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Neurotherapeutic effects of prodigiosin conjugated with silver-nanoparticles in rats exposed to cadmium chloride-induced neurotoxicity

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Abstract

Prodigiosin is a red pigment produced by *Serratia marcescens* strain. Bacterial prodigiosin and its synthetic derivatives are efficacious antioxidants and proapoptotic agents. This study illustrates a new approach for use of prodigiosin conjugated silver nanoparticles (PG-$AgNP_2$) against cadmium chloride ($CdCl_2$) induced neurotoxicity in rats. Rats were (ip) injected with Cd (6.5 mg/kg) for 7 days with or without PG-$AgNP_2$ (3 mg/kg). The concentration of Cd, DA, NE, 5-HT, amino acids, NO, MDA, SOD, GSH, catalase, TNF-α, IL-6, Bax, Bcl_2 and Caspase-3. The Cd-intoxicated group showed a significant increase in Cd concentration in brain tissue, in addition, to an increase in MDA and NO and a decrease in the content of neurotransmitters (DA, NE, and 5-HT), inhibitory amino acids, and level of all studied antioxidant enzymes. PG-$AgNP_2$ treatment, significantly reduced Cd-induced brain tissue injury as indicated by increased antioxidant molecules, neurotransmitters (DA, NE, and 5-HT), and inhibitory amino acids accompanied by lower oxidative stress indices (MDA and NO) and excitatory amino acids in brain tissue. PG-$AgNP_2$ decreased inflammatory mediators including pro-inflammatory cytokines and prevented the development of apoptosis in the brain tissue. Our findings suggest that PG-$AgNP_2$ can act as a therapeutic agent against neuronal impairments associated with Cd exposure.

Keywords: prodigiosin; nanoparticles; cd toxicity; brain; neurotransmitters.

Practical Application: Silver nanoparticles biosynthesized by prodigiosin pigment can be used as treatment for Cd induced brain toxicity.

1 Introduction

One of the environment's most toxic heavy metals and frequent industrial pollution is cadmium (Cd) (Almeer et al., 2018). Cigarette smoking, polluted water, and air pollution are the main sources of Cd exposure (Satarug et al., 2013). Moreover, exposure to pesticides, sludge, wastewater, metal plating, stains, polyethylene, silica product, and carbon cells are also other sources of Cd (Luparello et al., 2011). Since Cd cannot be broken down and has a long biological half-life (20 years), it is not easily eliminated from the body and builds up in several organs (Elkhadragy et al., 2018). The reproductive system, gastrointestinal tract, mucous tissues, and nervous system may all suffer serious harm as a result of Cd bioaccumulation in the living system (Gupta et al., 2015). Cd reaches the central nervous system (CNS) when inhaled through the nasal mucosa or olfactory pathways, resulting in neurotoxicity (Omairi et al., 2018).

In cultured rat cortical neurons (López et al., 2003) and rat main midbrain neuroglia cultures, Cd is neurotoxic, and it alters the typical neurochemistry of the animal brain (Méndez-Armenta & Ríos, 2007). The processes of Cd-induced neurotoxicity are yet unclear. In several organs, including the kidney (Chater et al., 2008), liver (Almeer et al., 2018), and brain (Salem, 2021) oxidative stress has been suggested as a potential mechanism for Cd toxicity. Through the inactivation of thiol groups in essential components, inhibition of antioxidant defences, and DNA repair mechanisms, Cd indirectly contributes to producing reactive oxygen species (ROS) (Shagirtha et al., 2017).

Natural pigments have recently seen a noticeable growth in application in various industrial sectors, including food, cosmetics, and health (Koyande et al., 2019). They are presented as a replacement for artificial synthetic colorants that are employed to demonstrate harmful side effects. Due to strong environmental concerns and evidence of their carcinogenic consequences, several have been taken out of industrial usage (Numan et al., 2018).

The rapid emergence of new antibiotic-resistant organisms represents a public health emergency and has reawakened the need to investigate novel compounds. On the other hand, the potential antimicrobial properties of some bio-dyes have emerged as promising alternatives. In particular, there are many benefits to producing pigments with microbes, including quick duplication times, high specific growth rates, straightforward purification procedures, biomass recovery, and production that is not dependent on environmental conditions (Venil, 2009).

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Additionally, bio-dyes may exhibit "extra" biological properties such as antioxidant, antiviral, antibacterial, and anticancer effects (Bernardes et al., 2010). The class of bioactive coloured compounds produced by microbial fermentation includes prodigiosin (PG). Red pigment PG, which is mostly produced by *Serratia marcescens* strains and other bacteria, has several intriguing potential medical applications (Han et al., 2021).

It was proven to be a powerful proapoptotic agent against a variety of cancer cell lines, including those that were resistant to numerous drugs, while having little to no impact on normal cell lines (Sudhakar et al., 2021). The antibacterial, antiparasitic, insecticidal, and immunomodulatory properties of PG are also demonstrated (Suryawanshi et al., 2017). Natural pigments like PG appear to be a desirable bioactive alternative and they have been the focus of extensive research over the past ten years.

The cytotoxic properties of prodigiosin have been known for many years. Fullan et al. (1977) observed the antitumor activity of prodigiosin in mice. Some cancer chemotherapeutic drugs work primarily by imposing apoptotic death in susceptible cancer cells. Each of these chemotherapeutic agents reacts with a specific target, causing targeting cancer cells to undergo apoptosis (Hannun, 1997).

Prodigiosin quickly and powerfully motivates cell death in hematopoietic cancer cells, breast cancer (Pan et al., 2012), digestive cancer cell line HGT-1 (Díaz-Ruiz et al., 2001), large intestinal cancer cells (Montaner & Pérez-Tomás, 2001), and respiratory cancer (Llagostera et al., 2005). However, non-malignant cells exhibited no obvious toxicity (Montaner et al., 2000).

The production of nanoparticles via biological metallic synthesis is a green, environmentally friendly process since it doesn't include hazardous chemicals or high temperatures (Sastry et al., 2003; Bhattacharya & Gupta, 2005; Rubilar et al., 2013).

Drug delivery, cancer and gene therapy, DNA analysis, antiviral, antibacterial, and antifungal agents, diagnostic tools, anticoagulant, thrombolytic, and nano-catalysis are just a few of the numerous biological, chemical, and physical uses for biosynthesized nanoparticles. (Ojo et al., 2016; Lateef et al., 2017). Nanoparticles of required shapes and sizes are accessible by controlling the synthesis conditions. In the same consideration, temperature and pH are reported to control AgNPs size in the supernatant of E. coli (Babu & Gunasekaran, 2013).

It was revealed that biological pigments, including those synthesized by bacteria, can be employed for green synthesis of nanoparticles (Manikprabhu & Lingappa, 2014). The present study aimed to investigate the possible therapeutic effect of prodigiosin-conjugated $AgNP_2$ on Cd-induced neurotoxicity in rats.

2 Materials and methods

2.1 Chemicals

Prodigiosin-conjugated $AgNP_2$

Bacterial isolation, Preparation, extraction, purification, and quantification of prodigiosin in addition to the formation of PG-conjugated $AgNP_2$ and its characterization were performed in the Microbiology Department, Faculty of Science, Helwan University according to Faraag et al. (2017) and El-Batal et al. (2017).

Cadmium chloride ($CdCl_2$) anhydrous was obtained from Sigma Chem. Co. (St. Louis, MO, U.S.A.).

2.2 Animals

The therapeutic effect of PG-AgNP2 on toxicity produced by $CdCl_2$ was investigated using sixty adult male Wister rats (weighing 130-160 g). The rats were purchased from the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). They were kept in polypropylene cages at room temperature (22 °C) with a 12-hour light/12-hour dark cycle. The rats were provided with water and a balanced diet ad libitum. Before starting the experiment, animals were allowed to adapt for two weeks without treatment.

2.3 Experimental protocol

After the acclimation phase, the animals were divided into six groups at random (n = 10 rats/group) as the following:

i- Control group: rats were intraperitoneal (i.p.) injected with 0.1 mL of 0.9% NaC.

ii- Prodigiosin-conjugated nano silver (PG-AgNP2) group: animals were i.p. treated with PG-AgNP2 (3 mg/kg) according to El-Batal et al. (2017).

iii- Cadmium (Cd) group: rats were i.p. injected with $CdCl_2$ (6.5 mg/kg) according to Elkhadragy et al. (2018).

iv- PG-AgNP2 + Cd group: animals were i.p. injected with PG-AgNP2 (3 mg/kg) after 2 h of Cd (6.5 mg/kg) exposure.

All the treated groups were treated for seven days. The animals were killed by sudden decapitation 24 h after the last treatment, brains were rapidly excised from skulls, blotted, and chilled. The brain tissue was rapidly wiped dry with filter paper. The first half, which was kept at (-80 °C), was utilized for the measurement of monoamines and free amino acids, while the second half was used for the measurement of other biochemical parameters.

2.4 Assay of dopamine, and norepinephrine

Weighing and homogenizing the tissue in 1/10weight/volume of 75% aqueous HPLC grade methanol is the first step in the HPLC method for determining the monoamines in the brain (hypothalamus). The homogenate was centrifuged at 3000 rpm for 10 min, and the supernatant was immediately used to determine the monoamine concentration after being extracted from the lipids and trace elements using a solid-phase extraction CHROMABOND column in the NH2 phase, Cat. No. 730031. After that, the sample was directly injected into an AQUAcolumn 15054.6 mm5 C18, which was obtained from Phenomenex in the USA, with the following operating parameters: mobile phase 97/3 20 Mm potassium phosphate, pH 3.0/methanol, flowrate

1.5 mL/min, UV 270 nm. After 12 minutes, the monoamines were separated. Each monoamine location and concentration from the samples were recognized by the ensuing chromatogram and compared to the standard and finally, the determination of the content of each monoamine as μg/gram of brain tissue (Pagel et al., 2000).

2.5 Determination of free amino acids

Using high-performance liquid chromatography (HPLC) and the precolumn PITC derivatization process developed by Heinrikson & Meredith (1984), free amino acid neurotransmitters GABA, Glycine, Taurine, Glutamate, Aspartate, and Serine were identified in the hypothalamus.

2.6 Determination of Acetylcholinesterase (AChE) and Monoamine Oxidase (MAO) activities

Using the colorimetric method described by Ellman et al. (1961), the activity of brain acetylcholinesterase (AChE) was evaluated. Using 5-hydroxytryptamine (500 mM) as a substrate, the MAO activity was determined fluorometrically at 550 nm (excitation wavelength) and 404 nm (emission wavelength) in accordance with the method outlined by Dar et al. (2005).

2.7 Oxidative stress marker

Using the technique outlined by Ohkawa et al. (1979) the concentration of malondialdehyde (MDA), a lipid peroxidation (LPO) biomarker, was measured in the brain tissue. 500 μL of supernatant was added with 0.67% thiobarbituric acid, 0.22% sulfuric acid, and distilled water. The prepared mixture was placed for 30 minutes at 95 °C, cooled to 25 °C, and then centrifuged for 15 minutes at 1000 g. Spectrophotometric determination at 540 nm. The data were obtained in terms of nanomoles MDA per milligram of protein. According to Green et al. (1982), the Griess reagent was used to measure the nitric oxide (NO) level. Griess reagent was combined with 100 μL of supernatant for 10 minutes at room temperature. At 540 nm, a spectrophotometric measurement of the created reddish purple azo dye was made. Using the Lodovici et al. (1997) approach, brain DNA was isolated and hydrolyzed to estimate 8-hydroxy-2-deoxyguanosine (8-OHdG).

2.8 Estimation of antioxidants

By reducing Elman's reagent (5,5′ dithiobis (2-nitrobenzoic acid; DTNB) with GSH to yield a yellow molecule, Glutathione (GSH) was measured. The absorbance of the reduced chromogen at 405 nm is directly proportional to the GSH content. According to Aebi (1984) catalase (CAT) activity was estimated. The Nishikimi et al. (1972) method was used to measure the superoxide dismutase (SOD) activity. Furthermore, the Paglia & Valentine (1967) method was used to test glutathione peroxidase activity. GPx activity was calculated using a reaction combined with glutathione reductase as the reduction in NADH per minute (GR). GPx activity was measured as a reduction in absorbance at 340 nm and expressed as U/mg protein. Additionally, the glutathione-dependent oxidation of NADPH at 340 nm was quantified and expressed as U/mg protein to determine GR activity.

2.9 Inflammatory markers in brain tissues

TNF (tumor necrosis factor) and interleukin-6 (IL-6) concentrations were measured using commercial ELISA kits (R&D System, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

2.10 Estimation of apoptotic markers in tissue

According to the manufacturer's instructions, a colorimetric caspase-3 assay kit (Sigma-Aldrich Co. USA) was used to examine brain tissue homogenates prepared in lysis buffer. By using ELISA kits, B cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax) levels in the tissue homogenate were determined (LifeSpan BioSciences, Inc., Seattle, WA, USA). The process was carried out by the manufacturer's instructions. The units of measurement were ng/mg of tissue protein.

2.11 Statistical analysis

Data analysis was done using the Statistical Package (SPSS) for the Social Sciences. The results were presented as the mean ± standard error of the mean (SEM). To ascertain significance, Duncan's test was used after a one-way analysis of variance (ANOVA). The acceptable level of significance was accepted at $p < 0.05$.

3 Results

Cd concentration in brain tissues significantly increased after treatment with $CdCl_2$ (6.5 mg/kg). Treatment with PG-AgNPs ameliorates this rise in Cd concentration (Figure 1).

The goal of the current investigation was to assess the potential therapeutic effects of PG-AgNPs (3 mg/kg) on neurotoxicity produced by $CdCl_2$ (6.5 mg/kg) exposure. Reduced levels of DA and NE in brain tissue were found in $CdCl_2$-exposed rats, which suggested a disruption in monoaminergic neurotransmission

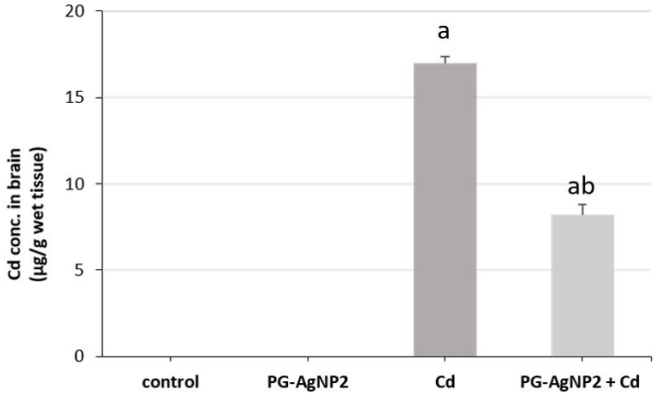

Figure 1. Bioaccumulation of Cd in brain tissue in response to PG-AgNPs and/or Cd treatment. Results are displayed as the mean ± SE (n = 10). a: $p < 0.05$ versus the control group; b: $p < 0.05$ versus the Cd-treated group.

and this was clear from the elevation in the activity of MAO and AChE as compared to control. Interestingly, the levels of these neurotransmitters were considerably restored ($p < 0.05$) by the injection of PG-AgNPs, suggesting the potent neuro-modulatory impact of PG-AgNPs against $CdCl_2$-mediated neurotoxicity in rats (Figure 2).

The studied excitatory amino acids (glutamate, aspartate, and glycine) markedly increased in the brain after $CdCl_2$ exposure ($p < 0.05$) compared to control groups. Following the induction of neurotoxicity by Cd, treatment with PG-AgNPs for 7 days led to a significant decrease in glutamate content as compared to the Cd group, but no significant changes were seen in the content of aspartate or glycine as compared to the $CdCl_2$ treated group as shown in Figure 3.

According to the data shown in Figure 3, rats exposed to $CdCl_2$ had significantly lower levels of the inhibitory amino acids GABA, taurine, and serene than animals in the control group. When compared to the $CdCl_2$ group, the inhibitory amino acids in brain tissue recorded significant amelioration in their contents after treatment with PG-AgNPs.

MDA, NO, and 8-OHdG production was elevated, indicating that the oxidative state of the brain tissue in $CdCl_2$-exposed rats was altered. These changes were followed by a significant decrease ($p < 0.05$) in the levels of endogenous antioxidant proteins such SOD, CAT, GSH, and its derived enzymes (GPx and GR) compared to the control group. The injection with PG-AgNPs considerably reduces the development of oxidative stress after exposure to $CdCl_2$ by increasing the levels of the examined antioxidant proteins and lowering the levels of pro-oxidants in brain tissue (Figures 4-5).

$CdCl_2$-induced Toxicity led to neuronal inflammation, which was detected by significantly higher tissue levels of pro-inflammatory cytokines (TNF-α and IL-6) compared to those found in the control group ($p < 0.05$). These brain inflammatory responses were dramatically reduced in PG-AgNPs-treated rats compared to the $CdCl_2$ group, demonstrating the anti-inflammatory effect of PG-AgNPs in the $CdCl_2$-induced neurotoxicity model (Figure 6).

To explore neuronal apoptotic events in the $CdCl_2$-induced neurotoxicity model rats and the potential anti-apoptotic role of PG-AgNPs treatment, the levels of Bcl-$_2$ and Bax and caspase-3 activity were examined in brain tissue. Compared with the control group, rats exposed to $CdCl_2$ exhibited significant elevations ($p < 0.05$) in the levels of apoptogenic proteins (Bax and caspase-3), whereas a significant reduction ($p < 0.05$) in the Bcl-2 level (anti-apoptotic protein) was observed. However, PG-AgNPs injection prevented the apoptotic cascade and reversed the $CdCl_2$-exposure-induced changes in apoptotic proteins compared with the untreated $CdCl_2$ levels, indicating the effective role played by, PG-AgNPs against neuronal loss following $CdCl_2$ exposure (Figure 7).

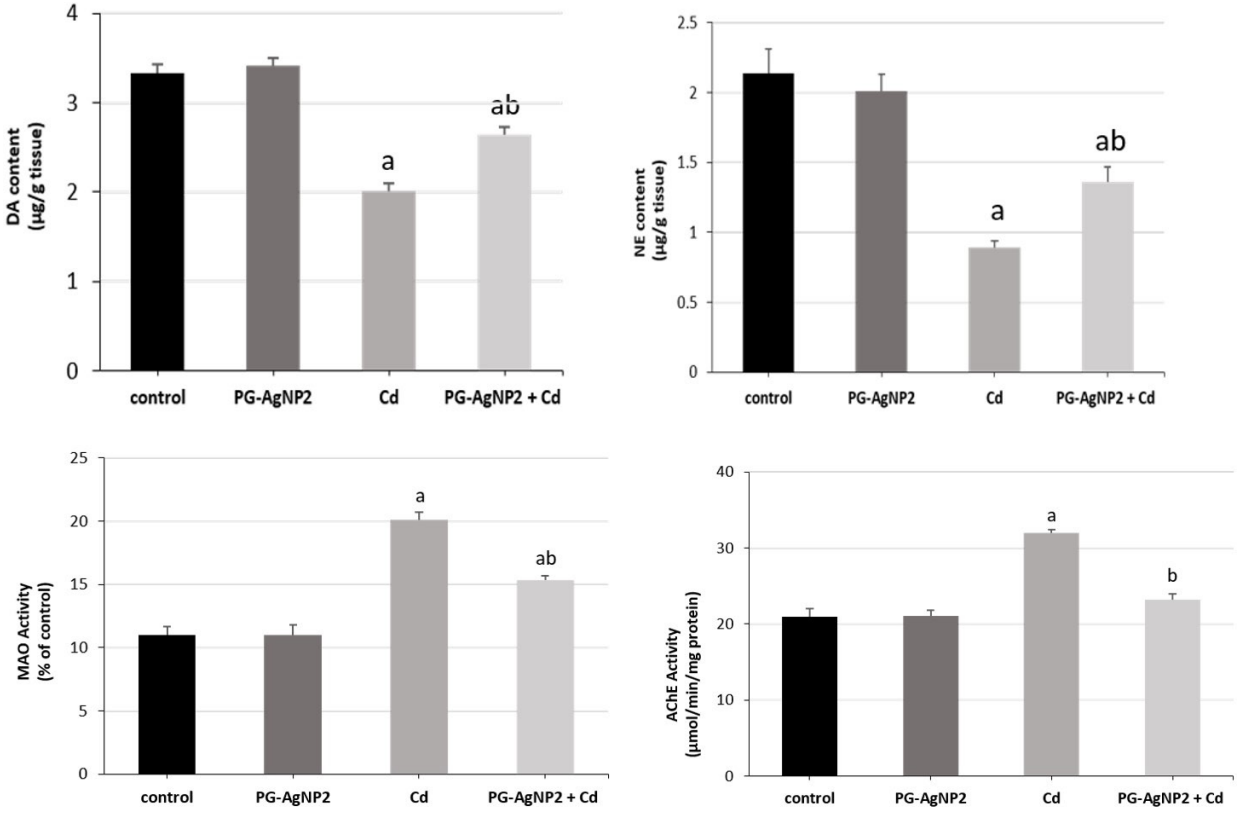

Figure 2. The effect of treatment with PG-AgNP2 (3 mg/kg) on the content of dopamine (DA) and norepinephrine (NE), monoaminoxidase (MAO) and acetylcholinesterase (AChE) in brain tissue in rats intoxicated with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

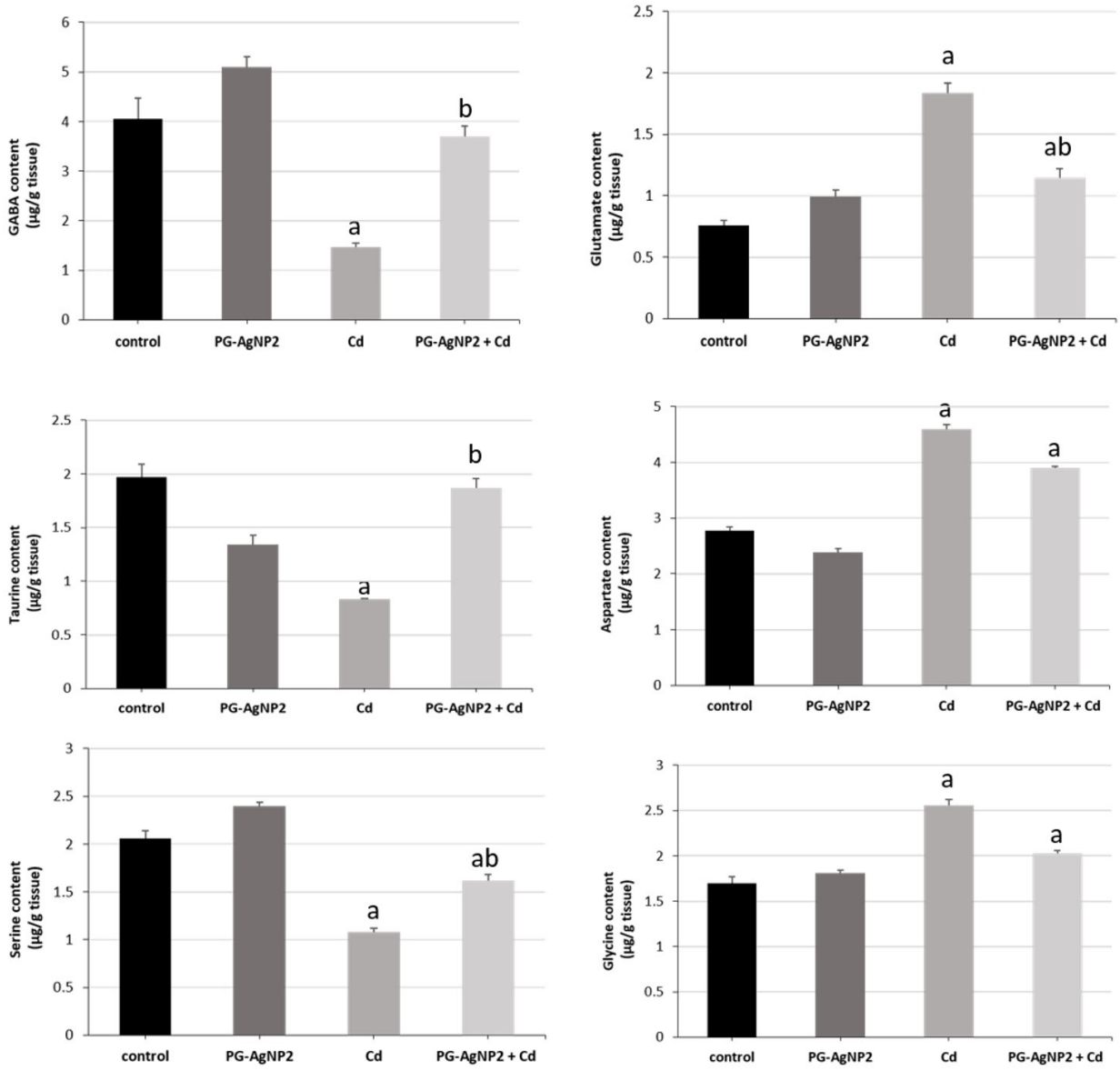

Figure 3. The effect of treatment with PG-AgNP2 (3 mg/kg) on the content of free excitatory (glutamate, aspartate and glycine) and inhibitory (GABA, taurine and serine) amino acids in brain tissue in rats intoxicated with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

4 Discussion

Prodigiosin, a red pigment derived from the *Serratia marcescens* strain, has been suggested to have a therapeutic effect against a variety of health issues connected to environmental toxins. Due to the improved bioavailability, delivery progression, and drug inflow to the target tissues provided by these treatment formulations compared to standard medication formulations, the employment of metal-based nanoparticles has emerged as a promising trend in the pharmaceutical industry. Several researchers demonstrated the accumulation of metals in the cells after treatment with metal-based nanoparticles in high doses for a long time. Patlolla et al. (2015) reported that a low dose of AgNP2 for 7 days does not cause any marked accumulation or toxicity in animal cells, it only enhances the delivery of the target treatment to the cells.

The purpose of the current investigation is to examine any potential therapeutic benefits of PG-AgNP2 for the neurotoxicity caused by Cd exposure. Cd is a harmful heavy metal that impairs both human and animal cellular and metabolic systems. Our findings demonstrated that after 7 days of treatment, levels of Cd in the brain tissue were high (approximately 600 times higher than control values) in the Cd-treated group. Because Cd can pass through the blood-brain barrier, it may have accumulated in the brain tissue (Shukla & Chandra, 1987). Following penetrating, Cd accumulates in several brain tissues and leads to cellular damage (Omairi et al., 2018; Sinha et al.,

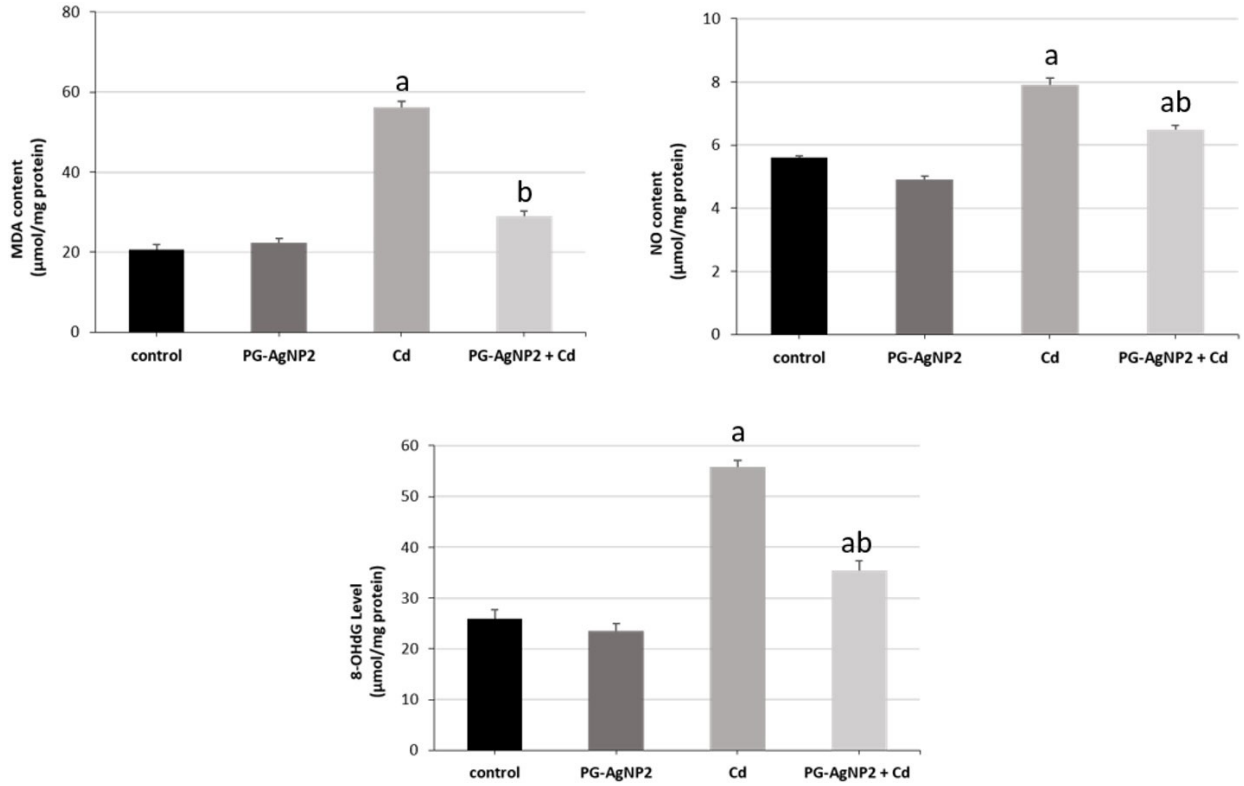

Figure 4. The effect of treatment with PG-AgNP2 (3 mg/kg) on brain levels of oxidative stress indicators in rats intoxicated with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

2008). Cd neurotoxicity is due to the production of ROS, which leads to oxidative stress (Chen et al., 2011). The ability of Cd in the production of ROS was confirmed by measuring the level of NO, MDA, and 8-OHdG in addition to determining the activity of antioxidant enzymes (GSH, GPx, GR, CAT, and SOD) in the brain homogenate of rats. Our results demonstrated that seven days of continuous exposure to Cd (6.5 mg/kg body weight) caused neuronal changes due to the depletion of antioxidant defence mechanisms, which disrupt cellular redox and cause oxidative stress. This conclusion was supported by a rise in MDA, 8-OHdG, and NO levels in addition to a fall in GSH levels as well as the activity of SOD, CAT, GR, and GPx.

The accumulation of Cd in the brain tissue, which consumes the GSH pool, may be responsible for the decline in antioxidant enzyme levels (Onyema et al., 2006). These enzymes become inactive when GSH levels drop, and Cd also inhibits oxidative enzymes by binding to their sulphydryl (-SH) groups (Renugadevi & Prabu, 2009). In our finding, the treatment with PG-AgNPs prevented Cd-induced changes in the redox status of brain tissue, as demonstrated by the inhibition of ROS production and MDA, 8-OHdG, and NO formation and the enhancement of the antioxidant system. These findings support the promising neuroprotective and antioxidative properties of PG-AgNPs. In their earlier study, Chang et al. (2011) reported that PG prevented neuronal oxidative and nitrative insults induced by hypoxia and ischemia by inhibiting NADPH oxidase2 activity and ROS production. Additionally, PG suppressed microcystin LR–mediated oxidative stress in HepG2 cells by inhibiting ROS production and activating 8-OHdG (Chen et al., 2019). Moreover, PG attenuated the development of oxidative damage associated with a gastric ulcer model, as demonstrated by decreased levels of lipid peroxidation and NO production and elevated levels of cellular antioxidant defense system components (Lapenda et al., 2020). This effect may be due to the free radical-scavenging activity of PG (Arivizhivendhan et al., 2018).

Cd has been found to increase the BBB's permeability, concentrating mostly in the brain's cortical tissue, which has been designated as a target for Cd-mediated toxicity (Yuan et al., 2013). In the current investigation of Cd accumulation in the brain, tissues may be related to the brain's susceptibility to Cd accumulation. Monoamines are essential for maintaining mood, motor control, and cognitive abilities, and it is impossible to ignore their significance in the formation and progression of neurodegenerative disorders. Consequently, xenobiotic substances that interfere with monoamines may cause changes in neurodevelopment (Felice et al., 2015; Kassab et al., 2019). According to our study, Cd is capable reduce the amounts of DA and NE in the brain tissue. Following Cd intoxication, the monoaminergic disturbance has been observed in animal models (Yıldız et al., 2022; Omairi et al., 2018).

The decrease in monoamine neurotransmitters following exposure to Cd may be caused by the production of ROS, which suppresses the enzymes involved in monoamine biosynthesis,

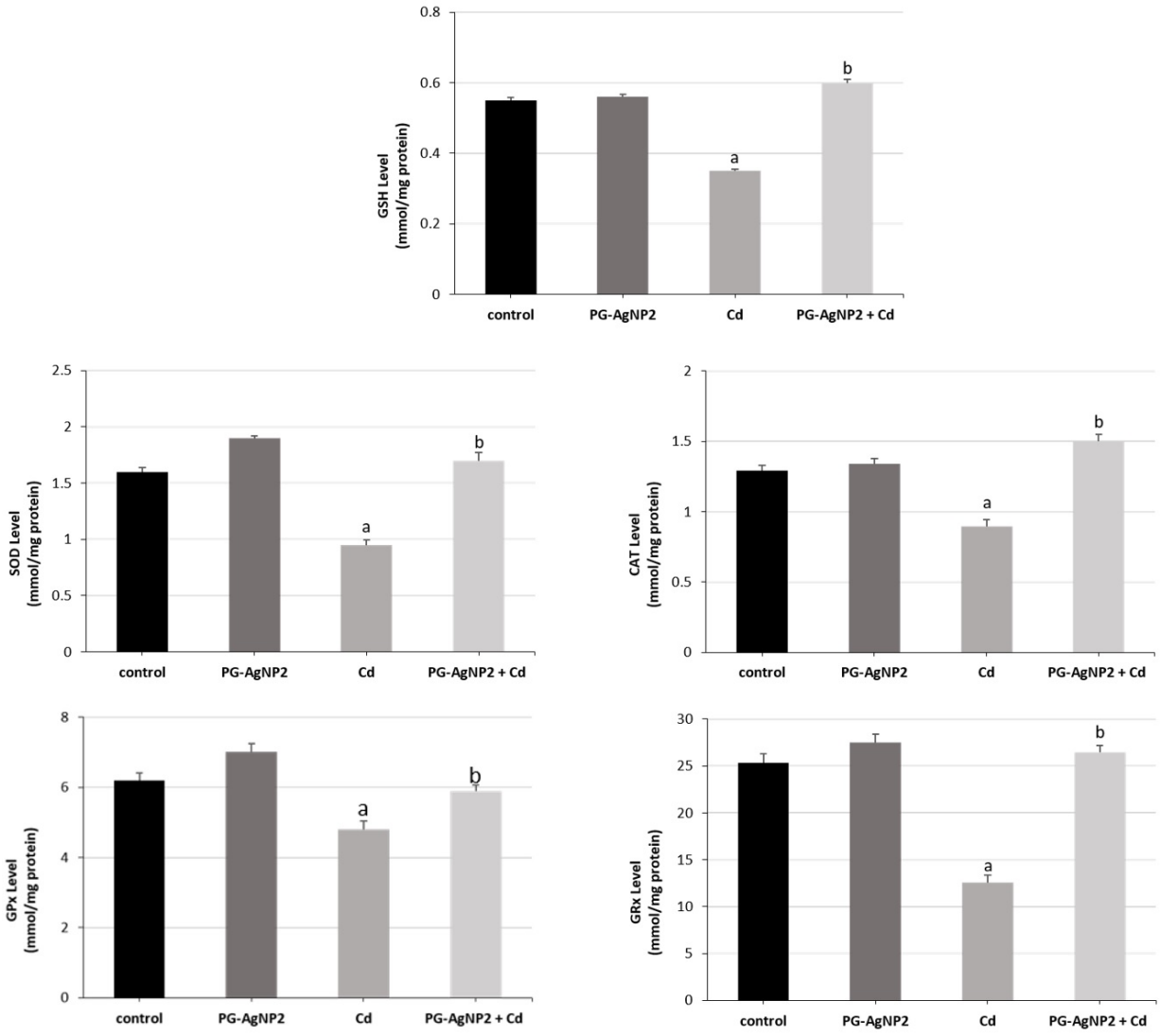

Figure 5. The effect of treatment with PG-AgNP2 (3 mg/kg) on brain antioxidant enzyme activities with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

disturbs monoamine metabolism by promoting their removal and breakdown, and inhibits the uptake of monoamines (Maodaa et al., 2016; Lizarraga et al., 2015). Additionally, it was recently found by Alnahdi & Sharaf (2019) that Cd toxicity activated monoamine oxidase (MAO), an enzyme that catalyzes the oxidative deamination of monoamines, which led to an increase in hydroxyl radical in the brain and a decrease in the contents of NE and DA in the brain (Štrac et al., 2016; Vitrac & Benoit-Marand, 2017). Interestingly, monoamine contents in the brain tissue were revived in PG-AgNP2 treated rats, indicating the neuroprotection effect of PG-AgNP2 against the disturbances that occurred following Cd intoxication. Our study is the first to investigate the possible neurotherapeutic effect of PGs-AgNPs, showing PGs' capacity to regulate neurotransmission in brain tissues, especially monoamines.

Inhibition of AChE activity in rats exposed to $CdCl_2$ is another sign of brain damage in the current research. The primary enzyme responsible for converting acetylcholine (ACh) into acetic acid and choline is known as AChE. ACh is deposited in central cholinergic synapses and neuromuscular junctions as a result of AChE being suppressed under oxidative stress, which may lead to neuronal dysfunctions such as neuromuscular cholinergic hyperactivity (Olayan et al., 2020). Carageorgiou et al. (2004) reported a considerable inhibition of AChE activity following Cd intoxication. Cd is one of the metal inactivators of AChE and can induce a conformational change in the enzyme-protein part, which leads to the formation of an inactive enzyme. Interestingly, PG-AgNP2 administration ameliorates the poisonous effect of Cd on the AChE activity. Although it is a promising therapeutic agent in the development of new anti-Alzheimer drugs due to its ability to regulate AChE activity (Ayaz et al., 2019), PG-AgNP2 was found to enhance the AChE activity in response to Cd intoxication, which may be due to its capability to quench ROS generated by Cd, or through the prevention of the interaction between Cd and AChE.

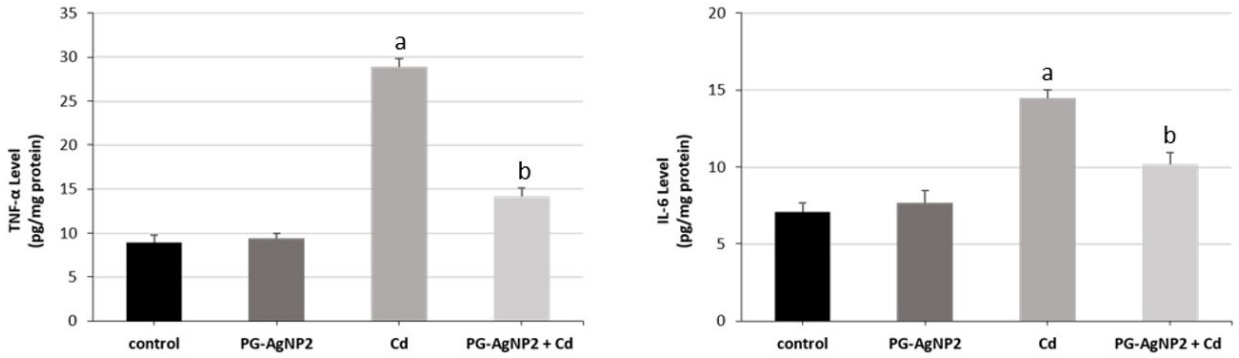

Figure 6. The effect of treatment with PG-AgNP2 (3 mg/kg) on brain neuroinflammatory markers in rats intoxicated with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

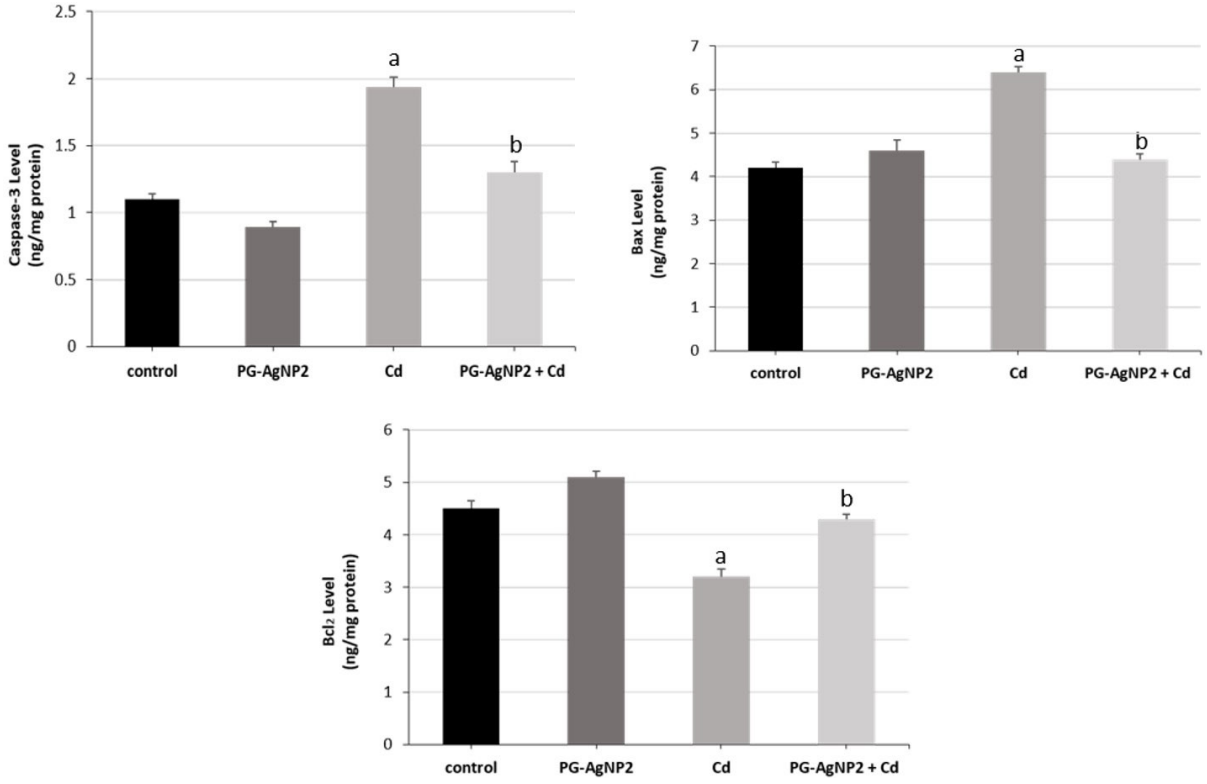

Figure 7. The effect of treatment with PG-AgNP2 (3 mg/kg) on brain level of apoptosis markers in rats intoxicated with $CdCl_2$ for 7 days. Data are expressed as means ± standard error (SE) for 10 animals/group. a: significance change at $P < 0.05$ in comparison with the control group; b: significance change at $P < 0.05$ with respect to $CdCl_2$.

In the present study, Cd injection for 7 days increased the production of proinflammatory cytokines specially TNF-α (trigger for other cytokines) and IL-6 (interface of inflammatory and immune response) which are the cause of damage in the brain tissue. TNF- α is a transmembrane protein/cytokine that is appear as a response to pathogen invasion in macrophages. It is also, used as the inflammatory interface of both local and systemic inflammation (Tracey, 2002). TNF- α is play a major role in the production of IL-6 and other mediators important in extending the inflammatory response and tissue damage (Aly et al., 2018). Yang et al. (2019), proved that inflammatory cytokines have a stimulating effect on the accumulation of neutrophils to increase the injury of inflammation in the tissues. The present results are also in harmony with Elkhadragy et al. (2018), who found an increase in TNF- α and IL-6 in the brain tissue of rats treated with $CdCl_2$. In the present study, the treatment with PG-$AgNP_2$ reduced the elevation of the inflammatory cytokines (TNF- α and IL-6) in brain tissues. The mechanism by which the PG-$AgNP_2$ repair the damage produced by Cd and ameliorate the studied cytokines may be due to its potent anti-inflammatory effect (Lin et al., 2019).

Cd induces apoptosis in various cells by interfering with protein kinase C, mitogen-activated protein kinase, and phospholipase C, as well as by suppressing calcium-dependent ATPase or by stimulating the inositol triphosphate pathway. According to the current findings, the amount of the apoptosis-inducing genes (Bax and caspase-3) increased while Bcl-2, which inhibits apoptosis, was decreased in the brain tissue. These results may be explained by the Cd capacity to increase the entry of Ca^{2+} into the mitochondria, which interferes with the normal metabolism of the mitochondria and causes apoptosis and growth arrest in neuronal cells (Xu et al., 2011; Yuan et al., 2013).

In the brain tissue of rats receiving PG-AgNP2 treatment, apoptosis was reduced. However, treatment with PG reduced the Cd-induced loss of neuronal cells as evidenced by a decrease in the production of pro-apoptotic proteins (Bax and caspase-3) and an increase in the expression of the anti-apoptotic protein Bcl-2. These findings are in agreement with those of Al Omairi, et al., (2022), who found that PG inhibited apoptosis in depressed rats. Lapenda et al. (2020), recorded the anti-apoptotic action of PG and found that it inhibited the apoptotic cascade linked to stomach lesions brought on by injections of acidified ethanol by upregulating Bcl-2 and downregulating Bax and caspase-3.

5 Conclusion

In conclusion, treatment with PG-AgNP2 exhibits significant neuroprotective effects against Cd-induced toxicity in rats by suppressing pro-oxidative insults (ROS, NO, and MDA), enhancing antioxidative defense systems (GSH, GPx, GR, SOD, and CAT), reducing neuronal inflammation (TNF-α, and IL-6), preventing neuronal apoptosis by lowering pro-apoptotic factors and increasing the anti-apoptotic protein, and modulating monoaminergic, amino-acidergic, and cholinergic transmission significantly in the brain tissue.

Ethical approval

All experiments were performed in accordance with the European Community Directive (95/701/EEC). The animal care procedures agreed with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, eighth edition, and were approved by the Institutional Animal Ethics Committee for Laboratory Animal Care at the Zoology Department, Faculty of Science, Helwan University (Approval number: HU/Z/010-19).

Conflict of interest

The authors declare no conflicts of interest.

Availability of data and material

The data used to support the findings of this study are included within the article.

Author contributions

All the authors contributed equally to this work.

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References

Aebi, H. (1984). Catalase. *Methods in Enzymology*, 105,121–126.

Almeer, R. S., Alarifi, S., Alkahtani, S., Ibrahim, S. R., Ali, D., & Moneim, A. (2018). The potential hepatoprotective effect of royal jelly against cadmium chloride-induced hepatotoxicity in mice is mediated by suppression of oxidative stress and upregulation of Nrf2 expression. *Biomedicine and Pharmacotherapy*, 106, 1490-1498. http://dx.doi.org/10.1016/j.biopha.2018.07.089. PMid:30119224.

Alnahdi, H. S., & Sharaf, I. A. (2019). Possible prophylactic effect of omega-3 fatty acids on cadmium-induced neurotoxicity in rats' brains. *Environmental Science and Pollution Research International*, 26(30), 31254-31262. http://dx.doi.org/10.1007/s11356-019-06259-8. PMid:31468353.

Aly, H. F., Elrigal, N. S., Ali, S. A., Rizk, M. Z., & Ebrahim, N. A. (2018). Modulatory effects of Casimiroa Edulis on aluminium nanoparticles - associated neurotoxicity in a rat model of induced Alzheimer's disease. *Journal of Material and Environmental Science*, 9(7), 1931-1941.

Al Omairi, N. E., Albrakati, A., Alsharif , K. F., Almalki, A. S., Alsanie, W., Abd Elmageed, Z. Y., Zaafar, D., Lokman, M. S., Bauomy, A. A., Belal, S. K., Abdel-Daim, M. M., Abdel Moneim, A. E., Alyami, H., Kassab, R. B. (2022). Selenium Nanoparticles with Prodigiosin Rescue Hippocampal Damage Associated with Epileptic Seizures Induced by Pentylenetetrazole in Rats. *Biology (Basel)*, 11(3), 354. https://doi.org/10.3390/biology11030354.

Arivizhivendhan, K. V., Mahesh, M., Boopathy, R., Swarnalatha, S., Mary, R. R., & Sekaran, G. (2018). Antioxidant and antimicrobial activity of bioactive prodigiosin produces from Serratia marcescens using agricultural waste as a substrate. *Journal of Food Science and Technology*, 55(7), 2661-2670. http://dx.doi.org/10.1007/s13197-018-3188-9. PMid:30042582.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Ovais, M., Ullah, I., Ahmed, J., & Shahid, M. (2019). Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Frontiers in Aging Neuroscience*, 11, 155. http://dx.doi.org/10.3389/fnagi.2019.00155. PMid:31293414.

Babu, M. M. G., & Gunasekaran, P. (2013). Extracellular synthesis of crystalline silver nanoparticles and its characterization. *Materials Letters*, 90, 162-164. http://dx.doi.org/10.1016/j.matlet.2012.09.029.

Bernardes, N., Seruca, R., Chakrabarty, A. M., & Fialho, A. M. (2010). Microbial-based therapy of cancer current progress and future prospects. *Bioengineered Bugs*, 1(3), 178-190. http://dx.doi.org/10.4161/bbug.1.3.10903. PMid:21326924.

Bhattacharya, D., & Gupta, R. K. (2005). Nanotechnology and potential of microorganisms. *Critical Reviews in Biotechnology*, 25(4), 199-204. http://dx.doi.org/10.1080/07388550500361994. PMid:16419617.

Carageorgiou, H., Tzotzes, V., Pantos, C., Mourouzis, C., Zarros, A., & Tsakiris, S. (2004). *In vivo* and *in vitro* effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na+, K+)- ATPase and Mg2+-ATPase activities: protection by L-cysteine. *Basic & Clinical Pharmacology & Toxicology*, 94(3), 112-118. http://dx.doi.org/10.1111/j.1742-7843.2004.pto940303.x. PMid:15049340.

Chang, C. C., Wang, Y. H., Chern, C. M., Liou, K. T., Hou, Y. C., Peng, Y. T., & Shen, Y. C. (2011). Prodigiosin inhibits gp91phox and

iNOS expression to protect mice against the oxidative/nitrosative brain injury induced by hypoxia–ischemia. *Toxicology and Applied Pharmacology*, 257(1), 137-147. http://dx.doi.org/10.1016/j.taap.2011.08.027. PMid:21925195.

Chater, S., Douki, T., Garrel, C., Favier, A., Sakly, M., & Abdelmelek, H. (2008). Cadmium-induced oxidative stress and DNA damage in kidney of pregnant female rats. *Comptes Rendus Biologies*, 331(6), 426-432. http://dx.doi.org/10.1016/j.crvi.2008.03.009. PMid:18510995.

Chen, J., Li, Y., Liu, F., Hou, D. X., Xu, J., Zhao, X., Yang, F., & Feng, X. (2019). Prodigiosin promotes Nrf2 activation to inhibit oxidative stress induced by microcystin-LR in HepG2 cells. *Toxins*, 11(7), 403. http://dx.doi.org/10.3390/toxins11070403. PMid:31336817.

Chen, L., Xu, B., Liu, L., Luo, Y., Zhou, H., Chen, W., Shen, T., Han, X., Kontos, C. D., & Huang, S. (2011). Cadmium induction of reactive oxygen species activates the mTOR pathway, leading to neuronal cell death. *Free Radical Biology & Medicine*, 50(5), 624-632. http://dx.doi.org/10.1016/j.freeradbiomed.2010.12.032. PMid:21195169.

Dar, A., Khan, K. M., Ateeq, H. S., Khan, S., Rahat, S., Perveen, S., & Supuran, C. T. (2005). Inhibition of monoamine oxidase–a activity in rat brain by synthetic hydrazines: structure-activity relationship (SAR). *Journal of Enzyme Inhibition and Medicinal Chemistry*, 20(3), 269-274. http://dx.doi.org/10.1080/14756360400026212. PMid:16119198.

Díaz-Ruiz, C., Montaner, B., & Pérez-Tomás, R. (2001). Prodigiosin induces cell death and morphological changes indicative of apoptosis in gastric cancer cell line HGT-1. *Histology and Histopathology*, 16(2), 415-421. PMid:11332697.

El-Batal, A. I., El-Hendawy, H. H., & Faraag, A. H. (2017). In silico and in vitro cytotoxic effect of prodigiosin-conjugated silver nanoparticles on liver cancer cells (HepG2). *Journal of Biotechnology, Computational Biology and Bionanotechnology*, 98(3), 225-243. http://dx.doi.org/10.5114/bta.2017.70801.

Elkhadragy, M. F., Kassab, R. B., Metwally, D., Almeer, R. S., Abdel-Gaber, R., Al-Olayan, E. M., Essawy, E. A., Amin, H. K., & Moneim, A. E. A. (2018). Protective effects of Fragaria ananassa methanolic extract in a rat model of cadmium chloride-induced neurotoxicity. *Bioscience Reports*, 38(6), BSR20180861. http://dx.doi.org/10.1042/BSR20180861. PMid:30291211.

Ellman, G. L., Courtney, K. D., Andres, V. Jr., and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7, 88–95.

Faraag, A. H., El-Batal, A. I., & El-Hendawy, H. H. (2017). Characterization of prodigiosin produced by Serratia marcescens strain isolated from irrigation water in Egypt. *Nature and Science*, 15(5), 55-68.

Felice, A., Ricceri, L., Venerosi, A., Chiarotti, F., & Calamandrei, G. (2015). Multifactorial origin of neurodevelopmental disorders: approaches to understanding complex etiologist. *Toxics*, 3(1), 89-129. http://dx.doi.org/10.3390/toxics3010089. PMid:29056653.

Fullan, N. P., Lynch, D. L., & Ostrow, D. H. (1977). Effects of bacterial extracts on Chinese-hamster cells and rat neoplasms. *Microbios Letter*, 5, 157-161.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical Biochemistry*, 126(1), 131-138. http://dx.doi.org/10.1016/0003-2697(82)90118-X. PMid:7181105.

Gupta, V. K., Singh, S., Agrawal, A., Siddiqi, N. J., & Sharma, B. (2015). Phytochemicals mediated remediation of neurotoxicity induced by heavy metals. *Biochemistry Research International*, 2015, 534769. http://dx.doi.org/10.1155/2015/534769. PMid:26618004.

Han, R., Xiang, R., Li, J., Wang, F., & Wang, C. (2021). High-level production of microbial prodigiosin: a review. *Journal of Basic Microbiology*, 61(6), 506-523. http://dx.doi.org/10.1002/jobm.202100101. PMid:33955034.

Hannun, Y. A. (1997). Apoptosis and the dilemma of cancer chemotherapy. *Blood*, 89(6), 1845-1853. http://dx.doi.org/10.1182/blood.V89.6.1845. PMid:9058703.

Heinrikson, R. L., & Meredith, S. C. (1984). Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenyl isothiocyanate. *Analytical Biochemistry*, 136(1), 65-74. http://dx.doi.org/10.1016/0003-2697(84)90307-5. PMid:6711815.

Kassab, R. B., Lokman, M. S., & Essawy, E. A. (2019). Neurochemical alterations following the exposure to di-n-butyl phthalate in rats. *Metabolic Brain Disease*, 34(1), 235-244. http://dx.doi.org/10.1007/s11011-018-0341-0. PMid:30446882.

Koyande, A. K., Show, P. L., Guo, R., Tang, B., Ogino, C., & Chang, J. S. (2019). Bio-processing of algal bio-refinery: a review on current advances and future perspectives. *Bioengineered*, 10(1), 574-592. http://dx.doi.org/10.1080/21655979.2019.1679697. PMid:31668124.

Lapenda, J. C. L., Alves, V. P., Adam, M. L., Rodrigues, M. D., & Nascimento, S. C. (2020). Cytotoxic effect of prodigiosin, natural red pigment, isolated from *Serratia marcescens* UFPEDA 398. *Indian Journal of Microbiology*, 60(2), 182-195. http://dx.doi.org/10.1007/s12088-020-00859-6. PMid:32255851.

Lateef, A., Ojo, S. A., Elegbede, J. A., Azeez, M. A., Yekeen, T. A., & Akinboro, A. (2017). Evaluation of some biosynthesized silver nanoparticles for biomedical applications: hydrogen peroxide scavenging, anticoagulant and thrombolytic activities. *Journal of Cluster Science*, 28(3), 1379-1392. http://dx.doi.org/10.1007/s10876-016-1146-0.

Lin, P. B., Shen, J., Ou, P. Y., Liu, L. Y., Chen, Z. Y., Chu, F. J., Wang, J., & Jin, X. B. (2019). Prodigiosin isolated from Serratia marcescens in the Periplaneta americana gut and its apoptosis-inducing activity in HeLa cells. *Oncology Reports*, 41(6), 3377-3385. http://dx.doi.org/10.3892/or.2019.7089. PMid:30942457.

Lizarraga, L. E., Cholanians, A. B., Phan, A. V., Herndon, J. M., Lau, S. S., & Monks, T. J. (2015). Vesicular monoamine transporter 2 and the acute and long-term response to 3,4-(±)-methylenedioxymethamphetamine. *Toxicological Sciences*, 143(1), 209-219. http://dx.doi.org/10.1093/toxsci/kfu222. PMid:25370842.

Llagostera, E., Soto-Cerrato, V., Joshi, R., Montaner, B., Gimenez-Bonafé, P., & Pérez-Tomás, R. (2005). High cytotoxic sensitivity of the human small cell lung doxorubicin-resistant carcinoma (GLC4/ADR) cell line to prodigiosin through apoptosis activation. *Anti-Cancer Drugs*, 16(4), 393-399. http://dx.doi.org/10.1097/00001813-200504000-00005. PMid:15746575.

Lodovici, M., Casalini, C., Briani, C., & Dolara, P. (1997). Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology*, 117(1), 55-60. http://dx.doi.org/10.1016/S0300-483X(96)03553-6. PMid:9020199.

López, E., Figueroa, S., Oset-Gasque, M. J., & González, M. P. (2003). Apoptosis and necrosis: two distinct events induced by cadmium in cortical neurons in culture. *British Journal of Pharmacology*, 138(5), 901-911. http://dx.doi.org/10.1038/sj.bjp.0705111. PMid:12642392.

Luparello, C., Sirchia, R., & Longo, A. (2011). Cadmium as a transcriptional modulator in human cells. *Critical Reviews in Toxicology*, 41(1), 73-80. http://dx.doi.org/10.3109/10408444.2010.529104. PMid:21073263.

Manikprabhu, D., & Lingappa, K. (2014). Synthesis of silver nanoparticles using the Streptomyces coelicolor klmp33 pigment: an antimicrobial agent against extended-spectrum beta-lactamase (ESBL) producing

Escherichia coli. *Materials Science and Engineering C*, 45, 434-437. http://dx.doi.org/10.1016/j.msec.2014.09.034. PMid:25491848.

Maodaa, S. N., Allam, A. A., Ajarem, J., Abdel-Maksoud, M. A., Al-Basher, G. I., & Wang, Z. Y. (2016). Effect of parsley (Petroselinum crispum, Apiaceae) juice against cadmium neurotoxicity in albino mice (Mus Musculus). *Behavioral and Brain Functions*, 12(1), 6. http://dx.doi.org/10.1186/s12993-016-0090-3. PMid:26846273.

Méndez-Armenta, M., & Ríos, C. (2007). Cadmium neurotoxicity. *Environmental Toxicology and Pharmacology*, 23(3), 350-358. http://dx.doi.org/10.1016/j.etap.2006.11.009. PMid:21783780.

Montaner, B., & Pérez-Tomás, R. (2001). Prodigiosin-induced apoptosis in human colon cancer cells. *Life Sciences*, 68(17), 2025-2036. http://dx.doi.org/10.1016/S0024-3205(01)01002-5. PMid:11388704.

Montaner, B., Navarro, S., Piqué, M., Vilaseca, M., Martinell, M., Giralt, E., Gil, J., & Pérez-Tomás, R. (2000). Prodigiosin from the supernatant of Serratia marcescens induces apoptosis in haematopoietic cancer cell lines. *British Journal of Pharmacology*, 131(3), 585-593. http://dx.doi.org/10.1038/sj.bjp.0703614. PMid:11015311.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46(2), 849-854. http://dx.doi.org/10.1016/S0006-291X(72)80218-3. PMid:4400444.

Numan, M., Bashir, S., Mumtaz, R., Tayyab, S., Rehman, N. U., Khan, A. L., Shinwari, Z. K., & Al-Harrasi, A. (2018). Therapeutic applications of bacterial pigments: a review of current status and future opportunities. *3 Biotech*, 8(4), 207. http://dx.doi.org/10.1007/s13205-018-1227-x. PMid:29623249.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351-358. http://dx.doi.org/10.1016/0003-2697(79)90738-3. PMid:36810.

Ojo, S. A., Lateef, A., Azeez, M. A., Oladejo, S. M., Akinwale, A. S., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Gueguim-Kana, E. B., & Beukes, L. S. (2016). Biomedical and catalytic applications of gold and silver-gold alloy nanoparticles biosynthesized using cell-free extract of Bacillus safensis LAU 13: antifungal, dye degradation, anti-coagulant and thrombolytic activities. *IEEE Transactions on Nanobioscience*, 15(5), 433-442. http://dx.doi.org/10.1109/TNB.2016.2559161. PMid:27164598.

Olayan, E. M., Aloufi, A. S., AlAmri, O. D., El-Habit, O. H., & Moneim, A. E. A. (2020). Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. *Science of the Total Environment*, 723, 137969. http://dx.doi.org/10.1016/j.scitotenv.2020.137969. PMid:32392679.

Omairi, N. E., Radwan, O. K., Alzahrani, Y. A., & Kassab, R. B. (2018). Neuroprotective efficiency of Mangifera indica leaves extract on cadmium-induced cortical damage in rats. *Metabolic Brain Disease*, 33(4), 1121-1130. http://dx.doi.org/10.1007/s11011-018-0222-6. PMid:29557530.

Onyema, O. O., Farombi, E. O., Emerole, G. O., Ukoha, A. I., & Onyeze, G. O. (2006). Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Indian Journal of Biochemistry & Biophysics*, 43(1), 20-24. PMid:16955747.

Pagel, P., Blome, J., & Wolf, H. U. (2000). High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. *Journal of Chromatography B: Biomedical Sciences and Applications*, 746(2), 297-304. http://dx.doi.org/10.1016/S0378-4347(00)00348-0. PMid:11076082.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine*, 70(1), 158-169. PMid:6066618.

Pan, M. Y., Shen, Y. C., Lu, C. H., Yang, S. Y., Ho, T. F., Peng, Y. T., & Chang, C. C. (2012). Prodigiosin activates endoplasmic reticulum stress cell death pathway in human breast carcinoma cell lines. *Toxicology and Applied Pharmacology*, 265(3), 325-334. http://dx.doi.org/10.1016/j.taap.2012.08.034. PMid:22982536.

Patlolla, A. K., Hackett, D., & Tchounwou, P. B. (2015). Silver nanoparticle-induced oxidative stress-dependent toxicity in Sprague-Dawley rats. *Molecular and Cellular Biochemistry*, 399(1-2), 257-268. http://dx.doi.org/10.1007/s11010-014-2252-7. PMid:25355157.

Renugadevi, J., & Prabu, S. M. (2009). Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. *Toxicology*, 256(1-2), 128-134. http://dx.doi.org/10.1016/j.tox.2008.11.012. PMid:19063931.

Rubilar, O., Rai, M., Tortella, G., Diez, M. C., Seabra, A. B., & Durán, N. (2013). Biogenic nanoparticles: copper, copper oxides, copper sulphides, complex copper nanostructures and their applications. *Biotechnology Letters*, 35(9), 1365-1375. http://dx.doi.org/10.1007/s10529-013-1239-x. PMid:23690046.

Salem, F. E. H. (2021). Modulatory effect of *Camellia sinensis* extract on the function of hypothalamic-pituitary-testicular axis in PTZ-induced epileptic seizures in rat model. *Egyptian Academic Journal of Biological Sciences (B. Zoology)*, 13(2), 185-195. http://dx.doi.org/10.21608/eajbsz.2021.205679.

Sastry, M., Ahmad, A., Islam, K. M., & Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current Science*, 85, 162-170.

Satarug, S., Swaddiwudhipong, W., Ruangyuttikarn, W., Nishijo, M., & Ruiz, P. (2013). Modeling cadmium exposures in low- and high-exposure areas in Thailand. *Environmental Health Perspectives*, 121(5), 531-536. http://dx.doi.org/10.1289/ehp.1104769. PMid:23434727.

Shagirtha, K., Bashir, N., & MiltonPrabu, S. (2017). Neuroprotective efficacy of hesperetin against cadmium induced oxidative stress in the brain of rats. *Toxicology and Industrial Health*, 33(5), 454-468. http://dx.doi.org/10.1177/0748233716665301. PMid:27803291.

Shukla, G. S., & Chandra, S. V. (1987). Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. *Archives of Environmental Contamination and Toxicology*, 16(3), 303-310. http://dx.doi.org/10.1007/BF01054947. PMid:3592755.

Sinha, M., Manna, P., & Sil, P. C. (2008). Cadmium-induced neurological disorders: prophylactic role of taurine. *Journal of Applied Toxicology*, 28(8), 974-986. http://dx.doi.org/10.1002/jat.1363. PMid:18548748.

Štrac, D. Š., Pivac, N., & Mück-Šeler, D. (2016). The serotonergic system and cognitive function. *Translational Neuroscience*, 7(1), 35-49. http://dx.doi.org/10.1515/tnsci-2016-0007. PMid:28123820.

Sudhakar, C., Shobana, C., Selvankumar, T., & Selvam, K. (2021). Prodigiosin production from *Serratia marcescens* strain CSK and their antioxidant, antibacterial, cytotoxic effect and in silico study of caspase-3 apoptotic protein. *Biotechnology and Applied Biochemistry*, 10.1002/bab.2261. Ahead of print. http://dx.doi.org/10.1002/bab.2261. PMid:34582588.

Suryawanshi, R. K., Patil, C. D., Koli, S. H., Hallsworth, J. E., & Patil, S. V. (2017). Antimicrobial activity of prodigiosin is attributable to plasma-membrane damage. *Natural Product Research*, 31(5), 572-577. http://dx.doi.org/10.1080/14786419.2016.1195380. PMid:27353356.

Tracey, K. J. (2002). The inflammatory reflex. *Nature*, 420(6917), 853-859. http://dx.doi.org/10.1038/nature01321. PMid:12490958.

Venil, C. (2009). An insightful overview on microbial pigment, prodigiosin. *The Electricity Journal*, 5, 49-61.

Vitrac, C., & Benoit-Marand, M. (2017). Monoaminergic modulation of motor cortex function. *Frontiers in Neural Circuits*, 11, 72. http://dx.doi.org/10.3389/fncir.2017.00072. PMid:29062274.

Xu, B., Chen, S., Luo, Y., Chen, Z., Liu, L., Zhou, H., Chen, W., Shen, T., Han, X., Chen, L., & Huang, S. (2011). Calcium signaling is involved in cadmium-induced neuronal apoptosis via induction of reactive oxygen species and activation of MAPK/mTOR network. *PLoS One*, 6(4), e19052. http://dx.doi.org/10.1371/journal.pone.0019052. PMid:21544200.

Yang, W., Tao, Y., Wu, Y., Zhao, X., Ye, W., Zhao, D., Fu, L., Tian, C., Yang, J., He, F., Tang, L. (2019). Neutrophils promote the development of reparative macrophages mediated by ROS to orchestrate liver repair. *National Communication*, 10(1), 1076. https://doi.org/10.1038/s41467-019-09046-8.

Yıldız, M. O., Çelik, H., Caglayan, C., Genç, A., Doğan, T., & Satıcı, E. (2022). Neuroprotective effects of carvacrol against cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. *Metabolic Brain Disease*, 37(4), 1259-1269. http://dx.doi.org/10.1007/s11011-022-00945-2. PMid:35316447.

Yuan, Y., Jiang, C. Y., Xu, H., Sun, Y., Hu, F. F., Bian, J. C., Liu, X. Z., Gu, J. H., & Liu, Z. P. (2013). Cadmium-induced apoptosis in primary rat cerebral cortical neurons culture is mediated by a calcium signaling pathway. *PLoS One*, 8(5), e64330. http://dx.doi.org/10.1371/journal.pone.0064330. PMid:23741317.