Research Article

Mohamed S. Othman*, Sofian T. Obeidat, Ghada M. Aleid, Mohamed M. Abdel-Daim, Ola A. Habotta, Laurent Schwartz, Amal H. Al-Bagawi, Manal M. Hussein, Ashraf Bakkar

Pergularia tomentosa coupled with selenium nanoparticles salvaged lead acetate-induced redox imbalance, inflammation, apoptosis, and disruption of neurotransmission in rats' brain

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Abstract: In this study, the neuroprotective potential of either *Pergularia tomentosa* leaf methanolic extract (PtE) alone or in combination with selenium nanoparticles (SeNPs-PtE) was investigated against lead acetate (PbAc)-induced neurotoxicity. Experimental rats were pretreated with PtE (100 mg/kg) or SeNPs-PtE (0.5 mg/kg) and injected intraperitoneally with PbAc (20 mg/kg) for 2 weeks. Notably, SeNPs-PtE decreased brain Pb accumulation and enhanced the level of dopamine and the activity of AChE compared to the control rats. In addition, elevated neural levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione along with decreased lipid peroxidation levels were noticed in pretreated groups with SeNPs-PtE. Moreover, SeNPs-PtE significantly suppressed neural inflammation, as indicated by lower levels of interleukin-1 beta, interleukin-6, tumor necrosis factor-alpha, nuclear factor-kappa B p65, and nitric oxide in the examined brain tissue. The molecular results also unveiled significant down-regulation in iNOS gene expression in the brains of SeNPs-PtE-treated rats. In addition, SeNPs-PtE administration counteracted the neural loss by increasing B-cell lymphoma 2 (Bcl-2) and brain-derived neurotrophic factor levels as well as decreasing BCL2-associated X protein and caspase-3 levels. To sum up, our data suggest that *P. tomentosa* extract alone or in combination with SeNPs has great potential in reversing the neural tissue impairment induced by PbAc via its antioxidant, anti-inflammatory, and anti-apoptotic activities. This study might have therapeutic implications in preventing and treating several lead-induced neurological disorders.

Keywords: *Pergularia tomentosa*, selenium nanoparticles, lead acetate, oxidative stress, neuroinflammation, apoptosis

*** Corresponding author: Mohamed S. Othman,** Basic Sciences Department, University of Ha'il, 2240, Hail, Saudi Arabia, e-mail: biostar55@yahoo.com
Sofian T. Obeidat, Ghada M. Aleid: Basic Sciences Department, University of Ha'il, 2240, Hail, Saudi Arabia
Mohamed M. Abdel-Daim: Department of Pharmaceutical Sciences, Phamacy Program, Batterjee Medical College, 6231 Jeddah, Saudi Arabia; Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, 41522 Ismailia, Egypt
Ola A. Habotta: Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Mansoura University, 35516, Mansoura, Egypt
Laurent Schwartz: Oncology Department, Assistance Publique des Hopitaux de Paris, 71150 Paris, France
Amal H. Al-Bagawi: Chemistry Department, Faculty of Science, University of Ha'il, 2240, Hail, Saudi Arabia
Manal M. Hussein: Zoology and Entomology Department, Faculty of Science, Helwan University, 11795, Cairo, Egypt
Ashraf Bakkar: Biochemistry Department, Faculty of Biotechnology, October University for Modern Science and Arts (MSA), 12585 Giza, Egypt

1 Introduction

Lead is a non-biodegradable environmental pollutant that has a wide range of harmful effects, particularly on brain-related disorders [1,2]. It is extensively found in the environment as it is used in pesticides, fertilizers, lead-containing gasoline, lead paints, cosmetics, and metal products as plumbing pipes [3]. The exposure of humans to lead may be occupational or through ingestion of drinking water, and also environmental through traffic pollution, coal burning, or high lead levels in some regions [2]. According to the Institute for Health Metrics and Evaluation, lead exposure was responsible for nearly one million mortalities in 2017 [3]. Lead can also accumulate and cause damage to various organs, such as the brain, kidney, liver, heart, and immune system [4].

The brain is especially vulnerable to lead poisoning. Multiple mechanisms underlie the pathophysiology of lead neurotoxicity, among which are inhibiting neurotransmitters' release, disrupting intracellular calcium homeostasis, and reducing neural synaptic plasticity [5]. In addition, lead exposure results in excess production of free radicals and disruption of antioxidant system hemostasis with consequent oxidative injury to neuronal cells [5,6]. It was also reported that lead induces a substantial neuroinflammatory response by triggering the transcription of nuclear factor-κB (NF-κB), which upregulates the secretion of inflammatory cytokines, such as tumor necrosis factor-alpha [5,7,8]. Moreover, former reports have documented neural cell death as a result of lead exposure in rats [7,9].

Unfortunately, the ordinarily used chelators for remedying lead toxicity have serious drawbacks, such as kidney and liver damage, gastrointestinal discomfort, hypocalcemia, hypotension, bone marrow damage, and longer bleeding time [6]. Moreover, some of these agents may chelate essential metals, such as calcium and iron. In addition, dimercaptosuccinic acid, a less toxic and most effective chelating agent, cannot pass the cell membrane and the blood–brain barrier [10]. Hence, it is urgent to find alternatives to mitigate the harmful effects of lead poisoning with low or minimal side effects.

Pergularia tomentosa L. (a member of the Asclepiadaceae family) is a medicinal plant native to Saudi Arabia, northern and southern Africa, and the Middle East [11]. It is frequently used in folk medicine to treat bronchitis, skin diseases, and asthma. Furthermore, the plant parts, especially the fruits and leaves, contain several chemicals, including cardiac glycosides, flavonoids, saponins, anthraquinones, alkaloids, and tannins [12]. Bioactivity studies have shown that *P. tomentosa* exerts antioxidant, antifungal, antibacterial, and cytotoxic activities [13,14]. Lahmar et al. [15] found that the fruits and leaf extracts of *P. tomentosa* exerted strong antioxidant properties as indicated by high free radical scavenging power and iron-reducing activity. Moreover, it has been shown that the cardenolide-rich fraction of *P. tomentosa* displayed *in vitro* and *in vivo* anti-angiogenic effects by diminishing the migration and viability of endothelial cells [16].

Phyto-manufactured selenium nanoparticles (SeNPs) are regarded as a unique medicinal form of selenium due to their high biocompatibility, low toxicity, and functioning as appropriate mediators for central drug delivery [17–19]. Nano-selenium reversed neural oxido-inflammatory stress and cell death in cypermethrin- [20], streptozotocin- [18], and deltamethrin- [21] exposed rats. Moreover, previous studies have demonstrated the neuroprotective efficacy of SeNP therapy against various neurodegenerative disorders, such as Alzheimer's disease, ischemic cerebral stroke, and epilepsy [17,22,23]. Albrakati et al. [24] reported that prodigiosin loaded with SeNPs decreased the brain oxidative stress, inflammation, and apoptosis induced by chronic unpredictable mild stress in rats.

Increased domestic or occupational exposure to lead has prompted researchers to seek effective therapies with better delivery systems. Hence, this study aimed to elucidate the potential neuroprotective role of *P. tomentosa*-synthesized SeNPs against lead neurotoxicity. The possible mechanisms underlying this effect are also explained, including oxidative stress, neuroinflammation, apoptosis, and neurotransmission in the brain tissue of intoxicated rats. These findings provide new insights for developing an effective and safe approach to lead detoxification.

2 Materials and methods

2.1 Preparation of *P. tomentosa* total extracts

P. tomentosa plants were harvested from the valleys east of Hail town, Hail region, KSA, in April 2022. A taxonomist from Ha'il University's Botany Department verified plant identification. After washing, the dried leaves (500 g) were macerated in methanol for 2 days at room temperature. The extract was concentrated and lyophilized using a rotary evaporator. Distilled water was utilized to achieve a final dilution of 200 mg/mL solution, which was then kept for future use [25].

2.2 Phytochemical studies of Hail *P. tomentosa*

The plant extract was exposed to qualitative phytochemical analysis using the technique of Mumtaz et al. [26] to evaluate the active compounds in the *P. tomentosa* plant. In addition, total phenolic compounds were measured by the Folin–Ciocalteu technique. Meanwhile, the total flavonoid compounds were identified utilizing aluminum chloride method and measured spectrophotometry at 430 nm [15].

2.3 Preparation and characterization of nanoparticles

A volume of 5 mL of the extract solution of *P. tomentosa* (200 mg/mL) was titrated with 5 mL of 5 mmol/mL

Na_2SeO_3 and stirred together for 24 h at 30°C [24]. The average size of SeNPs-PtE was investigated by a Zetasizer (Nano series, ZEN 3600, Malvern, England). Meanwhile, to determine the size range of the freshly generated SeNPs, a TEM investigation was conducted. Fourier transform infrared spectroscopy (FTIR) analysis was utilized for further characterization to identify the functional groups implicated in the production of nanoparticles (PerkinElmer Spectrum 10.5.4, US).

2.4 Animals and treatment protocol

Eight-week-old male Wistar albino rats weighing 175–191 g were obtained from VACSERA animal facility (Cairo, Egypt). The rats were acclimatized for 7 days under standard laboratory conditions of 12 h light/dark cycles at a temperature of 25 ± 2°C and a relative humidity of 50 ± 10% with access to pelleted rodent feed and water ad libitum. The experiment and the procedures and methodologies were designed in compliance with the standards of Helwan University's Institutional Animal Care and Use Committee (IACUC) (approval no. HU2021/Z/AEO0121-03).

The rats were divided into five groups of eight at random.

The first group (CTL) served as the control. Each rat in this group was given 0.3 mL of distilled water orally, followed by an intraperitoneal (i.p.) injection of 100 µL saline an hour later.

Group II (PtE group) received 100 mg/kg body weight (BW) of *P. tomentosa* extract orally, and an hour later, rats were injected i.p. with 100 µL saline.

Group III [lead acetate (PbAc)] received i.p. administration of PbAc at 20 mg/kg BW, as previously reported by Abdel Moneim [27].

Group IV (PbAc-PtE group) received 100 mg/kg BW of *P. tomentosa* extract orally, and an hour later, rats were intraperitoneally injected with PbAc at 20 mg/kg BW.

Group V (PtE-SeNPs) received 0.5 mg/kg BW of (PtE-SeNPs) orally and, after 1 h, rats received an i.p. injection of PbAc at 20 mg/kg BW. All respective doses were administered daily for 14 days.

The PtE dosages used were determined via a preliminary experiment that used 25, 50, and 100 mg/kg BW, respectively. Higher dosages were shown to be more beneficial in treating PbAc-induced neurotoxicity, with no toxic symptoms appearing until 100 mg/kg BW. Euthanasia and scarification of the mice were conducted within 24 h of the last treatment. Blood was instantly sampled, kept at 37°C for 24 min, and centrifuged at 3,300 rpm for 14 min, and serum was kept at −20°C. The rats' brains were taken out and longitudinally split into two parts for molecular and biochemical analyses. A part of brain tissue was homogenized with 10 mM PBS and centrifuged for 12 min at 3600 rpm. The resulting supernatant was kept at −80°C.

2.5 Assessment of protein and lead concentration

The total protein content of the homogenized brain was determined using the method of Lowry et al. [28]. A Perkin Elmer flame atomic absorption spectrophotometer 3100 was used to measure lead concentrations in the brain tissues at 283.3 nm. The amount of lead in the brain was represented as µg/g wet weigh brain tissue.

2.6 Analysis of markers of oxidative damage

In brain tissues, lipid peroxidation (LPO) concentrations were quantitated according to the method of Yagi [29], while nitric oxide (NO) concentrations were measured based on Green et al. [30] methodology.

2.7 Assessment of antioxidant status

Catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPx) enzymes activity, as well as glutathione (GSH) levels in brain tissue were evaluated using Aebi [31], Misra and Fridovich [32], Pinto et al. [33], Tappel [34], and Akerboom and Sies [35], respectively.

2.8 Quantitative real-time PCR

To extract total RNA, the RNeasy Plus Minikit was employed (Qiagen, Valencia, CA, USA). The cDNA was produced using Power SYBR® Green Master Mix (Life Technologies, CA, USA) and detected with the Applied Biosystems 7500 equipment. The inducible NO synthase (iNOS) gene expression levels were adjusted to GAPDH. Table 1 lists the primer sequences and gene accession numbers.

2.9 Evaluation of tissue pro-inflammatory

R&D Systems (MN, USA), ELISA kits were utilized to assess the levels of inflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), nuclear factor-κB p65 (NFkp65), and tumor necrosis factor-alfa (TNF-α) levels in the brain tissues according to the manufacturer's instructions using ETI-Max 3000 system (DiaSorin, MN, USA).

2.10 Assessment of neural apoptotic markers

The levels of apoptosis-related components, such as BCL2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), and caspase-3, were measured in brain tissues using rat ELISA kits (My BioSource, SD, USA) according to the manufacturer's instructions. While brain-derived neurotrophic factor (BDNF) was determined by ELISA kits obtained from BD Biosciences (USA), according to the manufacturer's instructions.

2.11 Biochemical studies

Acetylcholinesterase (AChE) enzyme activity of tissue homogenates was determined as described by Ellman et al. [36]. While dopamine (DA) levels were assessed spectrophotometrically based on DA's inhibitory action on thionine oxidation by bromate as described by Shishehbore et al. [37].

2.12 Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used for data analysis. The results were expressed as the mean ± standard deviation (SD). One-way analysis of variance followed by Duncan's test was applied to determine the significance. The acceptable level of significance was established at $P < 0.05$.

3 Results

3.1 Nanoparticles characterization

SeNPs-PtE had an average diameter of 67.3 nm and an average zeta potential of −14.1 mV (Figure 1). The participation of O–H, N–H, C═O, and C–O functional groups in forming SeNPs, which were connected to bioactive molecules coating their surfaces, was further confirmed by FTIR spectroscopy. A broad peak shows the O–H group at 3325.00 cm^{-1}. The absorption peak at 2121.81 cm^{-1} indicates C–H stretch alkynes. Carbon compounds cause the band at 1635.19 cm^{-1} with an asymmetric stretch called C–O. Due to C–X stretching, alkyl halides exhibit a band at 447.81, 431.98, and 415.77 cm^{-1}. Numerous functional groups were discovered in this work, which may be necessary for stabilizing and reducing SeNPs-PtE.

3.2 Effect of PtE and SeNPs-PtE on lead levels in the brain tissues

In the current study, the effects of different treatments on Pb bioaccumulation in the brain tissue of exposed rats were investigated (Figure 2). Lead levels (μg/g wet of cortical tissue) increased dramatically ($P < 0.05$) in the PbAc group than in the CTL group. Meanwhile, administration of PtE or SeNPs-PtE to Pb-exposed animals significantly ($P < 0.05$) reduced the Pb levels compared to the PbAc group, indicating that PtE and its combination with SeNPs were able to reduce Pb levels. SeNPs-PtE, on the other hand, was more successful in lowering Pb accumulation in brain tissues, which may explain why the combined nanoparticles-PtE was more efficient in reducing the neural Pb burden, as observed in the current study.

3.3 Effect of PtE and SeNPs-PtE on brain oxidative stress markers of PbAc-injected rats

As shown in Figures 3 and 4, PbAc injection considerably ($P < 0.05$) increased the levels of MDA and NO in the

Table 1: Primer sequences

Name	Accession number	Product length	Sense (5′–3′)	Antisense (5′–3′)
iNOS	NM_012611.3	241	GGTGAGGGGACTGGACTTTTAG	TTGTTGGGCTGGGAATAGC
GAPDH	NM_017008.4	70	CAGCCGCATCTTCTTGTGC	ATCCGTTCACACCGACCTTC

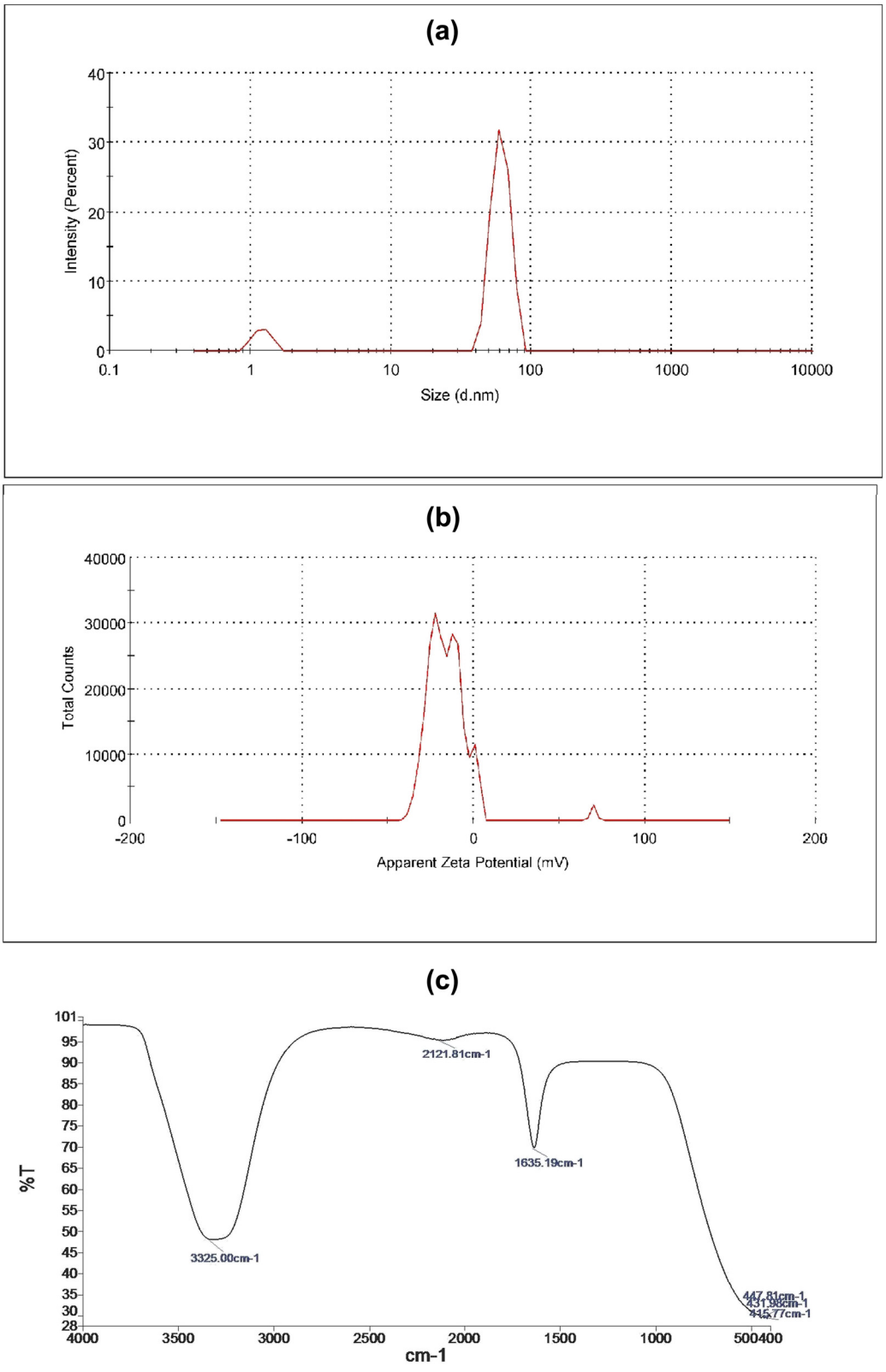

Figure 1: Characteristics of SeNPs coupled with PtE. (a) Hydrodynamic diameter of SeNPs-PtE by Zetasizer; (b) surface charge of SeNPs-PtE by zeta potential; (c) FT-IR spectra of SeNPs-PtE. FT-IR: Fourier transform infrared; SeNPs: selenium nanoparticles.

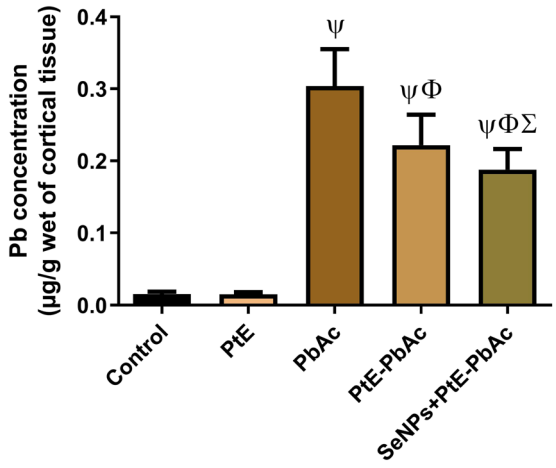

Figure 2: Effects of orally administered PtE or PtE-SeNPs on lead bioaccumulation in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

brain homogenates while also considerably ($P < 0.05$) lowered the levels of antioxidant markers (GR, GSH, GPx, SOD, and CAT) in comparison to the CTL rats. When compared to the PbAc group, PtE and SeNPs-PtE treatment caused significant reductions ($P < 0.05$) in MDA and NO levels accompanied with observable increases ($P < 0.05$) in the levels of the evaluated antioxidant indicators (GSH, GPx, GR, SOD, and CAT). Notably, SeNPs-PtE was linked to a significant increase in these antioxidant indices when compared to the PtE-PbAc group, indicating that it possesses a stronger antioxidant activity.

3.4 Effects of PtE and SeNPs-PtE on brain pro-inflammatory markers of PbAc-injected rats

There were no significant changes in the levels of TNF-α, NF-κB p65, IL-1β, IL-6, and iNOS mRNA expression rate in

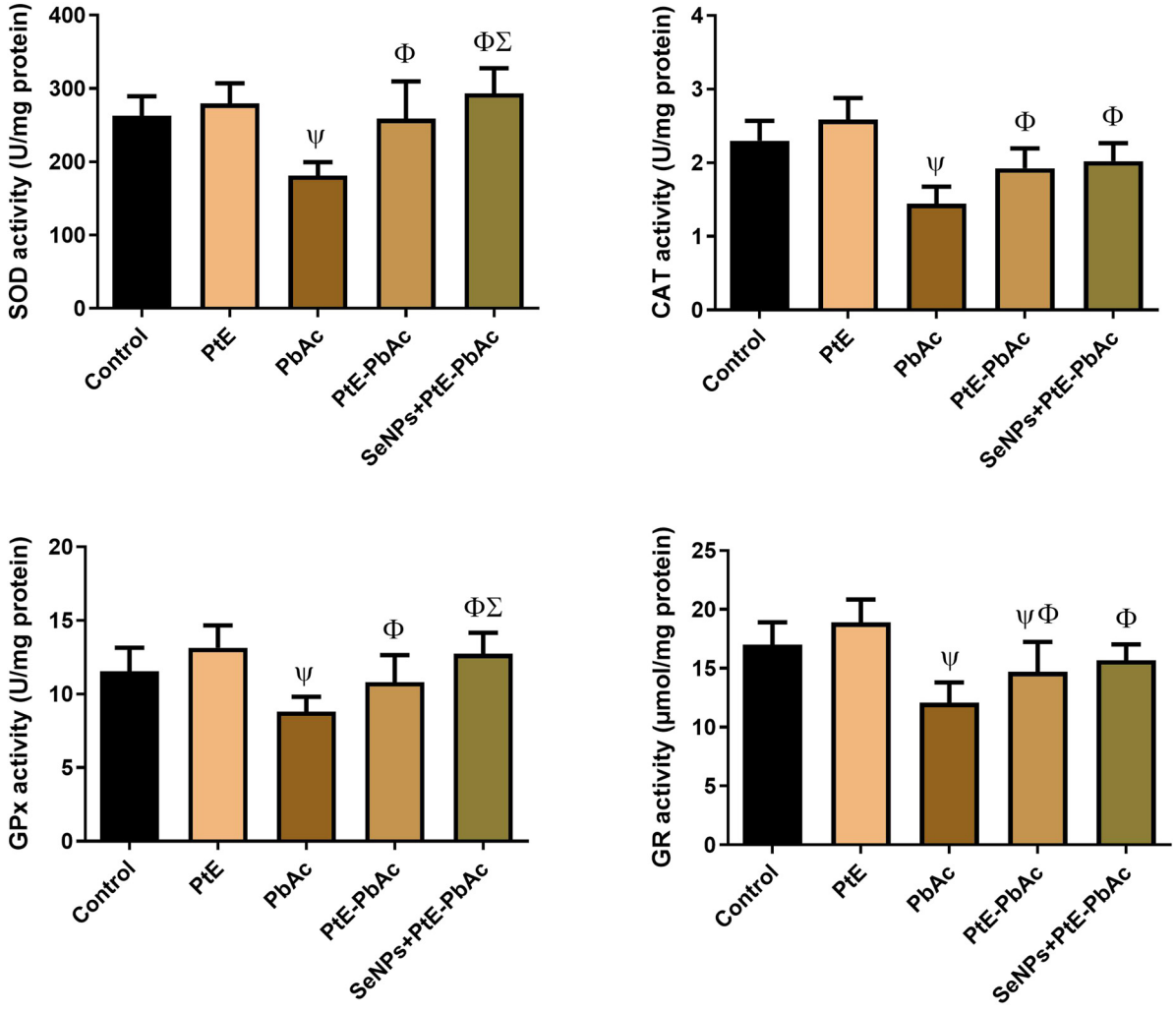

Figure 3: Effects of orally administered PtE or PtE-SeNPs on the antioxidant enzymatic activities of SOD, CAT, GR, and GPx in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

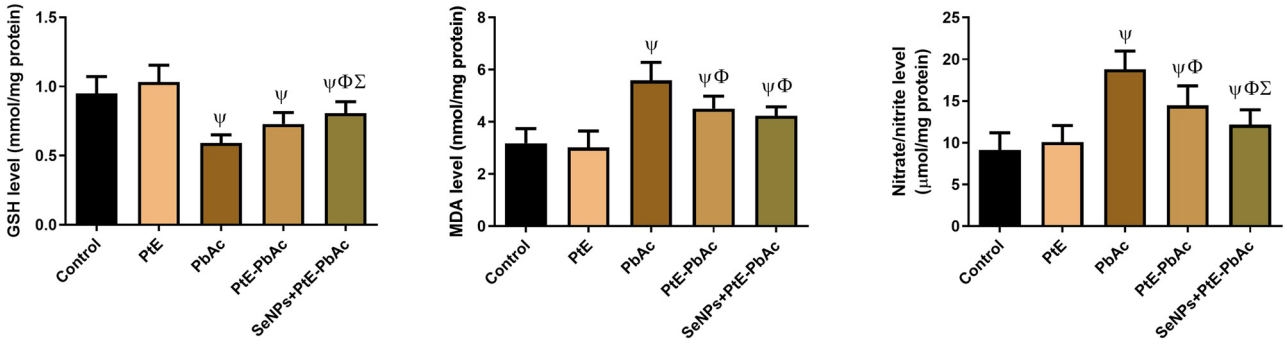

Figure 4: Effects of orally administered PtE or PtE-SeNPs on the levels of non-enzymatic oxidative stress markers (GSH, MDA, and NO) in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

the PtE group compared with those in the CTL group. In contrast, untreated rats exposed to PbAC exhibited a noticeable ($P < 0.05$) rise in the levels of these aforementioned pro-inflammatory indicators compared to the CTL group. In animals injected with PbAc and pretreated with PtE or SeNPs-PtE, the levels of these pro-inflammatory markers were significantly ($P < 0.05$) lower than those in the PbAc group, and this reduction was more noticeable in the SeNPs-PtE group (Figure 5).

3.5 Effects of PtE and SeNPs-PtE on apoptotic markers

As displayed in Figure 6, there was a notable decline ($P < 0.05$) in the anti-apoptotic factor Bcl-2, accompanied by a considerable increase ($P < 0.05$) in the levels of pro-apoptotic factors (Bax and caspase-3) in the PbAc group as contrasted to the CTL group. Meanwhile, when compared to the PbAc group, both PtE-PbAc and SeNPs-PtE

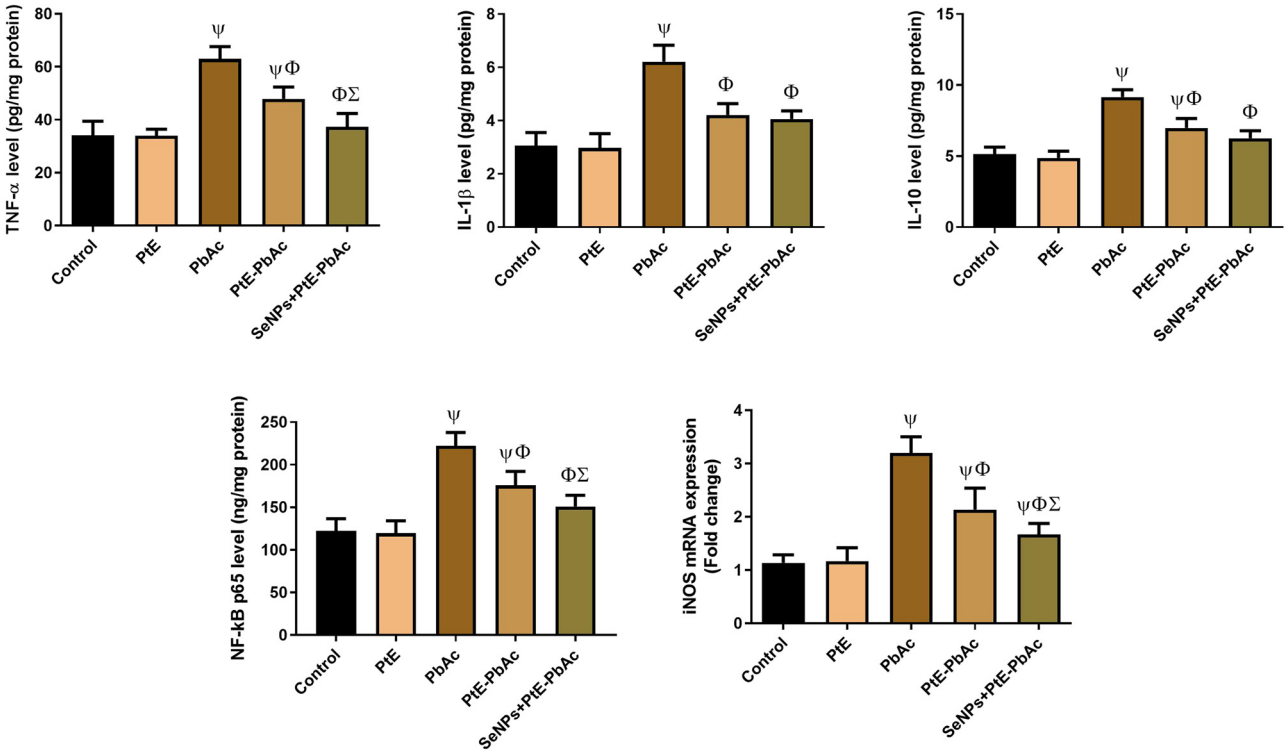

Figure 5: Effects of orally administered PtE or PtE-SeNPs on the levels of neuroinflammatory biomarkers (IL-1β, TNF-α, IL-6, and NF-κB) and the mRNA expression levels of *Nos2* in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

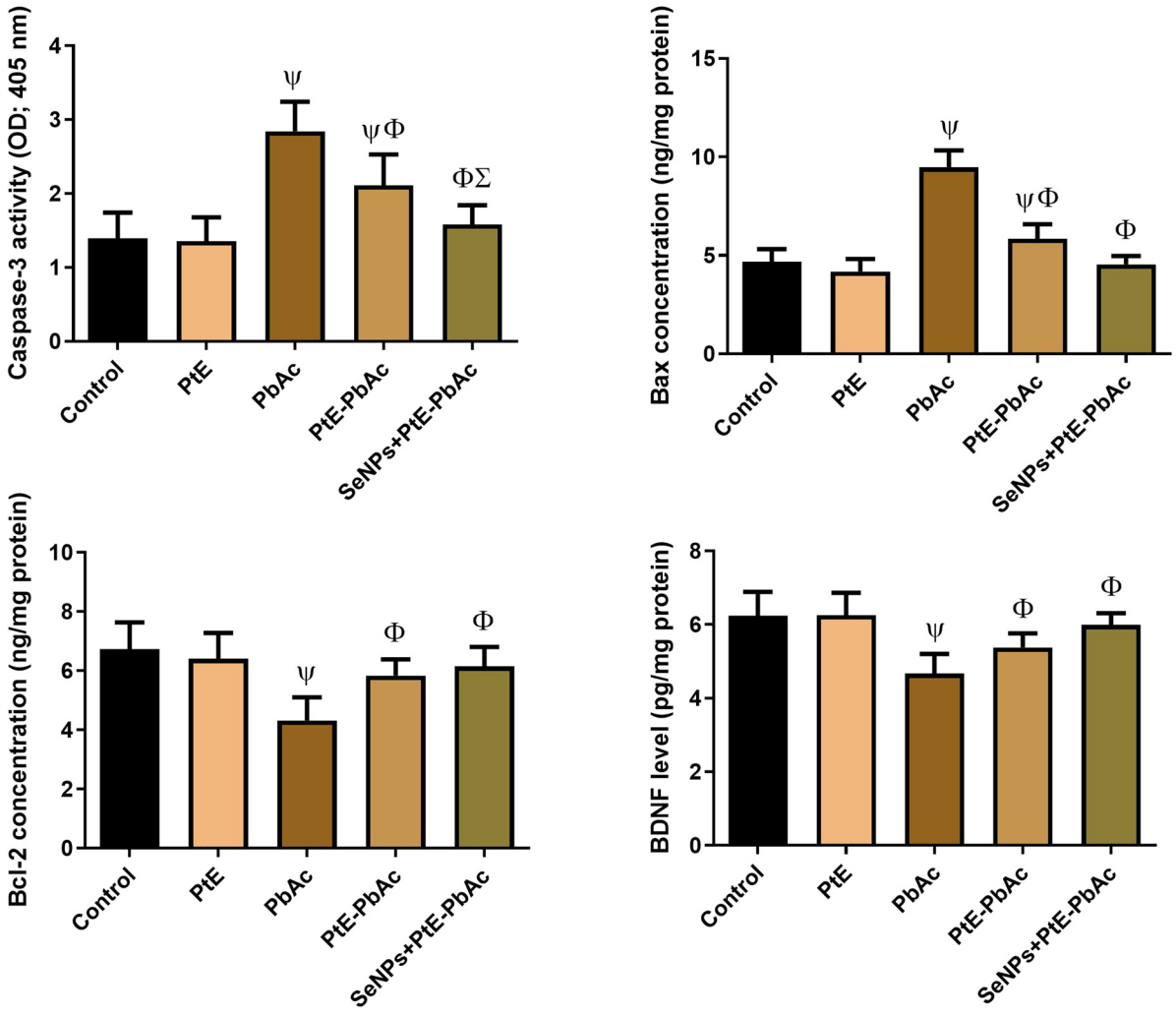

Figure 6: Effects of orally administered PtE or PtE-SeNPs on the levels of neural apoptotic markers (Bax, Cas-3, Bcl-2, and BDNF) in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

groups showed a notable rise ($P < 0.05$) in Bcl-2 levels, as well as a substantial decrease ($P < 0.05$) in Bax and caspase-3 levels. Furthermore, the current results revealed that SeNPs-PtE has more anti-apoptotic action than PtE alone. However, there were no significant changes in the levels of these apoptosis markers in the PtE group compared with those in the CTL group.

In addition, marked decreases ($P < 0.05$) were detected in the levels of BDNF in the PbAc group in respect to the control ones. However, the pre-administration of SeNPs-PtE or PtE resulted in a notable ($P < 0.05$) increase in BDNF concentration compared to the PbAc group. Meanwhile, no substantial change was found between the PtE and the CTL group.

3.6 Effect of PtE and SeNPs-PtE on AChE activities and DA concentration

Figure 7 depicts the impact of PtE and SeNPs-PtE on the activity of AChE in rats exposed to PbAc. AChE activity significantly increased ($P < 0.05$) in the PbAc group as contrasted to the CTL group. When compared to the PbAc group, the AChE activity of SeNPs-PtE and PtE-PbAc treated rats was considerably lower ($P < 0.05$), and this decline in AChE activity was more noticeable in the SeNPs-PtE group as contrasted to the PtE-PbAc group.

Furthermore, administration of PbAc significantly reduced ($P < 0.05$) the DA levels in the PbAc group in respect to the CTL group. In rats injected with PbAc and

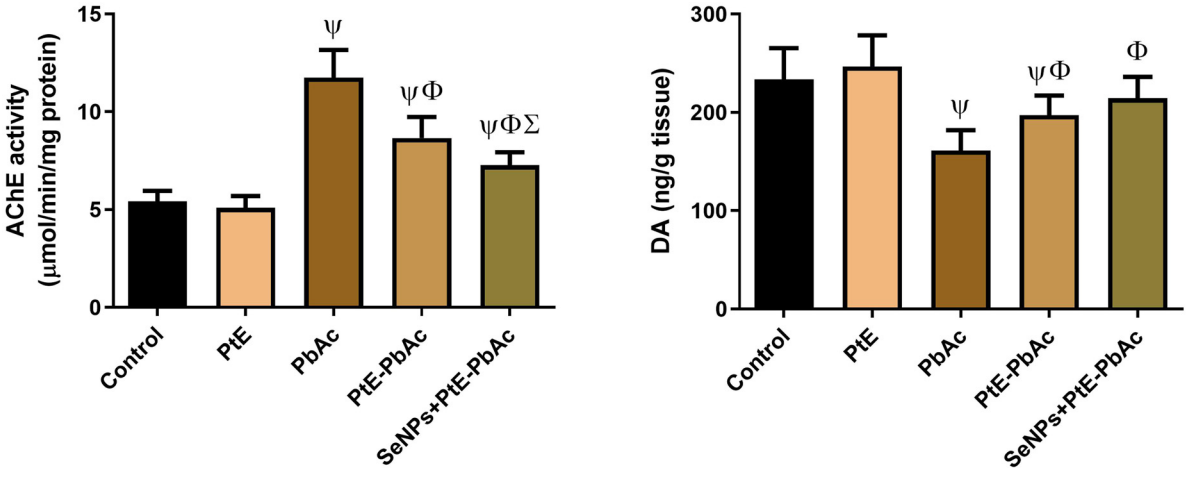

Figure 7: Effects of orally administered PtE or PtE-SeNPs on the levels of neural DA and AChE in the brain of PbAc-injected rats. $^{\psi,\Phi,\Sigma}$ superscript letters indicate statistical significance at $P < 0.05$ against control, PbAc, and PtE-PbAc group, respectively. All results are presented as the mean + SD.

pretreated with PtE or SeNPs-PtE, DA concentration demonstrated a statistically significant ($P < 0.05$) increase when compared with the PbAc group; such an increase in DA level was more pronounced in SeNPs-PtE than that observed in the PtE-PbAc group.

4 Discussion

Lead exposure evokes extensive damage to brain morphology and impairs cognition, especially in developing brains, thereby imposing serious health problems in children [38]. It can cross the blood–brain barrier to accumulate in the brain and cause neurotoxicity through various processes, including oxidative stress, apoptosis, and inflammation [39]. In the current study, the injection of animals with PbAc for 14 days caused Pb accumulation in the brain, as evidenced by the higher Pb concentrations in the rat brains. Similar results have been previously reported [40,41]. Meanwhile, treatment with *P. tomentosa* leaf extract, either alone or in combination with SeNPs, reduced the quantity of lead deposited in the brain tissues. It has been previously shown that *P. tomentosa* leaf extract is a chelator of heavy metals, including Pb [42]. According to Adhikari et al. [43], the Pb-morin (a flavonoid) complex is more sustainable than the Pb-free morin. In comparison to morin, the metal-morin chelate was also found to be substantially more soluble in aqueous solutions. It is also discovered that the soluble chelate complex's sustained antioxidant activity and heavy-metal chelation speed up the Pb detoxification (*in vitro*). El-Fakharany et al. [44] found that SeNPs' treatment for 12 weeks markedly lowered the lead levels in the blood and testes of PbAc-exposed rats. A notable reduction in lead levels was observed in the serum and thyroid tissues of PbAc-induced hypothyroidism in male rats [45]. These results indicate the chelating power of SeNPs-PtE via binding with Pb in the tissues and creating a compound that can be excreted in bile and urine.

AChE regulates cholinergic neurotransmitters and is crucial for the normal functioning of the cholinergic neural system [46,47]. Our results unveiled a dramatic increase in the brain's AChE activity in the Pb-treated group. These findings are consistent with previous studies [48]. Lead has been shown to affect cholinergic systems in the hippocampus and septum, impairing cholinergic nervous transmission [48]. Suleman et al. [49] reported that increased oxidative stress and LPO in the brain alter AChE activity, which impairs nervous transmission. Meanwhile, Brini et al. [50] concluded that lead has some similarities with calcium and that calcium ions promote AChE activity, suggesting that lead ions may also significantly influence AChE, affecting the sensitivity and neuromotor activities of the nervous system. In contrast, pretreatment with PtE or SeNPs-PtE considerably decreased AChE activity in brains of PbAc-treated rats, suggesting that *P. tomentosa* extract, alone or in combination with SeNPs, may protect against PbAc neurotoxicity by alleviating the defects in the AChE activity. The protective abilities of SeNPs and the extract may be due to their powerful antioxidant activities. Many previous reports support the idea that natural antioxidant-rich substances mitigate the abnormalities in AChE activities [50,51].

DA is involved in many cerebral functions, including locomotor activity, cognitive function, emotional stability, and endocrine regulation [52,53]. In harmony with the former study [6], our results showed that PbAc administration declined the DA levels, suggesting that Pb might cause neurotoxicity, at least in part, through the disruption of dopaminergic neurotransmission. Akinyemi et al. [54] reported that Pb administration damages dopaminergic neuron morphology, which may be linked to alterations in DA transporter that might lead to reduced extracellular DA concentration and consequent neurotoxicity. Interestingly, herein supplementation of either PtE or SeNPs-PtE to rats improved the level of brain DA. Similar to our findings, Yuan et al. [17] stated that treating epileptic animals with SeNPs reversed the alterations in the levels of neuromodulators, including DA. In another study, SeNPs conjugated with prodigiosin elevated DA contents in rats subjected to chronic unpredictable mild stress [55]. Selenium administration is associated with increased neurotransmitter levels via monoamine oxidase inhibition [56]. Furthermore, Anusha et al. [57] concluded that the flavonoid apigenin modulates DA neurotransmission by increasing DA biosynthesis and DA D2 receptor expression.

In the current study, exposure of rats to PbAc was accompanied by significant reductions in the antioxidant molecules, including CAT, GPx, SOD, and GR activity, as well as GSH levels, along with a considerable elevation of oxidative stress indices (LPO and NO) in comparison with the control rats. Lead can evoke neurotoxicity through the creation of hazardous complexes with the cellular compounds and the generation of highly ROS [7,8]. Our data support the finding of Abdel Moneim et al. [58], who concluded that PbAc increased LPO and NO generation in the brain while simultaneously lowering GSH and antioxidant enzymes' activities. Lead can disrupt the cellular antioxidant system by binding to the sulfhydryl groups or metal cofactors of antioxidant molecules with consequent disturbance in their physiologic roles [5]. Nevertheless, Pb not only promotes the production of ROS but also alters the antioxidant defenses, such as GSH interaction and reuse of catalysts, which further reduces the antioxidant activity of GSH enzymes [59].

Our findings demonstrated that pretreated rats with PtE or SeNPs-PtE before PbAc injection demonstrated a statistically significant improvement in the oxidant/antioxidant balance, as evidenced by rises in the antioxidant enzymes coupled with falls in the oxidative stress markers (LPO and NO). Compared with PtE alone, SeNPs-PtE produced much higher levels of antioxidant molecules and lower oxidative stress indicators. Selenium can quench ROS and thus inhibit oxidative stress as it is a component in selenoproteins and selenoenzymes [17]. This is consistent with the findings of the Othman et al. [60] study, which found a significant decrease in LPO and NO concentrations and an increase in GSH concentrations in mice treated with green-synthetized SeNPs with berberine. Moreover, nano-selenium re-established the antioxidant defense in the thyroid gland by decreasing the levels of MDA as well as elevating GSH, CAT, GPx, and SOD contents in PbAc-treated rats [45]. In a previous study, prodigiosin-SeNPs' administration decreased the hippocampal levels of NO and MDA, together with elevating GSH, GPx, GR, SOD, and CAT in stressed rats [55]. Similar results were reported in previous toxicity studies [21]. SeNPs' fabrication influences their bioactivity and biological availability. Thus, their surface functionalization using a biologically active compound will increase their bioavailability and boost their therapeutic power [61]. In this regard, *P. tomentosa* extract contains several bioactive components, such as flavonoids, alkaloids, and polyphenols, with antioxidants and other biologic activities. Our results agreed with Lahmar et al. [15], who reported that *P. tomentosa* extracts have potent antioxidant activity. Similarly, Yakubu et al. [62] concluded that *P. tomentosa*'s methanolic and aqueous extracts have antioxidant effects against DPPH, peroxyl, hydroxyl, and hydrogen peroxide radicals. Interestingly, *P. tomentosa* contains two significant sources of antioxidants: first, the high concentrations of phenolic compounds, which are known to be a good source of potent antioxidants, and second, the hydroxyl groups in flavonoids, which can give hydrogen atoms to free radicals.

Neuroinflammation has been identified as one of the mechanisms underlying lead neurotoxicity [5,7,8]. In the current study, rats exposed to PbAc had notably elevated levels of IL-1β, IL-6, TNF-α, NF-κB P65, and iNOS gene expressions, suggesting that neuroinflammation may be a possible mechanism underlying Pb-induced brain damage. According to earlier research, excess production of free reactive radicals induced by lead exposure boosted the release of inflammatory cytokines and NF-κB, which ultimately led to neuronal death [9]. NF-κB plays a central role during inflammation through the promotion of the release of inflammatory cytokines [63]. Moreover, iNOS enhances NO's production, contributing to inflammatory and immune responses [7]. Based on a previous study, Pb stimulates iNOS generation in brain endothelial cells [27]. Furthermore, Chen et al. [64] reported that enhanced iNOS expression might be the reason for the increased NO levels found in the frontal cortex and other brain regions of Pb-exposed rats.

In contrast, administration of *P. tomentosa* extract alone or in combination with SeNPs substantially decreased

the levels of IL-1β, IL-6, TNF-α, and NF-κB P65, as well as iNOS gene expression in the brain, indicating their anti-inflammatory action. These outcomes support the findings of Yuan et al. [17] that SeNPs decrease the inflammatory response in the epileptic brain, as indicated by lower TNF-α and IL-1β levels. Furthermore, Albrakati et al. [55] revealed that prodigiosin-SeNPs treatment reduced neuroinflammation in the hippocampus, as evidenced by a decrease in the production of pro-inflammatory markers (TNF-α, IL-1β, and IL-6). Furthermore, Farzadinia et al. [65] showed that *P. tomentosa* includes several bioactive compounds, including tannins, saponins, and steroid glycosides, which can lessen inflammation and hasten the healing of epidermal wounds in second-degree burns. The phytochemical analysis in this study also confirmed that *P. tomentosa* extract contains high levels of polyphenols and flavonoids. These compounds were reported to reduce inflammation via decreasing ROS production and downregulating several inflammatory mediators and pathways. Numerous flavonoids, including quercetin, genistein, apigenin, and kaempferol, have been demonstrated in recent research to limit the production of pro-inflammatory molecules, such as NF-κB and TNF-α, as well as to inhibit pro-inflammatory enzymes, such as iNOS and cyclooxygenase-2 [2,66].

Many reports have demonstrated that Pb exposure is associated with cellular apoptosis, resulting in the loss of valuable neurons [9]. The marked reduction in the anti-apoptotic marker Bcl-2 and the marked increase in the apoptotic markers Bax and caspase-3 levels in the PbAc group in the current study indicated that Pb could injure brain tissue through Pb-mediated apoptosis, which was in harmony with the former studies [6,7,9]. Lead is known to enhance the cytochrome release, a hallmark of apoptosis, through increasing ROS production and damaging the mitochondrial membrane [1,2]. Moreover, PbAc-induced activation of the caspase cascade triggers DNA breakage, chromatin condensation, and finally, neuronal apoptosis [67]. The current study also demonstrated that rats exposed to PbAc had considerably lower BDNF levels than rats in the other groups. These findings align with previous studies [4,68]. BDNF maintains the function and survival of neuronal cells. It controls synaptic transmission, plays a central role in cognitive function, and has neuroprotective effects against brain insults [46].

On the contrary, PtE or SeNps-PtE pretreatment counteracted the apoptotic changes induced by PbAc exposure, indicating the powerful anti-apoptotic properties of *P. tomentosa* plant extract and SeNPs. Such protective benefits have mostly been linked to the antioxidant properties of polyphenols and flavonoids in *P. tomentosa* [12]. These findings were comparable to the former studies [21,69]. SeNPs also reduced caspase-3 expression and inhibited the neural apoptosis through the mitochondrial pathway [19]. Furthermore, both PtE and SeNPs-PtE improved BDNF levels in the PbAc-treated animals. This could be attributed to the potent antioxidant and anti-inflammatory activities of Se and PtE. Similarly, Albrakati et al. [24] revealed that SeNPs-prodigiosin administration improved BDNF values in the brain tissues of stressed rats. Our data support the previous study by Rendeiro et al. [70], which showed that flavonoids could increase BDNF expression in the hippocampus. In addition, supplementation with flavonoid-rich blueberries enhances cognitive performance in animals by elevating hippocampal BDNF levels [71].

5 Conclusion

Collectively, *P. tomentosa* extract alone or in combination with SeNPs offered substantial neuroprotection against lead-induced neurotoxicity in rats. The tested extract or the formulated nanoparticles successfully diminished lead levels in the brain tissue of lead-exposed rats. Moreover, SeNPs-PtE restored the neural tissue content of AChE, DA, and antioxidant molecules. Significant anti-inflammatory action was noticed in SeNPs-PtE-treated animals and confirmed by biochemical and molecular analyses. These findings support the therapeutic value of SeNPs-PtE against lead-induced neurotoxicity. This study might have therapeutic implications in preventing and treating several lead-induced neurological disorders.

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