different doses of the nanoparticles (10 and the 25 mg/kg) and the phycocyanin, either individually or combined. To verify the structural and the morphological characteristics of the prepared nanoparticles, X-ray diffraction (XRD) and the scanning electron microscopy (SEM). To evaluate oxidative stress, the levels of the lipid peroxidation (MDA) and the antioxidant enzymes such as glutathione (GSH), superoxide dismutase (SOD), and the catalase (CAT) were measured in the blood serum.

Results: The important oxidative stress that cause by PP NPs was successfully mitigated by phycocyanin. The highest MDA levels (43.01 µmol/L) were recorded in T1, while T5 reduced it to 26.98 µmol/L. There are many antioxidant improvements, as shown by T3 reaching the highest CAT (51.13 IU/L) and GSH (73.32 µmol/L). In stark contrast, the control model firmly maintained peak SOD activity at 43.66 µmol/L. All this that mean neuroprotection is achieved.

Conclusion: phycocyanin provides a strong protective role against sever brain neurotoxicity that cause by polypropylene nanoparticles in the current rat study.

Keywords: Polypropylene nanoparticles, Phycocyanin, Neurotoxicity, Oxidative stress, Antioxidant enzymes, Juvenile rats

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Introduction

Plastics have fundamentally transformed industrial and the technological sectors during the twentieth century, primarily due to their cost-effectiveness, malleability, and the light weight compared to the traditional materials such as metal and the glass (1), this widespread utilization has resulted in the significant environmental accumulation of the plastic waste. Consequently, it has emerged as a primary pollutant, a situation exacerbated by the discovery of the micro- and the nano-sized plastic particles generated through environmental degradation processes (2).

Nanotechnology has emerged as a highly promising approach to the mitigate plastic-related challenges. This is achieved either by engineering advanced nanopolymers or by integrating nanoparticles into conventional plastic structures to the upgrade their inherent properties (3).

The advent of the nanoplastics as ultramicroscopic environmental pollutants has sparked extensive research interest. Their profound capacity to the permeate diverse ecosystems and the infiltrate the food web presents intricate health, regulatory, and the scientific challenges (4). Polypropylene (PP) stands as one of the most extensively utilized thermoplastic polymers globally, attributed to the versatile physicochemical properties and the broad industrial applicability (5).

On therapeutic front, Phycocyanin is a blue-colored proteinaceous pigment belonging to the phycobiliprotein family, predominantly found in the red algae and the cyanobacteria. It functions crucially in the photosynthesis by transferring light energy from the chlorophyll pigments to the reaction centers (6). This pigment is highly recognized for the capacity to the absorb light within the orange and the blue spectra, thereby maximizing the chemical energy conversion efficiency in the photosynthetic cells (7).

Due to its multifaceted biological properties, Phycocyanin has garnered substantial interest across the cosmetic, pharmaceutical, and the food industries. Its proven capabilities encompass immune system enhancement, alongside potent anti-inflammatory and the antioxidant activities (8). Recent empirical studies have further validated its potential as a highly safe, natural alternative to the synthetic dyes, highlighting its low toxicity and the robust stability across various nutritional conditions (9).

The primary objective is to the delineate the physiological alterations resulting from the exposure to the polypropylene nanoparticles in the juvenile white rats. Specifically, the

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study aims to the evaluate dynamic shifts in the critical biomarkers, including neurological enzyme levels, as well as renal and the hepatic function indicators.

Methods

Experimental Animals

The current study utilized forty-five male Albino rats that classified to an age range of the 4 to the 6 weeks. The animal weights were recorded between 67 to 103 grams by the researchers. The experiment was conducted in the animal house facility of the Biology Department, College of the Science, University of the Al-Qadisiyah. To make optimal conditions, a controlled environment was maintained, that consist of a 14-hour light and the 10-hour dark cycle. Plastic cages were utilized to the house the rats, covered with the metal grids, that contain specialized feeding and the watering dispensers. Water and the standard pellet diets were provided freely to the animals. The important hygienic protocols were followed, that mean wood shaving bedding was continuously replaced to the prevent contamination.

Experimental Design

Based on another study, the dosages of the polypropylene nanoparticles (PP NPs) were selected at 10 mg/kg and the 25 mg/kg (10). Also, Phycocyanin was administered at 10 mg/kg and the 25 mg/kg (11). The aim of this trial was to the observe physiological effects over 30 days. A total of the eight male rats were assigned to the each treatment group by the researchers, which were distributed as follows:

Control group (C): Four juvenile rats were given standard drinking water throughout the 30-day period.

First treatment (T1): Rats were orally administered PP NPs at a dose of the 10 mg/kg body weight for the 30 days.

Second treatment (T2): Rats received a higher dose of the PP NPs at 25 mg/kg body weight for the same duration.

Third treatment (T3): The Phycocyanin compound was administered at 25 mg/kg body weight for the 30 days.

Fourth treatment (T4): This group received a combined dose containing 10 mg/kg PP NPs and the 10 mg/kg Phycocyanin.

Fifth treatment (T5): Rats were treated with the 25 mg/kg PP NPs combined with the 25 mg/kg Phycocyanin.

As demonstrated in the Figure 1, the experimental design is visually detailed.

Animal Sacrifice and the Blood Collection

Following the 30-day trial, the final weights of the animals were recorded. The rats were anesthetized using chloroform before sacrifice. Five milliliters of the blood were drawn directly from the heart via syringe. For hematological tests, 2 ml of the blood was placed into anticoagulant tubes. While, the remaining 3 ml was deposited into plain tubes and the left to coagulate. Centrifugation was performed to the separate the serum. The extracted serum was stored in the Eppendorf tubes at -20°C until it could be utilized to the evaluate biochemical parameters.

Preparation of the Polypropylene Nanoparticles

The nanomaterial was prepared by the laboratory team following the protocol (12). Initially, mechanical crushing was employed to break down polypropylene pellets into smaller fragments using a Homogenizer. After, this process was repeated utilizing acetone as a dispersion medium. Larger polymer fragments were successfully removed via filtration utilizing a 0.45 µm syringe filter. Then, water was added, and the acetone was evaporated by placing the mixture in the incubator and the oven. Finally, the nanoscale size was confirmed by the researchers via SEM.

X-Ray Diffraction (XRD) Characterization

The structural variations and the layer uniformity of the extracts loaded on the nanomaterial were evaluated using X-ray diffraction as in Figure 2. This diagnostic tool has an important role in the verifying structural integrity and the homogeneity.

Scanning Electron Microscope (SEM) Characterization

The surface morphology of the loaded extracts was examined by researchers utilizing a Scanning Electron Microscope. The aim of the scan was to the visually assess the nanostructure shapes and the characteristics.

Determination of the Lipid Peroxidation (Malondialdehyde - MDA)

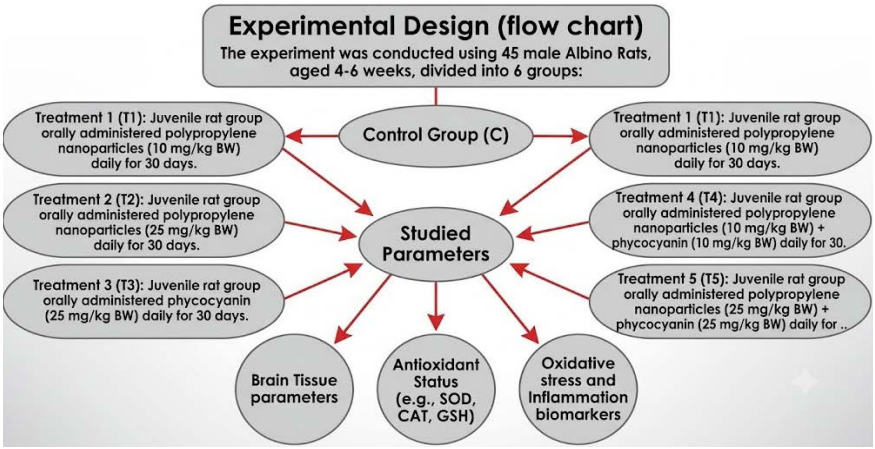

Figure 1. the experimental design

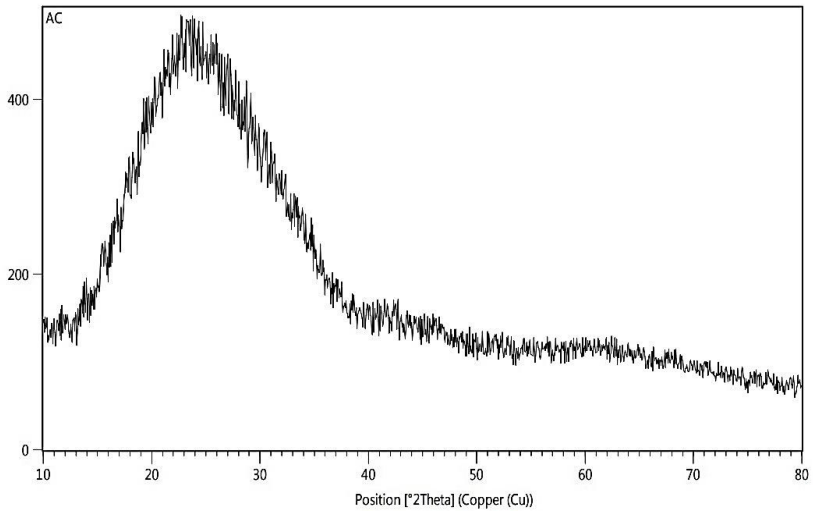

Figure 2. The XRD pattern of the sample that shown the formed crystalline phases

Based on the analytical method described by (13), the serum MDA levels were determined. The important principle of this assay relies on the chemical reaction between that MDA and the thiobarbituric acid (TBA) in the acidic environment that causes a pink-colored chromogen to form. Then, a UV-spectrophotometer was utilized to measure the absorbance of this specific complex at a wavelength of 532 nm. To make the reaction proceed optimally, equal volumes of the serum was mixed, TCA (17.5%), and the TBA (0.6%), after which they incubated the mixture in a water bath for 15 minutes. After adding TCA (70%), the samples were centrifuged at 2000 rpm for 20 minute before taking the reading, that lead to the accurate results.

Determination of Superoxide Dismutase (SOD)

The SOD activity was evaluated using a photochemical reduction reaction, that depending on the capacity of the enzyme to inhibit the reduction of NBT, which have important role in the general health. The serum was first extracted by centrifugation at 15000 rpm for 15 minute. A specific reaction mixture was utilized, that consist of methionine, NBT, riboflavin, EDTA, and a phosphate buffer (pH 7.8). After the serum was mixed with these reagents, the test tubes were incubated at 25 °C for 25 minutes, which can be considered a standard time. This procedure was completed when a spectrophotometer was used to record the absorbance at 560 nm against a blank solution.

Determination of Glutathione (GSH)

By employing the modified protocol of (14), the glutathione concentrations were estimated. The most critical reagent in this assay is Ellman's reagent (DTNB) that bind rapidly with the sulfhydryl (SH) groups present in GSH. This interaction produces a yellow-colored product that can be easily quantified spectrophotometrically. The extracted serum was mixed the with 4% sulfosalicylic acid, and centrifugation was applied to precipitate the proteins. Then, Ellman's reagent was introduced to the supernatant, and a spectrophotometer was employed to measure the final absorbance at 412 nm.

Determination of Catalase (CAT)

The catalase activity was determined according to the established kinetic method of (15). The rapid degradation of hydrogen peroxide (H2O2) into water and oxygen molecules by the CAT enzyme is cause of the absorbance change in this assay. To make the environment stable, the serum samples were diluted using a neutral phosphate buffer (pH = 7). After a freshly prepared 30% H2O2 solution was added to initiate the enzymatic reaction, the continuous absorbance decrease was monitored by a spectrophotometer at 240 nm. To calculate the reaction rate constant, the initial and final readings at 15 and 30 seconds were recorded, respectively, the kinetic measurement was executed.

Results

Nanoscale Examinations

X-Ray Diffraction (XRD)

The emergence of the multiple, sharp diffraction peaks was observed from the Search and the Match outputs that mean the synthesized compound possesses a crystalline nature. After comparing these peaks with the standard reference cards, a match with the several crystalline phases was revealed by the software. The most common among these was the "Rubidium silicide" phase (Rb12Si17), which recorded the highest match score (Score = 68). Then, a cobalt-molybdenum complex phase followed (Score = 63), alongside secondary phases of the ruthenium aluminum and the potassium aluminum sulfate compounds.

Scanning Electron Microscopy (SEM) and Surface Morphology Analysis

A highly granular and the dense structural arrangement is revealed by this investigation that consist of the closely packed particles at the nanoscale. The particles frequently agglomerate into broader continuous networks that mean a moderately rough texture can be observed across the

material at multiple magnification levels.

Specifically, larger spherical formations were highlighted in the Figure 3.A, displaying dimensions measured at approximately 118.83 nm, 152.87 nm, and the 169.12 nm. Conversely, the fine internal components of these agglomerates are clearly revealed by the high-magnification micrograph in the Figure 3.F, that depending on the high resolution capabilities of the electron beam. Much smaller dimensions, ranging from the 25.40 nm to the 30.84 nm, were measured for the distinct individual nano-grains in the referenced panel. That may be because the prominent spherical elements visible at lower magnifications directly form from the compact gathering of these primary minuscule entities.

Effect of the Polypropylene Nanoparticles and the Phycocyanin Compound on the Antioxidant Levels in the Brain of the Albino Rats

The statistical data presented in the Table 1 clarify the arithmetic means and the standard deviations, which are indicated by alphabetical letters to demonstrate the significant differences between the study groups. The statistical evaluation was based on the probability level of the less than or equal to the 0.05, utilizing the Least Significant Difference (LSD) value to the determine the effect of the polypropylene nanoparticles and the phycocyanin compound on the oxidative and the antioxidant parameters in the brain tissue, as detailed below:

Malondialdehyde (MDA) Levels

The statistical results in the Table 1 and the Figure 4 indicate evident significant differences in the malondialdehyde levels among the different treatments. The highest significant elevation in the biomarker values was recorded in the 10 mg PP NPs group (T1) and the 25 mg PP NPs group (T2), reaching 43.01 ± 5.03 µmol/L and the 40.68 ± 3.66 µmol/L respectively, that mean they are statistically classified under the top category (A).

Conversely, treatment T5 (25 mg PP NPs + 25 mg phycocyanin) demonstrated the lowest significant value,

Table 1. Effect of polypropylene nanoparticles and phycocyanin compound on some antioxidants in the brain of white rats

Group	Malondialdehyde (µmol/L)	Catalase (IU/L)	Glutathione (µmol/L)	Superoxide Dismutase (µmol/L)
C	36.38 ± 1.70 B	33.23 ± 1.88 C	53.92 ± 3.22 B	43.66 ± 0.33 A
T1	43.01 ± 5.03 A	36.05 ± 3.47 C	51.69 ± 2.60 B	30.62 ± 5.07 BC
T2	40.68 ± 3.66 A	44.96 ± 2.89 AB	57.27 ± 1.38 B	30.04 ± 1.40 BC
T3	31.26 ± 1.98 BC	51.13 ± 3.62 A	73.32 ± 2.38 A	32.96 ± 4.74 BC
T4	31.06 ± 3.84 BC	41.50 ± 2.58 B	68.57 ± 1.88 A	26.35 ± 1.65 C
T5	26.98 ± 3.89 C	42.08 ± 3.06 B	46.57 ± 1.61 C	37.98 ± 3.09 AB
LSD	8.93	7.94	5.72	8.14

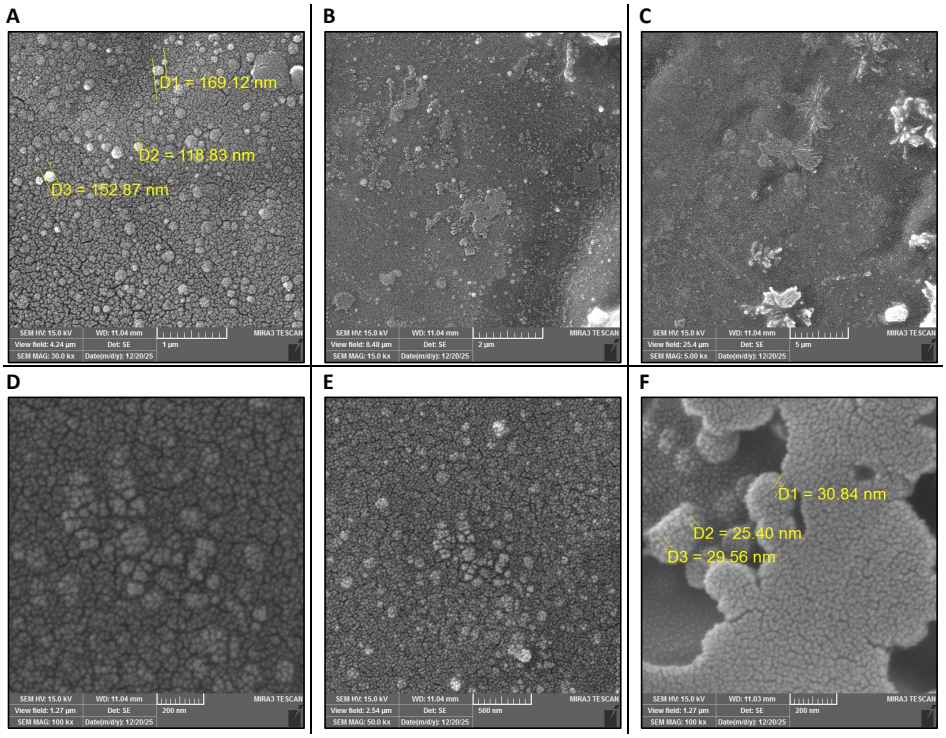

Figure 3. Scanning electron microscopy (SEM) micrographs of the synthesized sample's surface morphology, displaying large spherical agglomerations at lower magnification (A) and resolving individual primary nano-grains at high magnification (F)

reaching 26.98 ± 3.89 µmol/L and falling under category (C). This indicates that it does not differ significantly from the control group (C), which recorded 36.38 ± 1.70 µmol/L and falls within categories (B and the C). Meanwhile, the T3 and the T4 groups displayed intermediate values of the 31.26 ± 1.98 µmol/L and the 31.06 ± 3.84 µmol/L respectively, which classifies them under the statistical categories (B and the BC).

Catalase (CAT) Enzyme Levels

As demonstrated in the Table 1 and the Figure 5, the data regarding the catalase enzyme exhibited a significant variance that depending on the Least Significant Difference (LSD) value of the 7.94. The highest significant activity of the enzyme that classified to the isolated statistical category (A) was recorded by the 25 mg phycocyanin group (T3), reaching a mean of 51.13 ± 3.62 IU/L.

Then, the other treatments and the interaction groups (T2, T4, and the T5) followed this with the closely related values of the 44.96 ± 2.89, 41.50 ± 2.58, and the 42.08 ± 3.06 IU/L respectively, that mean they were categorized within the (AB) and the (B) classes.

While the control group (C) and the 10 mg polypropylene nanoparticles group (T1) demonstrated the lower enzyme activity values, recording 33.23 ± 1.88 IU/L and 36.05 ± 3.47 IU/L respectively. All this led to a significant decrease, stabilizing them within the statistical category (B).

Glutathione (GSH) Levels

A noticeable significant superiority was revealed by the statistical analysis results for the Glutathione levels, as demonstrated in the Table 1 and the Figure 6. The highest significant elevation in the biomarker concentration was recorded in the treatment T3 (25 mg phycocyanin) and the treatment T4 (10 mg polypropylene nanoparticles + 10 mg phycocyanin), with the values reaching 73.32 ± 2.38 µmol/L and the 68.57 ± 1.88 µmol/L, respectively. That mean both groups are shared within the top statistical category (A).

Then, treatment T2 followed this rank, which recorded a value of 57.27 ± 1.38 µmol/L that classified to the category (B).

While the control group (C), treatment T1, and the treatment T5 shared the lowest statistical category (C). Close values of 53.92 ± 3.22, 51.69 ± 2.60, and 46.57 ± 1.61 µmol/L were achieved by these groups, respectively. All this indicates no difference significantly among them.

Superoxide Dismutase (SOD) Enzyme Levels

As demonstrated in the Table 1 and the Figure 7, the highest significant enzyme activity was achieved by the standard model of the control group (C). It reached a mean of 43.66 ± 0.33 µmol/L, that classified to the prime statistical category (A), that depending on the a Least Significant Difference (LSD) value of the 8.14. Then, the T5 treatment recorded a value of 37.98 ± 3.09 µmol/L, which positioned it statistically between the (AB) categories.

Also, a closely related decrease in the values was

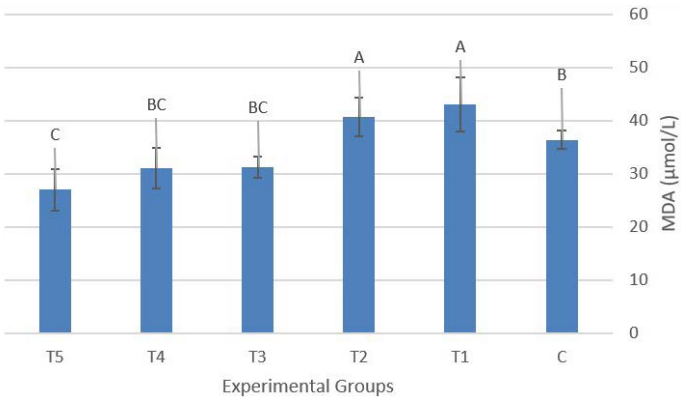

Figure 4. Effect of the treatments on the malondialdehyde (MDA) levels in the brain

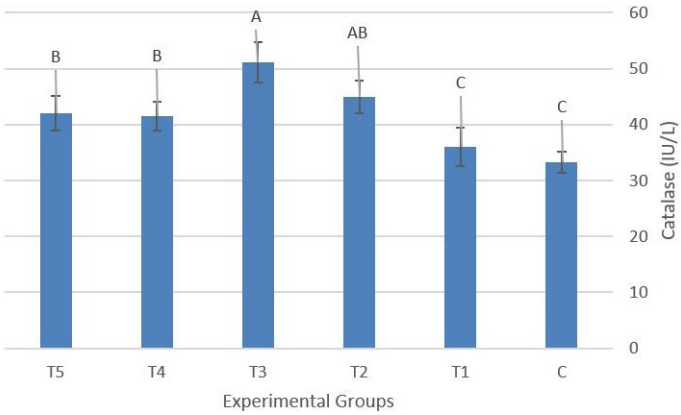

Figure 5. Effect of the treatments on the catalase enzyme levels in the brain

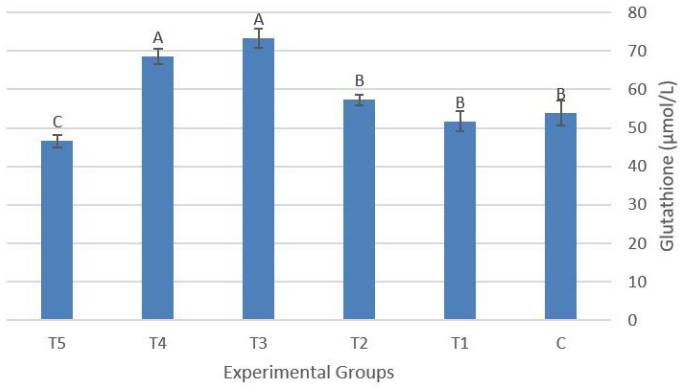

Figure 6. Effect of the treatments on the glutathione levels in the brain

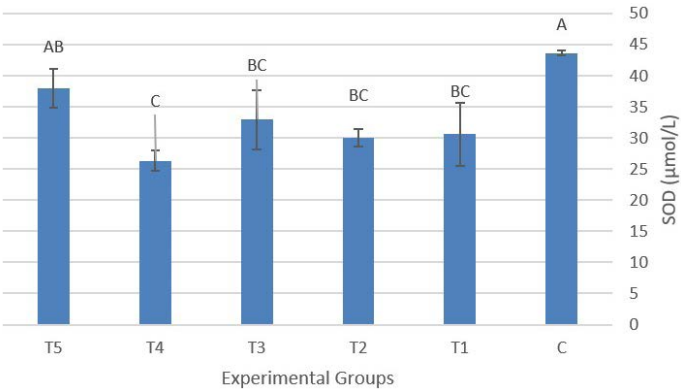

Figure 7. Effect of the treatments on the superoxide levels in the brain

demonstrated by the remaining treatments (T1, T2, and the T3). They recorded 30.62 ± 5.07, 30.04 ± 1.40, and 32.96 ± 4.74 µmol/L respectively, meaning they all shared the (BC) statistical symbols. All this indicates a moderate reduction across these specific groups. While the lower average activity for this enzyme was noted in the treatment T4. It presented a strict value of 26.35 ± 1.65 µmol/L, placing it firmly within the minimum statistical category (C) in the analyzed data.

Discussion

A direct biomarker of the severe oxidative stress was clearly indicated by the significant elevation in the Malondialdehyde (MDA) levels within both brain tissues and the blood serum for the polypropylene nanoparticle groups (T1 and the T2). That mean massive structural and the peroxidative damage afflicted the cellular lipids of the brain cell membranes after exposure to these nanoplastics. Opposite to this trend, an effective neuroprotective role is firmly established by the distinct and the significant reduction in this parameter's levels in the intervention group (T5) when compared to the isolated polypropylene groups, safely returning to the control group's (C) range. That may be because the Phycocyanin compound actively suppressed the oxidative stress cycle within the neural tissue. The important molecular mechanisms of the Phycocyanin and its linked derivative (Phycocyanobilin - PCB) can explain this fundamental protection. In a previous study, it was shown by (16) that a superior ability to inhibit NADPH oxidase gene expression is possessed by this biological compound. Specifically, NOX2 expression, which is the primary enzyme responsible for the generating lethal superoxide free radicals in the brain, is strongly targeted. That lead to the complete prevention of the free radical formation causing lipid peroxidation from the very beginning, as the Phycocyanin derivative (PCB) binds with this enzyme through a remarkably high molecular binding affinity. All this directly prevents the accumulation of the MDA compound in the brain tissue.

The current findings and their associated protective mechanisms strongly align with the comprehensive reference study by (17). The exceptional biological potential possessed by the Phycocyanin compound as a potent neuro-inflammatory and the antioxidant agent was confirmed by their extensive research. Neurons are actively protected from the death and the cellular degradation through multiple molecular mechanisms that consist of the reducing oxidation products and the trapping free radicals inside the complex brain environment. Another study published by (18) further enhances these readings. The immense therapeutic value of the Phycocyanin and the derivatives (C-Phycocyanin and the Phycocyanobilin) in the stimulating neural recovery and the protecting astrocytes from the damage caused by cytotoxicity and the brain oxidative stress was comprehensively illustrated in their work.

The massive significant leap in the catalase levels in the isolated phycocyanin group (T3) and the interaction groups (T2, T4, T5) reveals an advanced molecular mechanism possessed by this biological compound to the enhance

antioxidant expression. Based on the mechanism proposed by (19), the phycocyanin compound and its chromophoric derivative (Phycocyanobilin - PCB) act as activators for the Aryl hydrocarbon receptors (AhR). They have important role in the directly stimulating the transcription of the nuclear factor (Nrf-2), which is responsible for the regulating and the activating phase II antioxidant enzymes. That refer to the exact reason for this substantial elevation and the rapid compensation of the catalase enzyme within the brain tissue.

The current results positively align with the findings of (20), that mean the phycocyanin treatment possesses an exceptional ability to the mitigate neurotoxicity caused by environmental pollutants. To make this happen, it effectively penetrates the cerebral cortex, reduces the levels of the reactive oxygen species (ROS), and the reorganizes and the activates the enzymes linked to the redox pathway. Also, another study by (21) biologically supports these readings. It was laboratory proven that the PCB compound balances the oxidative defect in the damaged brain by restoring the normal levels of the CAT and the SOD enzymes, that lead to the protecting the cells from the degradation.

Furthermore, this protective enzymatic behavior completely matches the scientific conclusions of the (22). They confirmed that the extracted phycocyanins strongly facilitate the construction and the formation of the antioxidant defense system inside the mitochondrial parts of the neuron.

These results indicate that treatment with the phycocyanin led to a significant increase in the brain glutathione levels, likely due to the compound's potent antioxidant properties, which neutralize free radicals and the reduce oxidative stress. A previous study, conducted in the 2021 to the evaluate the antioxidant potential of the phycocyanin extracted from the Spirulina platensis algae, demonstrated that the compound exhibited dose-dependent efficacy in the improving levels of the oxidative stress biomarkers (such as GSH) in the cortex and the hippocampus (23). This suggests that phycocyanin possesses potent neuroprotective properties, limiting the excessive generation of the oxygen free radicals that damage biological molecules such as proteins and the lipids (23). The aforementioned study aligns with the current one in the demonstrating the high efficacy of the phycocyanin in the raising endogenous antioxidant levels.

The results demonstrated a decline in the superoxide dismutase (SOD) activity in the brain for the most treatments compared to the standard control group, while one treatment exhibited a significant recovery in the enzyme activity. These findings suggest a metabolic imbalance and the generation of the high oxidative stress, leading to the depletion of the natural cellular defense mechanisms. This may be due to the excessive accumulation of the free radicals and the reactive oxygen species, requiring continuous enzyme consumption to the neutralize toxic effects (24).

A previous study investigating the role of the antioxidants in the brain protection (2022) concluded that neuronal damage is directly linked to the depletion of the defense enzymes such as SOD as a result of the oxidative stress. This finding is consistent with the reduction observed in the current treatments (24). The aforementioned study is consistent with the current study, while a second study, aimed at determining therapeutic role of the phycocyanin (2021), demonstrated the ability of these bioactive compounds to the effectively suppress free radicals and the inhibit oxidative enzymes (25-27). Furthermore, a third study, aimed at reviewing the beneficial effects of the spirulina on the brain health (2025), highlighted the importance of the active compounds in the enhancing cognitive abilities and the reducing the decline in the defensive enzyme activity (3).

This significant statistical variability between treatments may indicate that the neurons had suffered severe oxidative damage due to the exposure to the neurotoxins that impaired defensive enzyme activity. The relative improvement observed in the higher-value treatment after the control group is likely due to the intervention of the compounds with the free radical scavenging properties, which contributed to the reducing oxidative stress, allowing the cells to the partially restore enzymatic activity and the protect brain tissue from the further deterioration (3).

Conclusions

This study demonstrated that exposure to the polypropylene nanoparticles causes' acute oxidative stress and the neurotoxicity in the rat brain tissue, leading to the increased lipid peroxidation and the impaired levels of the protective enzymes. Phycocyanin exhibited a strong and the effective protective role for the neurons by neutralizing free radicals and the boosting levels of the endogenous antioxidants such as glutathione and the catalase, making it a promising therapeutic agent for the mitigating neuronal damage caused by nanoplastic pollutants.

Authors' Contribution

Conceptualization: Ahmed Jassim Hassan
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Formal Analysis: Noor Abdultateef Abbas
Funding Acquisition: All Authors
Investigation: Noor Abdultateef Abbas
Methodology: Noor Abdultateef Abbas
Project Administration: Ahmed Jassim Hassan
Resources: All Authors
Software: Ahmed Jassim Hassan
Supervision: Ahmed Jassim Hassan
Validation: Ahmed Jassim Hassan
Visualization: Noor Abdultateef Abbas
Writing–Original Draft: Noor Abdultateef Abbas
Writing–Review & Editing: All Authors

Competing Interests

The authors declare no specific conflict of interest.

Ethical Approval

Not applicable.

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