RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 6: 953-962 (June 2025)

Potential Neuromodulatory Effects of Prodigiosin-conjugated Silver Nanoparticles: Implications for Epilepsy Management and Their Impact on Obesity in Rat Model

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10.18805/IJAR.BF-1915

ABSTRACT

Background: Exposure to pentylenetetrazole (PTZ) is capable of inducing experimental epilepsy in rats. As a result of the use of anticonvulsant treatment, obesity and metabolic syndrome are commonly observed in patients with epilepsy. Prodigiosin (PG) is a red pigment synthesized by bacterial species with important pharmaceutical and biological activities. Here, we investigated the neuroprotective and anticonvulsant activities of silver nanoparticles conjugated with PG (PG-AgNPs) versus pentylenetetrazole (PTZ)-induced epileptic seizures.

Methods: Rats were assigned into six experimental groups: control; PG-AgNP (300 mg/kg); PTZ (60 mg/kg, epileptic model); sodium valproate (600 mg/kg) + PTZ; PG-AgNP + PTZ and sodium valproate (VPA)+PG-AgNP+ PTZ. The treatment duration is extended to 7 days.

Result: Induction of epilepsy resulted in a significant decrease in monoamines (5-HT, DA, NE), glutamate, aspartate, serine and a significant increase in GABA, glycine and taurine in the brain. In addition, it causes an increase in serum insulin and leptin and a significant decrease in serum glucose and disturbance in oxidative and antioxidant system. Additionally, PG-AgNP enhanced the antioxidant capacity of brain tissue by activating antioxidants and decreasing the levels of pro-oxidants. In addition, it caused decrease in insulin resistant and leptin level in serum accordingly caused increase in the content of blood glucose level which enhance the metabolic rate and minimize the increase in the body weight as a result of anticonvulsant (VPA). Interestingly, PG-AgNP restored the PTZ-induced imbalance between excitatory and inhibitory amino acids and improved monoaminergic and cholinergic transmission.

Key words: Apoptosis, Epilepsy, Obesity, Oxidative stress, PG-AgNPs, Prodigiosin.

INTRODUCTION

Epilepsy is a neurodevelopmental disorder that is hypothesized to arise due to exposure to a broad range of toxicants or alternation in a constellation of genetic factors. These etiological factors can disrupt neuronal development leading to the manifestation of epileptic symptoms. Epilepsy is growing at an alarming pace with 50 million people being affected globally. Abnormalities in several brain regions and pathways could be responsible for this disorder (Devinsky *et al*., 2018). Epileptic seizures can be produced by the disturbance in neuronal excitation and inhibition with amino acid neurotransmitters (Morimoto *et al*., 2004). Treatment of epilepsy was improved by several third-generation of antiepileptic drugs during the past decades. Valproic acid is one of the most widely used antiepileptic drugs and is also used to treat bipolar disorder (Natasha *et al*., 2008). Many reports have shown that common antiepileptic drugs can cause behavioral disorders, learning difficulties and multiple birth defects (Štefánik *et al*., 2015).

Currently available antiepileptic medications cause many adverse events, including memory disturbances, gastrointestinal distress, osteoporosis, depression, fatigue, nausea and weight gain (de Kinderen *et al*., 2014). Advances in nanotechnology have led to the development of various metal-based nano formulations, which have recently been used to treat neurodegenerative diseases (Gupta *et al*., 2019). The effects of nanoparticles (NPs) on target tissues depend on the size, shape, concentration and type of NPs, as well as the tissue structure and exposure time. Silver nanoparticles (AgNPs) are absorbed more effectively and their relatively small sizes allow them

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How to cite this article: Almohawes, Z.N., El-Khadragy, M.F., Al-Megrin, W.A.I. and Salem, F.E.H. (2025). Potential Neuromodulatory Effects of Prodigiosin-Conjugated Silver Nanoparticles: Implications for Epilepsy Management and their Impact on Obesity in Rat Model. Indian Journal of Animal Research. 59(6): 953-962. doi: 10.18805/IJAR.BF-1915.

Submitted: 31-10-2024 **Accepted:** 08-12-2024 **Online:** 02-01-2025

to cross membrane barriers and accumulate in tissues, leading to increased reactivity.

Prodigiosin (PG) is a red dye naturally produced by various bacterial species and is characterized by a typical pyrrolyl pyrromethene skeleton. PG has several biological and pharmaceutical activities, including antioxidant, antimalarial, antibacterial, anticancer, anti-inflammatory and immunosuppressive. It was revealed that biological pigments, including those synthesized by bacteria, can be employed for green synthesis of nanoparticles (Manikprabhu and Lingappa, 2014; El-khadragy *et al*., 2023). The present study aimed to investigate the possible ameliorative effect of prodigiosin-conjugated AgNP2 alone or by combination with anticonvulsant drug (VPA) on pentylenetetrazole (PTZ)-induced epileptic seizures.

MATERIALS AND METHODS

Chemicals

Pentylenetetrazole (PTZ, Sigma Aldrish Co. PVT Ltd, USA) was injected intraperitoneal at one single dose (60 mg/kg) according to Arafa *et al*. (2013).

Sodium valproate (Depakine) was obtained from Sanofiaventis, Paris, France. VPA was injected intraperitoneal at one single dose (60 mg/kg) according to (Al-Amin *et al.,* 2015).

Prodigiosin-conjugated AgNP2: Bacterial isolation, Preparation, extraction, purification and quantification of prodigiosin in addition to the formation of PG-conjugated AgNP2 and its characterization were performed in the Microbiology Department, Faculty of Science, Helwan University according to Farag *et al*. (2017) and El-batal *et al*. (2017).

Animals

The therapeutic effect of PG-AgNP2 on toxicity produced by epilepsy 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 balanced diet ad libitum. Before starting the experiment, animals were allowed to adapt for two weeks without treatment. By the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition, all protocols and animal handling at the Department of Zoology, Faculty of Science, Helwan University were approved by the Committee on Research Ethics for Laboratory Animal Care (approval number: HU/Z/010-19).

Experimental protocol

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

Control group: Rats were intraperitoneally (i.p.) injected with 0.1 ml of 0.9% NaCl.

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).

PTZ group: The animals of this group were i.p injected with a single dose of PTZ (60 mg/kg b.wt.).

Sodium valproate (VPA): Animals were i.p. injected with VPA (600 mg/kg) for 7 days. At the 7th day, the animals received acute single dose of PTZ (60 mg/kg ip).

PG-AgNP+PTZ group: Animals were i.p. injected with PG-AgNP2 (3 mg/kg) for 7 days. At the 7th day, the animals received acute single dose of PTZ (60 mg/kg ip).

Sodium valproate+PG-AgNP+ PTZ: Animals were i.p. injected with PG-AgNP2 (3 mg/kg) and after 1 h animals were i.p. injected with VPA (600 mg/kg) for 7 days. At the 7th day, the animals received acute single dose of PTZ (60 mg/kg ip).

All the treated groups were treated for seven days. The animals were killed by sudden decapitation 24 h after the last treatment, serum was collected and 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.

Measurement of body weight

Each rat was weighed daily (9:00 a.m.) using an electronic scale. Total body weight was calculated as the mean of daily body weights (g).

Assay of monoamines, free amino acids, acetylcholinesterase and monoamine activity

Monoamines and free amino acids in the neuronal homogenate were determined using HPLC following the illustrated techniques performed by (Pagel *et al*., 2000 and Henrikson and Meredith, 1984), respectively. The Acetylcholinesterase (AChE) activity was estimated following the protocol described by Ellman´s, (1959), protocol. And monoamine oxidase activity was determined fluorometrically in accordance with the method outlined by Dar *et al*. (2005).

Estimation of insulin, leptin and glucose

For the assessment of biochemical parameters, including insulin, leptin and glucose levels, serum was used using commercial kits from Randox Laboratories, UK, according to the manufacturer's protocol.

Oxidative stress marker

Malondialdehyde (MDA) was mixed with thiobarbituric acid (TBA) according to the method of Ohkawa *et al.* (1979). The Biodiagnostic Nitrite Test Kit was used to measure neuronal nitric oxide (NO) levels according to Berkels *et al*. (2004). Using the method of Lodovici *et al*. (1997), brain DNA was isolated and hydrolyzed to determine 8-hydroxy-2-deoxyguanosine (8-OHdG). The protocol described by

Ellman and Lysko (1979), was used to determine reduced glutathione (GSH).

Estimation of antioxidants

Superoxide dismutase (SOD) and catalase (CAT) were assayed based on the protocols described by Sun *et al*. (1988) and Aebi (1984), respectively.

Inflammatory markers in brain tissues

Nuclear factor kappa-B (NF-kB), TNF-α (tumor necrosis factor-α) and interleukin-1β (IL-1β) concentrations were measured using commercial ELISA kits (R and D System, Minneapolis, MN, USA) in accordance with the manufacturer's instructions.

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 (Life Span Bio-Sciences, Inc., Seattle, WA, USA). The process was carried out by the manufacturer's instructions.

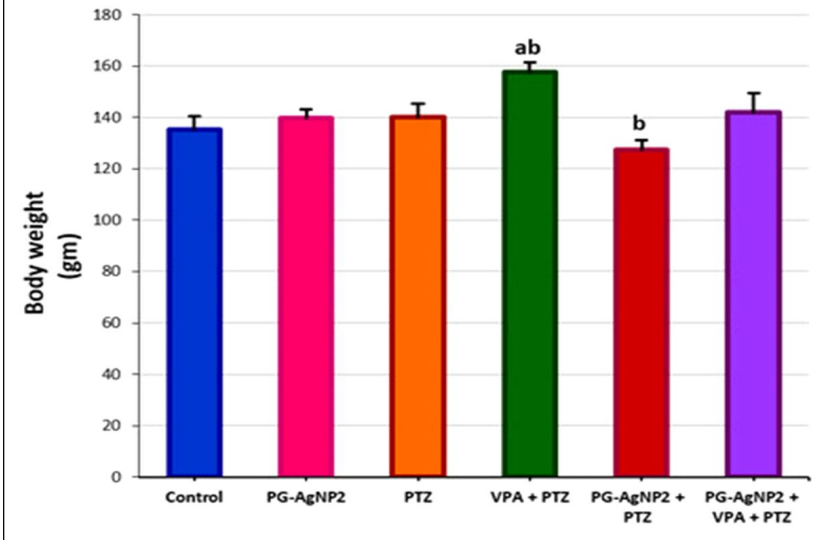
Statistical analysis

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 $p<0.05$.

RESULTS AND DISCUSSION

The use of metal nanoparticles has become a promising trend in the pharmaceutical industry as these therapeutic agents offer improved bioavailability, delivery schedule and drug delivery to target tissues compared to standard drug formulations. Several researchers have demonstrated accumulation of metals in cells after long-term treatment with high-dose metal nanoparticles. Patlolla *et al*. (2015) reported that low doses of AgNP2 for 7 days did not cause significant accumulation or toxicity in animal cells, but only enhanced targeted drug delivery to cells. The aim of this study was to investigate the possible therapeutic effect of prodigiosin conjugated silver nanoparticles (PG-AgNP2) on neurotoxicity induced by epilepsy. The goal of the current investigation was to assess the potential protective effects of PG-AgNPs (3 mg/kg) on neurotoxicity produced as a results of epilepsy induction. After treatment with anticonvulsant (VPA), the body weight of the PTZ-exposed group increased significantly ($p<0.05$) compared with the control group. PG-AgNP treatment significantly reduced the body weight of PTZ-exposed rats even when combined with VPA ($p<0.05$) (Fig 1).

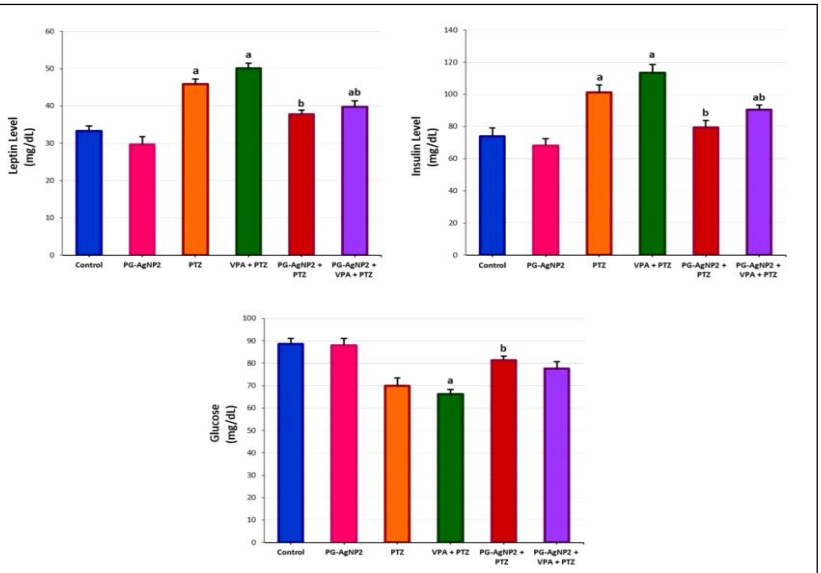
Prospective and retrospective studies have shown that PTZ is widely used as a convulsant drug in experimental studies (Morimoto *et al*., 2004). PTZ competitively interacts with the picrotoxin binding site of the GABA-A receptor, reducing the transmembrane chloride flux and causing generalized tonic-clonic seizures (Seo *et al*., 2020). In addition, PTZ leads to increased oxidative stress, which mainly affects the brain compared to other organs (Yang *et al*., 2017).



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 PTZ.

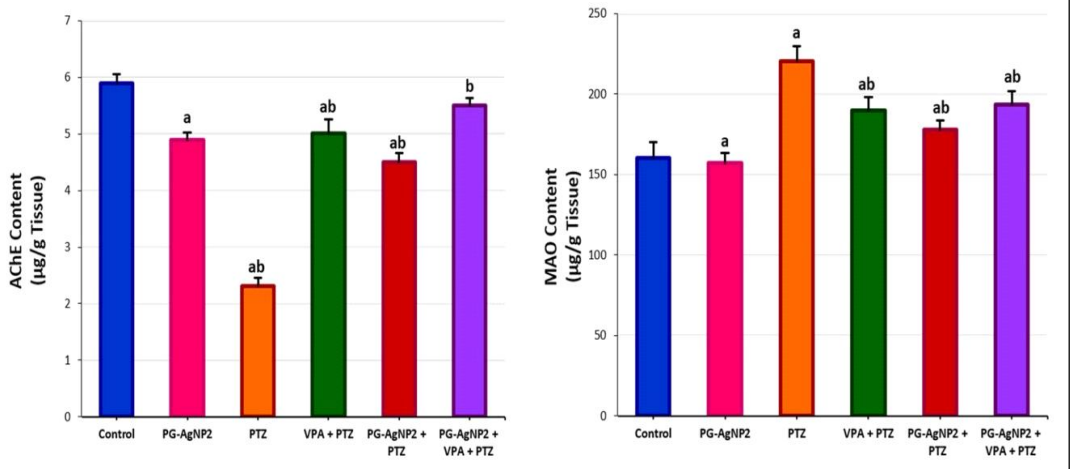
Fig 1: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the body weight of PTZ induced epileptic seizures in rat model for 7 days.

The treatment with anticonvulsant (VPA, 600 mg/kg) in PTZ treated animals resulted in significant increase in the insulin resistance and leptin in blood of the treated animals which consequently caused marked decreased in blood glucose level. The pretreatment with PG-AgNPs with or without combination with VPA for 7 days led to a significant amelioration in the metabolic studied parameters (Fig 2). One of the most frequent side effects of VPA treatment in epileptic patients is weight gain, which has been shown in several studies. Because weight gain and insulin resistance are known to be strongly associated with the occurrence of metabolic syndrome and type 2 diabetes mellitus, especially in children, the risk of acquiring these conditions is quite concerning. Changes in insulin and leptin levels brought on by VPA are also linked to weight gain. In this study, the treatment with VPA (600 mg/kg) for 7 days caused an increase in the level of insulin and leptin in addition to increase in glucose content in serum. VPA is



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 PTZ group.

Fig 2: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the metabolic rate, insulin, leptin and glucose level in serum of PTZ induced epileptic seizures in rat model 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 PTZ group.

Fig 3: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the content of monoaminoxidase (MAO) and acetylcholinesterase (AChE) in brain tissue of PTZ induced epileptic seizures in rat model for 7 days.

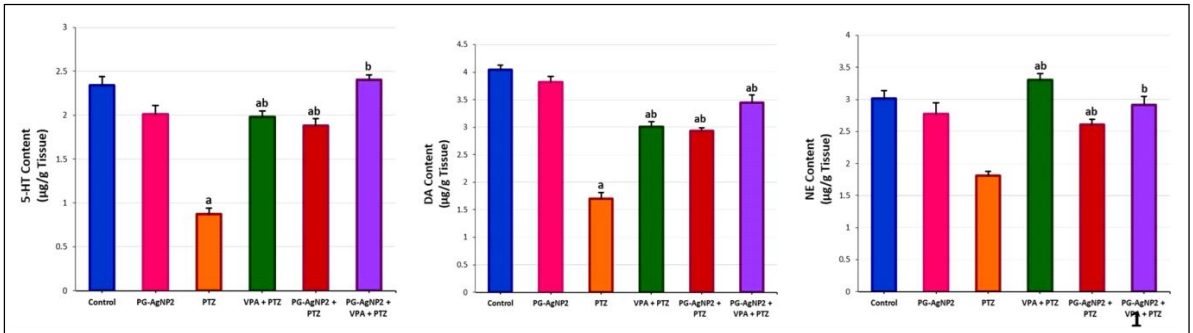
a product of fatty acids and by inhibiting many metabolic processes, including glucose uptake, glycogenesis and glucose oxidation, this rise in free long-chain fatty acids dysregulates the action of insulin and encourages the development of insulin resistance (Grill and Qvigstad, 2000; Prajapati *et al*., 2024). It is also believed that VPA's suppression of hepatic insulin metabolism raises serum insulin. It was also suggested that VPA's direct effect on the pancreatic beta cells was the cause of elevated insulin levels (Luef *et al*., 2003). In addition, Adipose tissue secretes the hormone leptin, which mainly controls body weight and energy expenditure (Lakhanpal *et al*., 2007).

They showed that obese patients' leptin levels were noticeably greater than controls' after VPA therapy. In the present study treatment with PG-AgNp2 with or without VPA treatment resulted in significant decrease in insulin and leptin levels as compared to PTZ group. Prodigiosin can indirectly affect leptin signaling, since it may modulate inflammation and oxidative stress. Indeed, its anti-inflammatory action supports better leptin signaling, especially in regulating appetite and energy metabolism in obesity or metabolic disorders (Tran *et al.,* 2021). Simliarly, prodigiosin has been suggested to play a potential role in the regulation of glucose metabolism, apparently through reductions in insulin resistance. It has extra anti-inflammatory and antioxidant properties that provide extra protection to pancreatic β-cells against oxidative injury, which is very important for proper insulin production and secretion. Some animal studies suggest prodigiosin improves insulin sensitivity; thus, it has a potential application in metabolic disorders such as type 2 diabetes, although the mechanisms involved are not yet completely investigated (Abbas and Hegazy, 2020).

As a result of epileptic induction, MAO and AchE activity may also be affected by neuroinflammation and oxidative stress (Fig 3). Oxidative damage and inflammatory mediators in the brain can alter their function, which in turn alters monoaminergic and cholinergic signaling, affecting seizure activity and the development of epilepsy. The reduction in monoamine neurotransmitters after PTZ exposure may be caused by the production of ROS, which inhibit enzymes involved in monoamine biosynthesis, disrupt monoamine metabolism by promoting the removal and degradation of monoamines and inhibit the uptake of monoamines (Maodaa *et al*., 2016). In addition, Alnahdi and Sharaf (2019) recently found that ROS activate monoamine oxidase (MAO), leading to an increase in brain hydroxyl radicals and a decrease in 5-HT, NE and DA content in the brain (Vitrac and 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 induction of epilepsy (Fig 4).

Most theories regarding these amino acid neurotransmitters in epilepsy suggest that the GABAergic system is inhibited, leading to an increase in the glutamate system. Overactivity of glutamate may lead to excitotoxicity, which can cause abnormal neuronal development (Bittigau and Ikonomidou, 1997). When this system is over functional, neuronal growth and connectivity may be impaired during critical periods of development. Excessive glutamatergic stimulation has also been associated with seizures, which are common in patients with epilepsy. These results could explain our current findings of increased glutamate levels and decreased GABA levels in animal models of epilepsy. Monoamine content and free amino acids were restored in brain tissue of rats treated with PG-AgNP2 and PG-AgNP2+VPA+PTZ, indicating that PG-AgNP2 has a neuroprotective effect against disorders that occur after epilepsy induction (Fig 5).

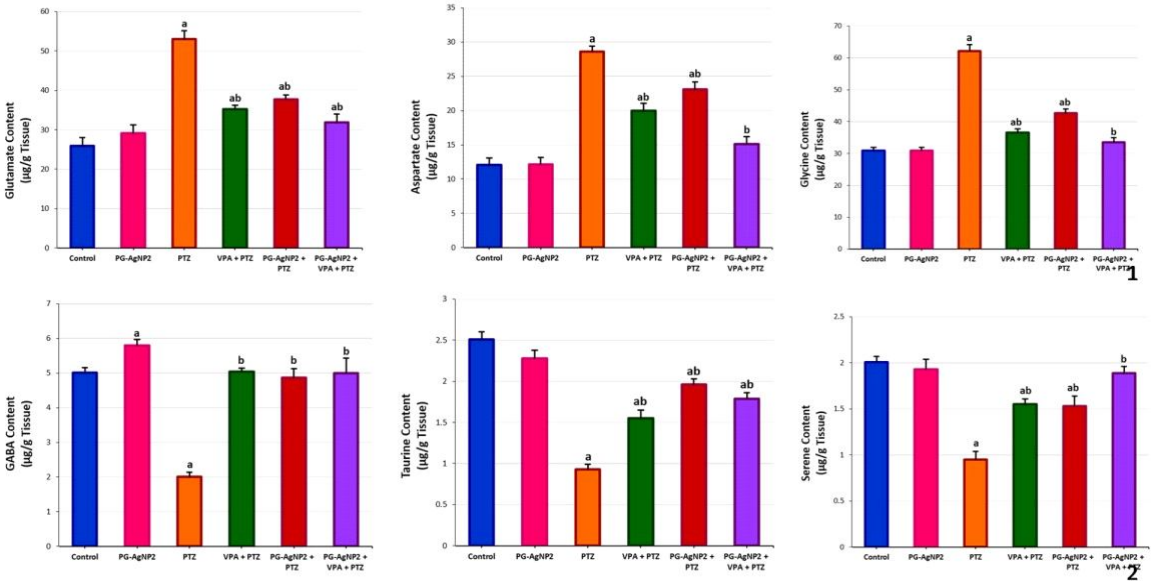
MDA, NO and 8-OHdG production was elevated, indicating that the oxidative state of the brain tissue in epileptic rats was altered. These changes were followed by a significant decrease ($p<0.05$) in the levels of endogenous antioxidant proteins such as SOD, CAT and GSH compared to the control group. The injection with PG-AgNPs considerably reduces the development of an oxidative stress epileptic model by increasing the levels of the examined antioxidant proteins and lowering the levels of pro-oxidants in brain tissue (Fig 6 and 7). According to



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 PTZ group.

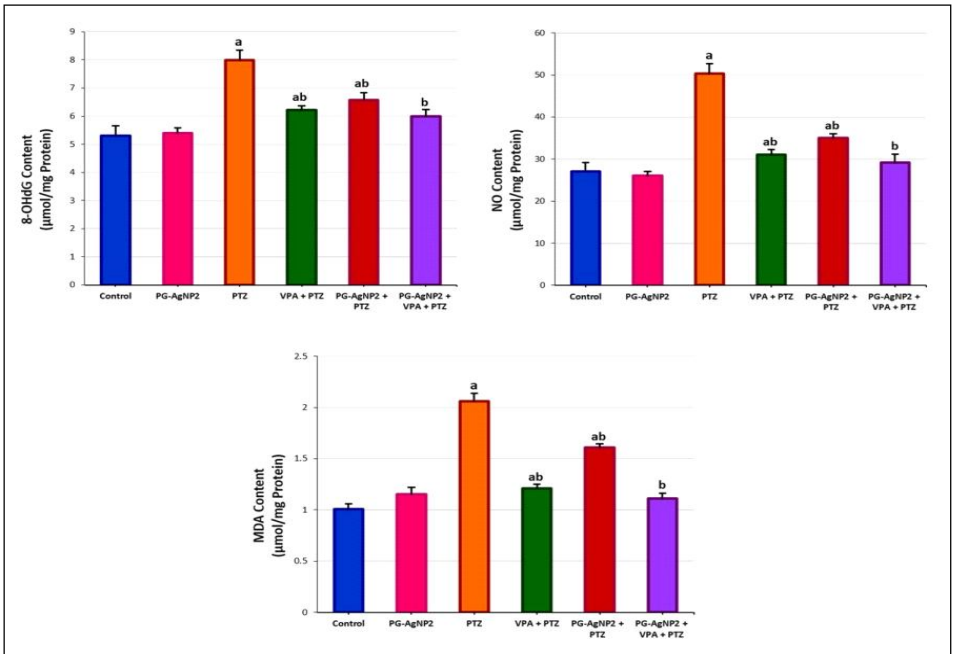
Fig 4: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the content of serotonin (5-HT), dopamine (DA) and norepinephrine (NE), in brain tissue of PTZ induced epileptic seizures in rat model for 7 days.

our findings, PG-AgNP therapy prevented epileptic-induced alterations in the redox state of brain tissue, as evidenced by the antioxidant system being strengthened and ROS generation, MDA, 8-OHdG and NO creation being inhibited. These results provide credence to PG-AgNPs' encouraging neuroprotective and antioxidant qualities. Chang *et al*. (2011)



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 PTZ group.

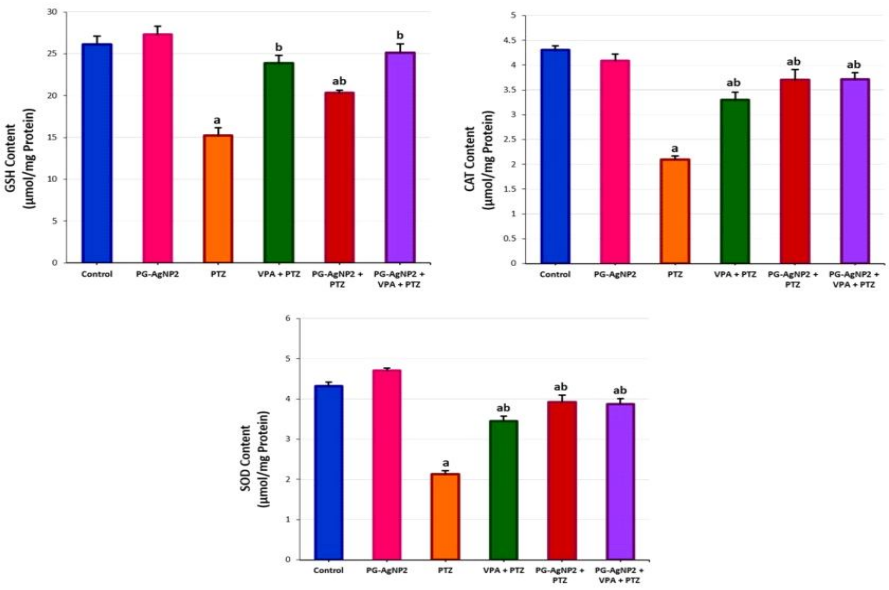
Fig 5: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the content on the content of free excitatory (Glutamate, Aspartate and Glycine) and inhibitory (GABA, Taurine and Serene) amino acids in brain tissue of PTZ induced epileptic seizures in rat model for 7 days.



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 PTZ group.

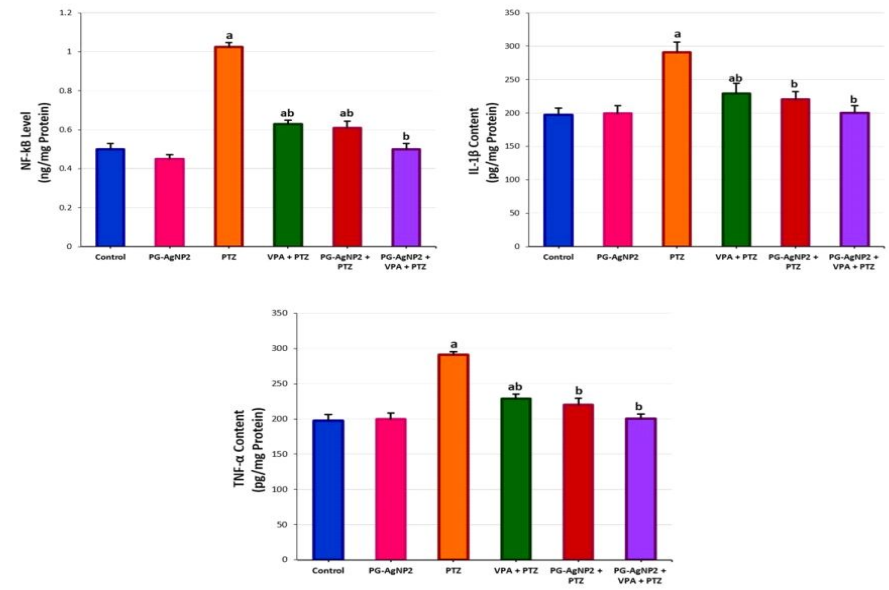
Fig 6: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the oxidative stress indicators in the brain tissue of PTZ induced epileptic seizures in rat model for 7 days.

found that PG inhibited ROS generation and NADPH oxidase2 activity, hence preventing neuronal oxidative and nitrative damages caused by hypoxia and ischemia. Furthermore, by preventing ROS generation and activating 8-OHdG, PG reduced microcystin LR-mediated oxidative stress in HepG2 cells (Chen *et al*., 2019).



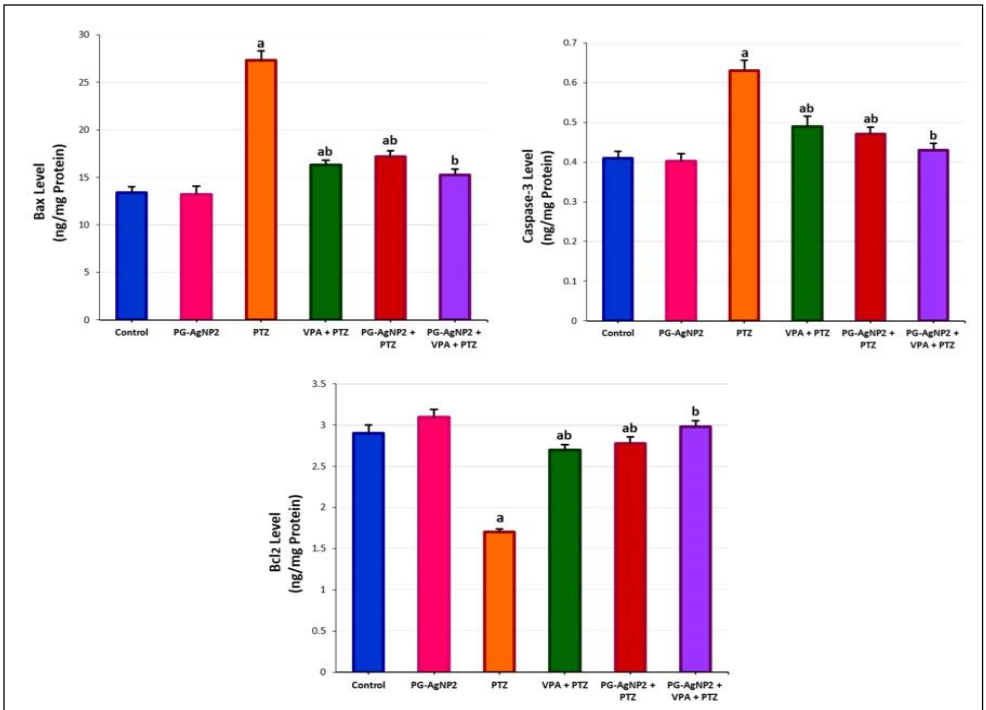
Data are expressed as means±standard error (SE) for 10 animals/ group. a: Significance change at P<0.05 in comparison with the control group. b: Significance change at P<0.05 with respect to PTZ group.

Fig 7: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the antioxidant enzymes activities in the brain tissue of PTZ induced epileptic seizures in rat model 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 PTZ group.

Fig 8: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the inflammatory biomarker's levels in the brain tissue of PTZ induced epileptic seizures in rat model 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.

Fig 9: The effect of treatment with PG-AgNP2 (3 mg/kg) with or without VPA (200 mg/kg) on the on the brain level of apoptosis markers of PTZ induced epileptic seizures in rat model for 7 days.

In this study, the induction of epilepsy led to a rise in the production of proinflammatory cytokines, specifically NF-κB, TNF-α and IL-1β, which are responsible for brain tissue damage (Fig 8). According to Yang *et al.* (2017), inflammatory cytokines stimulate neutrophil accumulation, which exacerbates tissue inflammation and damage. In the current investigation, PG-AgNP2 therapy decreased the increase of inflammatory cytokines (NF-κB, TNF-α and IL-1β) in brain tissues. The strong anti-inflammatory impact of PG-AgNP2 may be the mechanism by which it reduces the inflammation brought on by seizures and improves the cytokines under study (Lin *et al.,* 2019; Alahmari *et al.,* 2024).

By interfering with protein kinase C, mitogen-activated protein kinase and phospholipase C, as well as by inhibiting calcium-dependent ATPase or activating the inositol triphosphate pathway, epilepsy causes apoptosis in a variety of cells. The new findings show that whereas Bcl-2, which suppresses apoptosis, decreased in the brain tissue, the number of the genes that cause apoptosis (Bax and caspase-3) rises at p<0.05 (Fig 9). These findings might be explained by the way epilepsy increases Ca^{2}+ entrance into the mitochondria, which disrupts the mitochondria's regular metabolism and leads to neuronal cell death and growth arrest (Yuan *et al.,* 2013). Rats treated with PG-AgNP2 showed less apoptosis in their brain tissue. However, PG therapy decreased the loss of neuronal cells, as shown by an increase in the expression of the anti-apoptotic protein Bcl-2 and a decrease in the synthesis of pro-apoptotic proteins (caspase-3 and Bax). These results are consistent with those of Al Omairi *et al.* (2022), who discovered that PG prevented mice with depression from dying. By upregulating Bcl-2 and downregulating Bax and caspase-3, PG prevented the apoptotic cascade associated with tomach lesions caused by injections of acidified ethanol.

CONCLUSION

By inhibiting pro-oxidative insults (ROS, NO and MDA), boosting antioxidative defense systems (GSH, SOD and CAT), lowering neuronal inflammation (TNF-α and IL-1β), preventing neuronal apoptosis by lowering pro-apoptotic factors and increasing the anti-apoptotic protein and significantly modulating monoaminergic, amino-acidergic and cholinergic transmission in the brain tissue, the study's findings demonstrated that PG-AgNP2 has neuroprotective effects on epilepsy in rats.

ACKNOWLEDGEMENT

The present study was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R39), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Disclaimers

The views and conclusions expressed in this article are solely of the authors and do not necessarily represent the views of our affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures of these experiments were approved by the Committee of Experimental Animal Care and handling techniques were approved by the University of Animal Care Committee. By the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, 8th edition, all protocols and animal handling at the Department of Zoology, Faculty of Science, Helwan University were approved by the Committee on Research Ethics for Laboratory Animal Care (approval number: HU/Z/010-19).

Conflict of interest

There are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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