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Green Synthetized Selenium Nanoparticles Using *Syzygium aromaticum* (Clove) Extract Reduce Pentylenetetrazol-Induced Epilepsy and Associated Cortical Damage in Rats

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Abstract: We aimed to investigate the potential anticonvulsant effect of green synthetized selenium nanoparticles (SeNPs) using *Syzygium aromaticum* extract (SAE) (SAE-SeNPs) against epileptic seizures and cortical damage induced by pentylenetetrazole (PTZ) injection in rats and its mechanism. A total of 84 rats were divided into six groups; control, PTZ-exposed group, SAE + PTZ-treated group, sodium selenite (Na_2SeO_3) + PTZ-treated group, SAE-SeNPs + PTZ-treated group, and diazepam + PTZ-treated group. SAE-SeNPs significantly increase ($p < 0.05$) the latency time to seizures and reduce both the seizure duration and death rate, which were enhanced by the PTZ injection. SAE-SeNPs counteracted the PTZ-induced changes in the oxidants and antioxidants. Furthermore, SAE-SeNPs significantly restored ($p < 0.05$) the pro-inflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor-α) to their normal levels and suppressed the activity of the glial fibrillary acidic protein showing their inhibitory effect on the epilepsy-associated inflammation. In addition, SAE-SeNPs significantly reduced ($p < 0.05$) PTZ-induced cortical cell apoptosis, as revealed by a reduction in the pro-apoptotic Bax and caspase-3 levels, and an elevation of the anti-apoptotic Bcl-2 level. Moreover, SAE-SeNPs significantly modulate ($p < 0.05$) the PTZ-induced changes in the neurotransmitter norepinephrine level and acetylcholinesterase enzymatic activity. These data concluded the anticonvulsant activity of SAE-SeNPs via their antioxidant, anti-inflammatory, and anti-apoptotic effects, along with their ability to modulate neurotransmitters.

Keywords: *Syzygium aromaticum*; selenium nanoparticles; epilepsy; oxidant; neuroinflammation; apoptosis; neurotransmitter; cerebral cortex

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1. Introduction

Epilepsy is a worldwide chronic disease that affects the central nervous system (CNS) of all ages [1]. Epileptic seizures are the common manifestation of epilepsy, which appears as an abnormal neuronal activity in the brain [2]. Seizures and their resulting conditions can cause premature mortality and lifelong disability [3]. Both the hippocampus and cerebral cortex are the most epileptogenic areas of the brain; thus, the epileptic severity and symptoms are related to the brain areas affected by overactivity [4]. Epileptic seizures are usually associated with neurodegenerative disorders [5]. However, the exact molecular mechanism of epileptic seizures' development is not clearly underlined yet. The balance

between neuronal excitation and inhibition is considered as a crucial key in epilepsy development with an impact on the excitatory response of neurons, as reported in many in vivo studies [6]. Moreover, neurotransmitter dysregulation, neuronal cell inflammation, and oxidative stress can affect the pathophysiology of epileptic seizures [3].

The involved molecular mechanism in epilepsy development is mostly related to neural injury as a result of oxidative stress, inflammation, and apoptosis of the neural tissues [7]. One of the main molecular events in epilepsy is the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS), with the resultant reduction in antioxidant molecules in the neural tissues [8]. Programmed neural cell death (apoptosis) is mediated by ROS overproduction and can develop epileptic seizures as well [7]. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the antioxidant activity by expressing different antioxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH)-related enzymes. Therefore, the Nrf2 molecule was targeted in the development of anti-epileptic drugs [9]. In addition, neuroinflammation is evidenced by neuroglial cell activation and pro-inflammatory cytokine release, which was suggested to enhance epileptic seizures Glial fibrillary acidic protein (GFAP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-κB) were some of the reported cytokines that induce neuronal hyperexcitability and result in seizures development [10,11].

To date, there are many available antiepileptic drugs (AEDs) such as valproic acid, phenytoin, phenobarbital, and newer drugs, including topiramate, tiagabine, and oxcarbazepine. AEDs can treat epileptic seizures through different mechanisms, including sodium and calcium channel blockers, gamma-aminobutyric acid (BABA) enhancers, glutamate blockers, and neurotransmitter modulators, resulting in different efficacies and side effects [12]. Most AEDs are associated with side effects such as liver damage, teratogenicity, gastrointestinal disturbances, drowsiness, fatigue, depression, sleep disorders, and continued or recurrent seizures [13]. Since the treatment goal is having no seizures with no adverse effects, finding an efficient novel antiepileptic drug with no side effects is essential.

Incorporating nanomaterials in the biological and medical fields is a good way to obtain the highest efficiency and safety; metal-based nano therapies have been used to treat neurodegenerative diseases such as Alzheimer's disease [14]. The cellular responses to nanoparticles and their uptake are directly affected by their size, shape, coating, agglomeration, and dissolution rate [15]. Selenium (Se) possesses an antioxidant activity with a main role in the immune response and thyroid gland function [16]. Selenium nanoparticles (SeNPs) have been involved in the therapeutic field because of their bioactivity and low toxicity; they have been applied as an antioxidant, antifungal, antimicrobial, and anticancer [15,17]. In addition, SeNPs showed antioxidative, anti-inflammatory, anti-apoptosis, and neuro-modulatory effects, as concluded in previous in vivo studies of epilepsy treatment [3,18]. Biogenic SeNPs were more biocompatible and stable than the chemical-based SeNPs, which enabled their applications in the medical field [19,20]. Many researchers have already synthesized SeNPs using extracts of different plants, such as Hawthorn (berries), Allium (garlic), and Berberis vulgaris (barberry) successfully [15,21,22]. Plant-based SeNPs were reported to treat hepatic, renal, and neuronal toxicities [18,19]. *Syzygium aromaticum* (clove) is one of the widely used medicinal plants in treating many disorders; this is owing to its therapeutic roles, including antimicrobial, anti-cancer, and antioxidant effects [23,24]. *Syzygium aromaticum* extract (SAE) possesses these therapeutic properties because of the presence of eugenol, carvacrol, thymol, and cinnamaldehyde [25]. To date, the antiepileptic effect of SAE either delivered alone or with SeNPs has not been investigated yet. Furthermore, we recently confirmed the antiepileptic effect of SeNPs biosynthesized with prodigiosin, a red dye naturally produced by various bacterial species [26]. Thus, the current study aimed to find the potential antiepileptic effect of green synthetized SeNPs using SAE against the epileptic seizures and cortical damage induced by pentylenetetrazole (PTZ) injection in rats and its mechanism.

2. Materials and Methods

2.1. Preparation of Syzygium aromaticum Extracts

In September 2021, *S. aromaticum* flower buds were purchased from a local market in Hail, KSA. The plant's identity was confirmed by a taxonomist Prof. Dr. Abdel Moneim E.S. Hamad from Ha'il University. The flower buds (50 g) were macerated in 70% methanol for two days at room temperature. The extract was concentrated, lyophilized, and stored for future use in a rotary evaporator as reported previously [23].

2.2. Drugs and Nanoparticles

Pentylenetetrazole and sodium selenite were purchased from Sigma Chemical Co. (St. Louis, MO, USA). SeNPs were synthetized using SAE. Then 10 mL of 10 mM sodium selenite (Na_2SeO_3) was stirred with 10 mL SAE (5 mg/mL) for 24 h. The change in color from colorless to red indicated the successful formation of SeNPs. Then, the mixture (SAE-SeNPs) was lyophilized by a vacuum freeze dryer (Marshall Scientific, Hampton, NH, USA), and the nanoparticles powder was kept for further use in the experiment.

Zeta sizer (ZEN 3600, Malvern, UK) was utilized to measure the mean size and surface charge of the SeNPs. The molecular structure of SAE-SeNPs was analyzed using Fourier Transform Infra-Red spectroscopy (FTIR; PerkinElmer, Akron, OH, USA). Moreover, transmission electron micrographs were recorded using a high-resolution transmission electron microscope (HR-TEM; JEOL Ltd., Tokyo, Japan) equipped with an electron diffraction pattern.

2.3. Animals

Male albino Wistar rats weighing 180–200 g and 11 weeks of age were taken from VACSERA, Cairo, Egypt. Rats were housed under standard lab settings (12-h light/dark cycle; 25 ± 2 °C). Required food and water were delivered ad libitum. Before beginning the experiment, rats were acclimatized to the living conditions for 7 days. Experimental protocol was assigned by the Committee of Research Ethics for Animal Care, Helwan University (approval no. HU2021/Z/AEO0121-01).

2.4. Study Groups

Rats were grouped into six groups (n = 14 per each) and treated as follows:

Group 1, CNTR group: Rats received physiological saline orally for 14 days. On the 14th day, rats were injected intraperitoneally (i.p.) with normal saline, one hour following the oral administration of saline.

Group 2, PTZ-treated group (PTZ): Rats received normal saline orally for 2 weeks. On day 14, rats received a single i.p. injection of PTZ (60 mg/kg) one hour following the oral administration of saline, as reported previously [27].

Group 3, SAE+ PTZ-treated group (SAE + PTZ): Rats received a daily dose of SAE (250 mg/kg, orally) for 14 days, as reported previously [28]. On day 14, rats received a single i.p. injection of PTZ (60 mg/kg) one hour following the oral administration of SAE.

Group 4, Na_2SeO_3 + PTZ-treated group (Na_2SeO_3 + PTZ): Rats received a daily dose of Na_2SeO_3 (0.5 mg/kg, orally) for 14 days, as previously reported [29]. On day 14, rats received a single i.p. injection of PTZ (60 mg/kg) one hour following the oral administration of Na_2SeO_3.

Group 5, SAE-SeNPs + PTZ-treated group (SeNPs + PTZ): Rats received a daily dose of SAE-SeNPs (0.5 mg/kg, orally) for 14 days, as reported previously [30]. On day 14, rats received a single i.p. injection of PTZ (60 mg/kg), one hour following the oral administration of SAE-SeNPs.

Group 6, diazepam + PTZ-treated group (diazepam + PTZ): Rats received a daily dose of diazepam (20 mg/kg, orally) for 14 days, as reported previously [31]. On day 14, rats received a single i.p. injection of PTZ (60 mg/kg) one hour following diazepam administration.

Normal saline was used to dissolve PTZ, Na_2SeO_3, SAE-SeNPs, and diazepam for oral administration. The doses of SAE and SAE-SeNPs were selected according to the previous study of Hegazy et al. [28] and Dkhil et al. [30], respectively, and to a preliminary study using different SAE doses (150, 200, and 250 mg/kg) and SAE-SeNPs doses (0.1, 0.25, and 0.5 mg/kg). The potent anti-seizure effects were observed at a higher dose of SAE (250 mg/kg) and SAE-SeNPs (0.5 mg/kg) (data were not shown). After one day of the last treatment, eight rats were euthanized and sacrificed, and the remaining six rats were kept to calculate the death rate within 7 days post-seizures. The cerebral cortex was immediately dissected and rinsed with physiological saline and divided into small parts. To obtain a 10% (*w*/*v*) homogenate for biochemical examination, parts of cortical tissues were homogenized in 10 mM phosphate buffer (pH 7.4). In order to evaluate norepinephrine (NE) neurotransmitter levels, another parts cortical tissues were blended in high-performance liquid chromatography (HPLC)-grade methanol and centrifuged for 10 min at 12,000 rpm at 4 °C, the supernatant was exposed to HPLC. For histopathological studies, some cortical tissues were fixed in 10% formalin, and the tissue was examined for histopathological changes.

2.5. Induction of Seizures by PTZ Injection

In order to induce epileptic seizures in rats, 60 mg/kg of PTZ was intraperitoneally injected. After 40 min of PTZ injection, the animals were observed carefully, and a seizure index was recorded following the 5 phases of the modified Racine scale [32]. Additionally, latency, duration of seizures, and the percent of death after PTZ injection were recorded.

2.6. Assessment of Cortical Oxidant/Antioxidant Status

In order to estimate the oxidative stress in cerebral cortex tissues, lipid peroxidation was evaluated and expressed by the protein levels of malondialdehyde (MDA) as described previously [33] that formed in the form of thiobarbituric acid reactive substances (TBARSs). Additionally, nitric oxide (NO) levels were measured at 540 nm as described previously [34]. Moreover, the levels of reduced glutathione were assessed following Ellman's method [35] relying on the ability of glutathione to convert 5,5-dithiobis (2-nitrobenzoic acid) into yellow-colored 5-thionitrobenzoic acid.

On the other side, to estimate the antioxidant capacity in the cerebral cortex tissues, the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) were determined using the methods of Misra and Fridovich at 560 nm [36], Aebi at 240 nm [37], Paglia et al. [38], and Factor et al. [39], respectively.

2.7. Assessment of Cortical Inflammatory Biomarkers

ELISA kits from Cusabio Technology CO. (Hu, USA) were used to measure the pro-inflammatory cytokines levels in cerebral cortex tissues. The levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), NF-κB p65, a subunit of the NF-kappa-B (NF-κB) transcription complex, and interleukin-1β (IL-1β) were determined using ELISA kits with Cat. No (CSB-E11987r), (CSB-E04640r), and (CSB-E08788r), respectively, according to the manufacturer's protocols.

2.8. Assessment of Cortical Apoptosis Biomarkers

The protein levels of Bcl-2 and Bax were determined in cerebral cortex tissues using ELISA kits (Cat. No. CSB-E08854r and E4513, respectively) purchased from BioVision)Waltham, MA, USA) following the supplier's protocol. Meanwhile, caspase-3 activity was measured using a colorimetric method with commercially accessible kits from Sigma Aldrich (St. Louis, MO, USA) following the manufacturer's protocol of kit number (CASP3C-1KT).

2.9. Gene Expression Analysis of Nrf2

Total RNA was extracted from cerebral cortex tissue using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was produced from an aliquot of total RNA using reverse transcriptase. Real-time PCR reactions were conducted using Power SYBR Green PCR Master Mix, (Thermo Fisher Scientific, Waltham, MA, USA), and the analysis was performed using Applied Biosystems 7500. β-actin was used as a reference gene. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative changes in Nrf2 expression. Table 1 lists the primer sequences.

Table 1. Primer sequences of Nrf2 and β-actin genes.

Gene	Sense (5′–3′)	Antisense (5′–3′)
Nrf2	5′-GGTTGCCCACATTCCCAAAC-3′	5′-GGCTGGGAATATCCAGGGC-3′
β-actin	5′-GCAGGAGTACGATGAGTCCG-3′	5′-ACGCAGCTCAGTAACAGTCC-3′

2.10. Assessment of Acetylcholinesterase and Neurotransmitter

Acetylcholinesterase (AChE) activity in cerebral cortex tissues was determined using a colorimetric assay of Ellman et al. [40]. AChE activity was measured depending on the yellow color after addition of thionitrobenzoic acid at 412 nm. To assess NE as a neurotransmitter, the chromatograms of HPLC were obtained using ChemStation data system. Solid-phase extraction of cerebral cortex samples utilizing a CHROMABOND (Macherey-Nagel, Düren, Germany) column was performed (Cat. No. 730031). The mobile phase flow rate was 0.2 mL/min using a gradient mobile phase (methanol and water acidified with 0.1% formic acid that applied from 10% to 30% in 5 min, then from 30% to 70% in 10 min, then from 70% to 90% in 5 min, then holds the gradient for 3 min, then from 90% to 10% in 3 min). NE was isolated after 12 min and its concentrations (ng/g tissue) in the samples were determined according to Pagel et al. [41].

2.11. Histopathological Examination

Specimens of cerebral cortex tissues were fixed in 10% buffered formalin for one day. After that, the specimens were dried and paraffinized in wax at room temperature. Using the microtome, the tissues were then sectioned into 4–5 µm thick sections, which were then stained with hematoxylin and eosin (H and E) and kept for further light microscopy examination [42]. Nikon microscope (Eclipse E200-LED, Tokyo, Japan) with a magnification lens of 400× was used for examination. The cortical histopathology lesions were scored semi-quantitatively for the various treatment groups. The severity of each pathological lesion was graded based on the percentage of the affected area as follows: 0 = absence of lesion, = 5–25%, 2 = 26–50%, 3 = 51–75%, and 4 = ≥75%. Ten fields (about x200) from three rats in each treated group were randomly selected, and the most obvious pathological lesions were selected for the scoring.

2.12. Estimation of Glial Fibrillary Acidic Protein

To assess glial fibrillary acidic protein (GFAP) as a main marker for brain astrocytes, GFAP was determined in cerebral cortex tissues using ELISA kit obtained from Merck (Cat. No. NS830, Darmstadt, Germany) following the supplier's instructions.

2.13. Statistical Analysis

The SPSS software application (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA: IBM Corp.) was used to statistically evaluate all the data. The mean and standard deviation (SD) of the data were calculated. The difference between mean values of distinct groups was calculated using a one-way ANOVA with Duncan multiple comparison test. Statistical significance was defined as a P value of less than 0.05.

3. Results

3.1. SAE-SeNPs Characterization

The average diameter of SAE-SeNPs was 135.4 nm (Figure 1) and the average zeta potential was −26.9 mV. These data demonstrate the degree of stability and aggregation of SAE-SeNPs. Furthermore, the figure shows the results of FT-IR analysis of manufactured SAE-SeNPs. The O–H group is demonstrated by a broad peak at 3307.78 cm^{-1}. C–H stretch alkynes are shown by the absorption peak at 2112.01 cm^{-1}. C–O asymmetric stretch carbon compounds are accountable for the band at 1635.19 cm^{-1}. In alkyl halides, C–X stretching creates a band at 442.74, 433.96, 419.19, and 410.18 cm^{-1}. This study revealed the presence of many functional groups that may be required for SAE-SeNPs reduction and stability. Moreover, HR-TEM is performed to evaluate the character shape of the SAE-SeNPs green synthetized with SAE. HR-TEM image of SAE-SeNPs revealed spherical crystals within the diameter < 200 nm. These crystals were well-distributed with low to mild aggregation (Figure 1D).

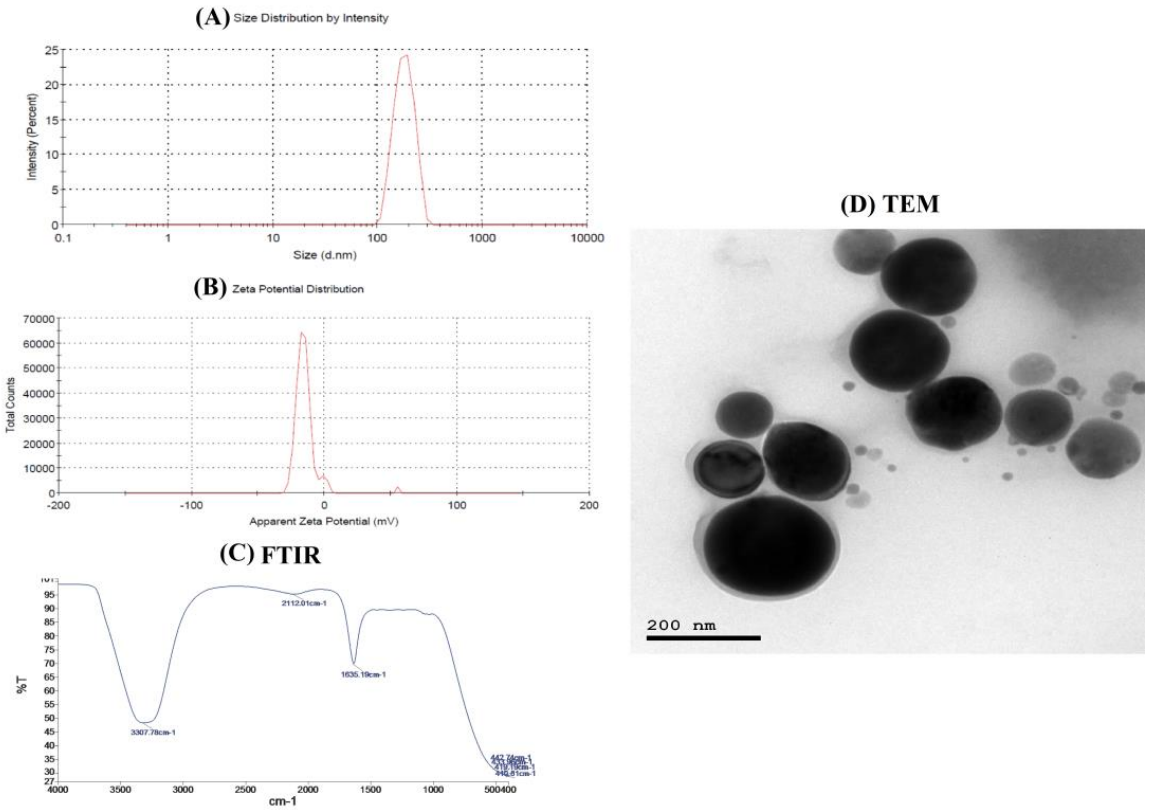

Figure 1. Characterization of *Syzygium aromaticum* extract coated selenium nanoparticles (SAE-SeNPs). (**A**): Hydrodynamic diameter of SAE-SeNPs by Zetasizer. (**B**): Surface charge of SAE-SeNPs by Zeta potential. (**C**): FT-IR spectra of SAE-SeNPs. (**D**): HR-TEM of SAE-SeNPs.

3.2. PTZ-Induced Epileptic Seizures

In this study, PTZ injection stimulated tonic, myoclonic, and general seizures, as recorded by the Racine scale. As presented in Figure 2, resembling the common antiepileptic drug (diazepam), pre-treatment with Na_2SeO_3 or SAE-SeNPs for 4 weeks considerably lowered seizure duration. The latent period until the start of seizures was notably elongated in the treated groups compared with the PTZ-exposed rats. Moreover, the death rate was considerably reduced in the treated groups in contrast to that in the PTZ-exposed group. These data suggest the anticonvulsant effects of SAE, Na_2SeO_3, and SAE-SeNPs against PTZ-induced seizures.

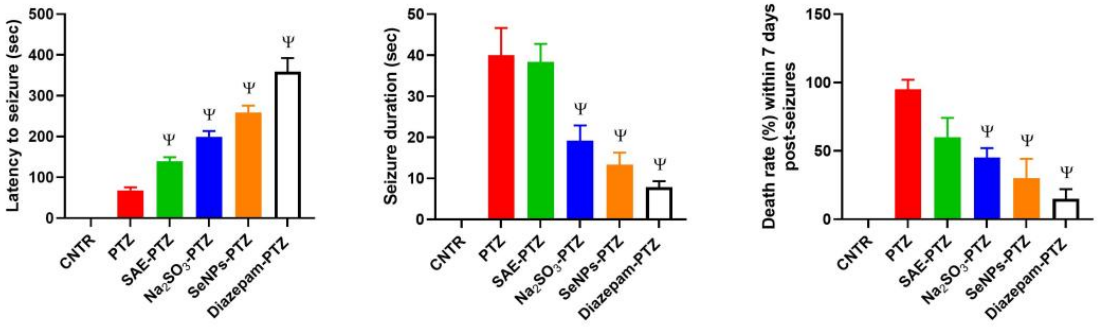

Figure 2. Effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) on the seizure duration, latency to seizures, and death rate following pentylenetetrazol (PTZ)-induced epileptic seizures (n = 8). $^{\Psi}$ indicates significant differences ($p < 0.05$) compared with the PTZ-exposed group. All data are presented as the mean ± standard deviation (SD).

3.3. Antioxidant Effect of SAE-SeNPs against the PTZ-Induced Oxidative Stress

In order to analyze the oxidant/antioxidant capacity in the cerebral cortex, the levels of both oxidant and antioxidant particles were assessed in all groups. The administration of PTZ can induce oxidative damage in cerebral cortex tissue, which is indicated by increased lipid peroxidation (expressed by MDA) and NO levels, along with the reduction in the GSH level. On the other side, the antioxidant capacity was reduced, as indicated by the decreased levels of CAT, GPx, and GR enzymes compared with control levels. Interestingly, all changes in the measured oxidant/antioxidant markers were significantly restored to induce the balance between oxidants and antioxidants molecules in SAE and SAE-SeNPs groups compared with the PTZ-exposed animals as presented in Figure 3. These data suggest the antioxidant effect of both SAE alone and SAE-SeNPs against PTZ-induced oxidative stress.

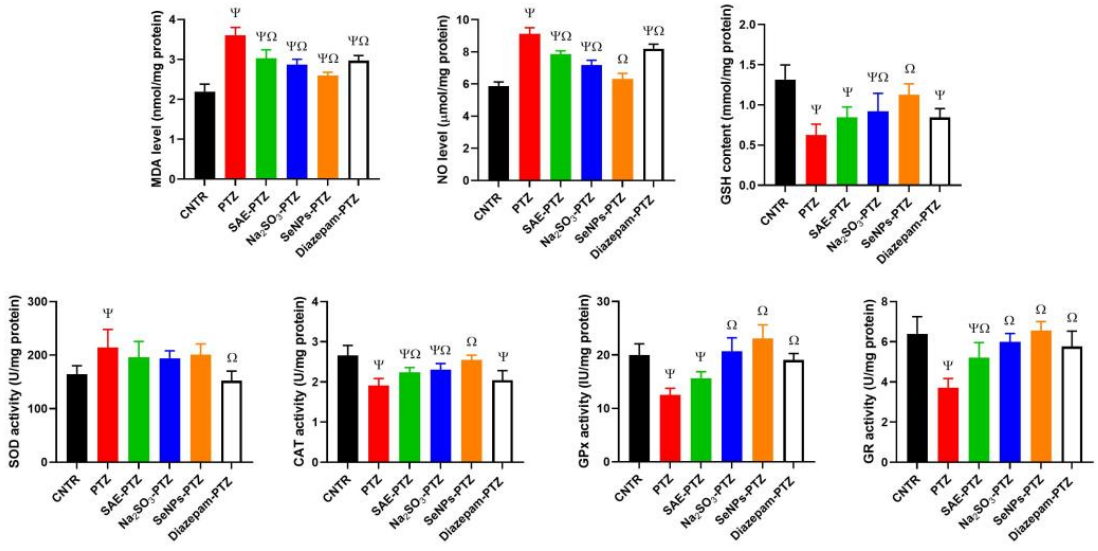

Figure 3. Antioxidant effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) indicated by the cortical levels of malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) against pentylenetetrazol (PTZ)-induced neuronal oxidative stress (n = 8). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

3.4. Anti-Inflammatory Action of SAE-SeNPs against the PTZ-Provoked Neuroinflammation

In order to investigate the anti-inflammatory activity of SAE-SeNPs against PTZ-induced neuroinflammation, the protein levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and NF-κB) were measured and presented in Figure 4. The PTZ-exposed group showed significant elevation of all measured cytokines in cortical tissue when contrasted to the control group. While the SAE-SeNPs-administrated group showed normal levels of TNF-α, IL-6, and NF-κB compared with the control and PTZ-exposed groups. These findings show that SAE-SeNPs have an anti-inflammatory effect on PTZ-induced neuroinflammation.

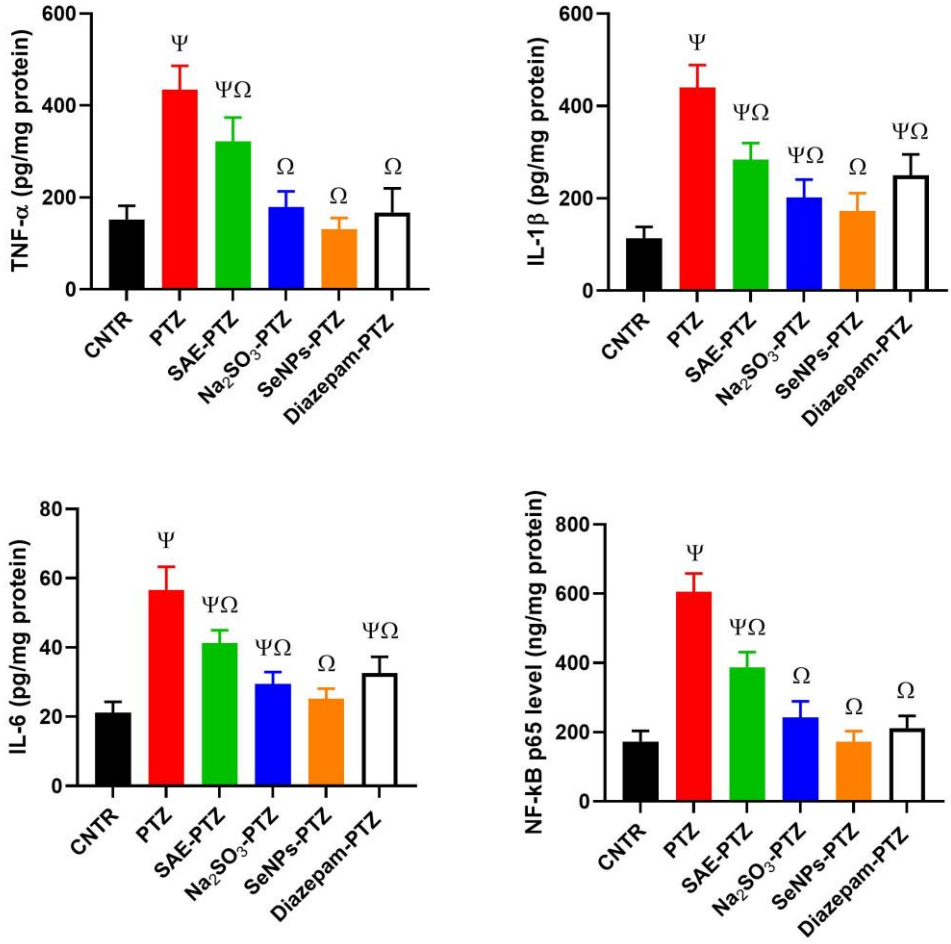

Figure 4. Anti-inflammatory effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs), indicated by the cortical levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and NF-kappa-B (NF-κB) transcription complex subunit (NF-κB p65), against pentylenetetrazol (PTZ)-induced neuro-inflammatory response (n = 8). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

3.5. Anti-Apoptotic Effect of SAE-SeNPs against PTZ-Stimulated Apoptosis and Neuronal Loss

Neuronal apoptosis was evaluated by measuring the amounts of the anti-apoptotic marker (Bcl-2) and the pro-apoptotic proteins (Bax and caspase-3) in the cortical tissues. The results showed that PTZ administration induced neuronal cell apoptosis in the cerebral cortex as recorded by the significant elevations in Bax and caspase-3 levels, along with a significant decrease in the levels of Bcl-2 compared with the control group. SAE-SeNPs-treated rats showed the best restoration of the apoptotic biomarker levels to normal levels compared with the control group as shown in Figure 5. These data suggest the anti-

apoptotic effect of SAE-SeNPs on PTZ-induced neuronal cell apoptosis in the cortical tissue of rats.

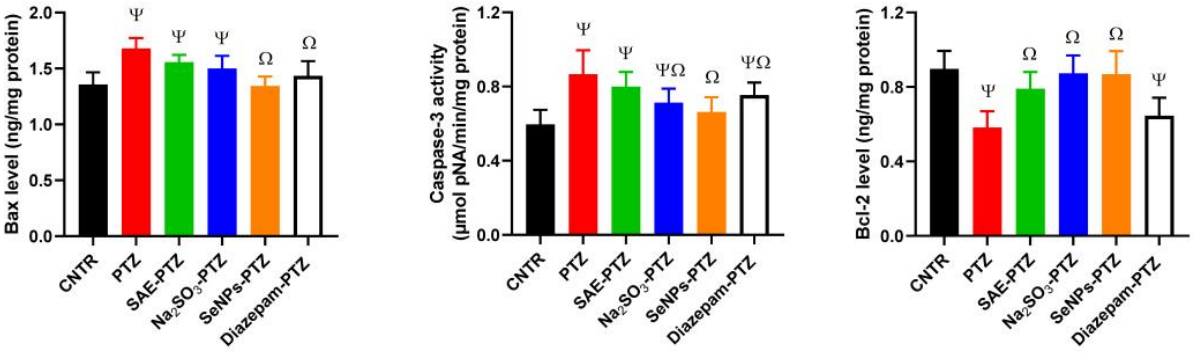

Figure 5. Anti-apoptotic effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs), indicated by the cortical levels of pro-apoptotic Bax and caspase-3 activity, and anti-apoptotic Bcl-2, against pentylenetetrazol (PTZ)-induced neuronal cells apoptosis (n = 8). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

The transcription factor Nrf2 controls the antioxidant cellular response and the consequent anti-inflammatory and anti-apoptotic responses. Thus, to know whether Nrf2 had a part in the mechanism of the SAE-SeNPs' effects on PTZ-induced neuronal damage or not, Nrf2 mRNA expressions in the cortical tissues of all groups were analyzed. As shown in Figure 6, the PTZ-exposed group showed a noticeable downregulation in the Nrf2 mRNA expression compared with the control group. All treated groups can restore the Nrf2 expression to the normal level with the highest expression in the SAE-SeNPs-treated group. These data suggest that Nrf2 had a clear role in the ameliorative effects of SAE-SeNPs on PTZ-induced neural damage.

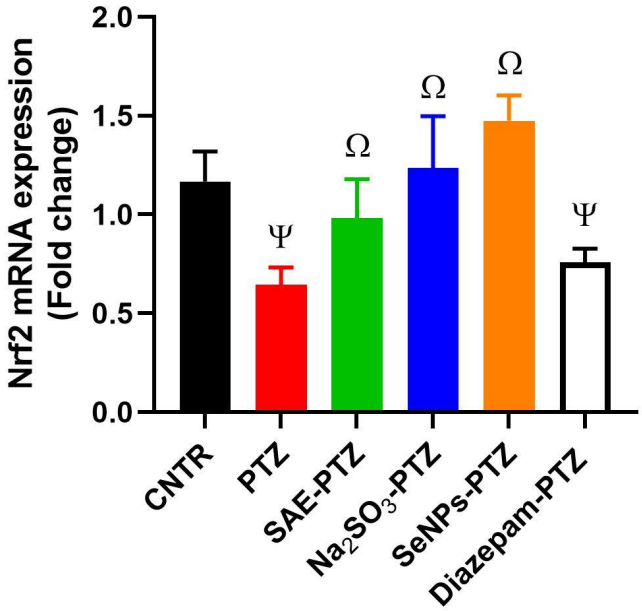

Figure 6. Effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) on the nuclear factor erythroid 2-related factor 2 (Nrf2) mRNA expression against pentylenetetrazol (PTZ)-induced Nrf2 downregulation (n = 8). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

3.6. The Effect of SAE-SeNPs Treatment on the Neurochemical Levels in Cortical Tissue

In the PTZ-exposed group, there was a significant reduction in the hippocampal concentrations of neurotransmitter (NE) accompanied by a significant elevation in the AChE activity as contrasted to the control group. In the SAE-SeNPs-treated group, NE levels were notably elevated and the AChE activities were considerably reduced compared with the PTZ-exposed group, as presented in Figure 7. These data suggest the ability of SAE-SeNPs to modulate the levels of neurotransmitters in the cerebral cortex during epileptogenic molecular mechanisms.

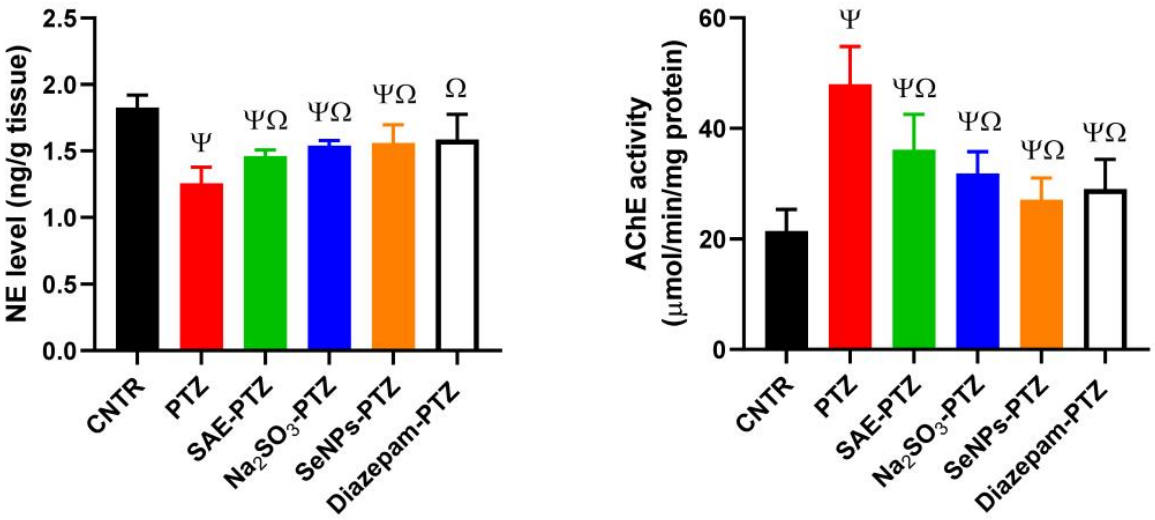

Figure 7. Effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) on the neurotransmitter norepinephrine (NE), and acetylcholinesterase (AChE) activity in cerebral cortex tissue against pentylenetetrazol (PTZ)-induced neurotransmission modulation ($n = 8$). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

3.7. The Protective Role of SAE-SeNPs on the PTZ-Induced Histopathological Changes during the Epileptic Seizures

The control rats displayed intact healthy cortical architecture, while the PTZ-exposed animals showed degenerated and necrotic neurons, with pyknosis in the cortical region. On the other hand, the rats treated with SAE, SAE-SeNPs, and diazepam showed almost normal histological architecture as shown in Figure 8. Furthermore, a significant decrease in semi-quantitative histopathological analysis score was observed in SAE-SeNPs and diazepam-treated groups compared with the PTZ-treated group (Supplementary data; Figure S1).

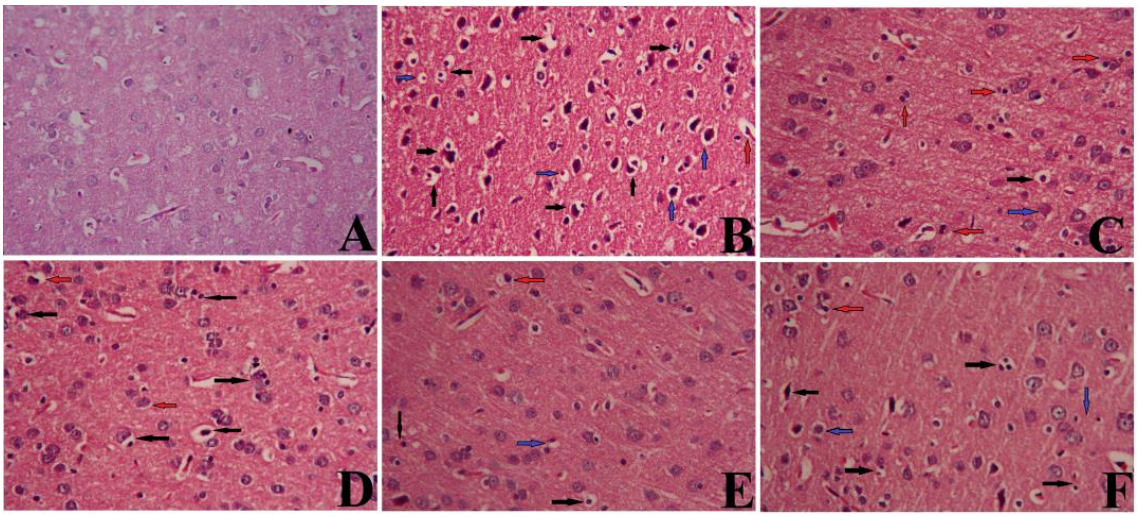

Figure 8. Effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) on histopathology of cortical tissue following pentylenetetrazole (PTZ)-induced epileptic seizures (n = 8). (**A**): control group; (**B**): PTZ-treated group; (**C**): SAE + PTZ-treated group; (**D**): Na_2SeO_3 + PTZ-treated group; (**E**): SAE-SeNPs + PTZ-treated group; and (**F**): diazepam + PTZ-treated group. Scale bar = 80 µm. Black arrows indicate apoptotic neurons, blue arrows indicate vacuolated neurons, and red arrows indicate inflammatory cells infiltration.

3.8. The Protective Role of SAE-SeNPs on the PTZ-Induced GFAP Expression in the Epileptic Seizures

Moreover, The PTZ-exposed group revealed a significant increase in GFAP levels in cerebral cortex tissue, indicating astrocyte activation and corroborating the discovery of elevated inflammatory markers in this group. However, the SAE-SeNPs-treated group showed a remarkable decline in GFAP levels compared with the PTZ-exposed animals as presented in Figure 9.

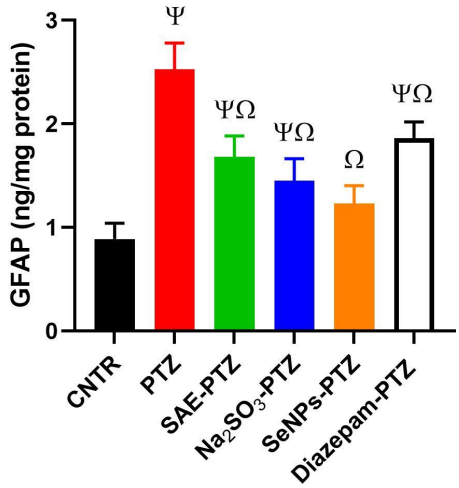

Figure 9. Effects of oral administration of *Syzygium aromaticum* extract (SAE), sodium selenite (Na_2SeO_3), and green synthetized SeNPs using SAE (SAE-SeNPs) on protein expression of glial fibrillary acidic protein (GFAP) in cerebral cortex tissue responding to pentylenetetrazole (PTZ)-induced epileptic seizures (n = 8). $^{\Psi}$ and $^{\Omega}$ indicate significant differences ($p < 0.05$) compared with the control and PTZ-exposed groups, respectively. All values are presented as the mean ± standard deviation (SD).

4. Discussion

Selenium nanoparticles have been recommended to be used in many therapeutic protocols of different diseases as a co-therapy because of their biocompatibility and improved cellular uptake [43]. In this study, we hypothesized that green synthetized SeNPs using SAE might protect against PTZ-stimulated epileptic seizures in rats. This hypothesis was based on the reported therapeutic properties of both SeNPs and SAE separately [3,44]. Therefore, the synthesis of SeNPs using SAE potentiate their protective efficiency, and this was investigated in the current study on cortical damage and epileptic seizures in rats.

The data obtained in this study confirmed that a single injection of PTZ was able to induce a convulsive effect, as indicated by the recorded seizures and cortical oxidative stress (elevated MDA and NO levels). Moreover, the antioxidant molecules (CAT, GPx, and GR) were exhausted and reduced following PTZ injection. The neuronal antioxidant response is controlled by the transcriptional factor Nrf2 gene expression, which was downregulated as well following the PTZ injection. The pathogenesis of PTZ-induced epileptic seizures is related to the ROS production and the depletion of enzymatic and non-enzymatic antioxidants during epileptogenesis [8]. Moreover, the high level of NO can interact with superoxide anions producing peroxynitrite radicals that lead to deleterious neurological effects [45,46]. The oxidative stress and the associated neuronal hyperexcitability result in neuronal cell death (apoptosis) at the end [47,48], which is indicated in our findings by the elevated pro-apoptotic molecules (Bax and caspase-3) and reduced anti-apoptotic molecule (Bcl-2) following PTZ injection. The PTZ-induced signal transduction and molecular changes are confirmed by our findings of apoptotic markers.

SeNPs with their nanosize and large surface area were reported for their potent antioxidant effects by enhancing free radicals scavenging, and inhibiting ROS generation [49]. SeNPs can reduce PTZ-induced neuronal oxidative damage by inhibiting ROS generation and upregulating Nrf2 and heme oxygenase-1 enzyme (HO-1) in the cerebral cortex tissue [3]. Moreover, neurobehavioral disorders and neuronal lipid peroxidation in the brain of diabetic rats were alleviated by a combination of SeNPs and metformin [50]. Our findings revealed the antioxidant effect of SAE-SeNPs against the PTZ-induced oxidative stress in the cortical tissues of rats that might explain the reduction in seizure duration after SAE-SeNPs supplementation. The ability of SAE-SeNPs to reduce the MDA, NO levels, and to increase the activities of enzymatic and non-enzymatic antioxidants (GSH, SOD, CAT, GPx, and GR) can be related to the flavonoid and phenolic content of SAE [51]. Shekhar et al. [44] reported that *S. aromaticum* ethanolic extracts have potent antioxidant properties and can alleviate oxidative stress in brain tissues in amyloid beta-induced Alzheimer's disease-like pathology in rats. Moreover, SeNPs itself might be responsible for the resultant antioxidant capacity via inhibiting ROS accumulation, cytotoxicity, and protecting the antioxidant enzymes activities [52]. Therefore, the current oxidant/antioxidant profile of SAE-SeNPs showed potential and significant antioxidant activity in the epileptic cortical tissues.

The release of pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β is the result of NF-κB signal transduction that is activated by ROS generation [53,54]. IL-1β can upregulate the cyclooxygenase-2 (Cox-2) enzyme's gene expression that converts arachidonic acid to prostaglandins. Prostaglandin is a precursor of prostacyclin that is expressed in the inflammatory response and stimulates astrocytes to produce glutamate, resulting in seizure-associated neuro-excitability [55]. The obtained findings in this study revealed the neuro-inflammatory response to the PTZ injection, which is indicated by the markedly elevated protein levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and NF-κB) in the cerebral cortex tissue. Moreover, GFAP was significantly increased following PTZ injection, indicating the astrocytes activation that leads to inflammatory and apoptotic responses in the brain [56]. It was obvious in this study that SAE-SeNPs reduced the levels of the measured pro-inflammatory cytokines and GFAP expression, resulting in its anti-inflammatory activity against PTZ-induced neuroinflammation. This protective effect is mostly attributed to the flavonoids, phenolics, and GSH components

of the SAE, which may prevent the free radical's accumulation and reduce the oxidative stress and its consequent inflammation and apoptosis [57,58]. Furthermore, SeNPs were reported to reduce the gamma aminobutyric acid levels in the brain of rats, resulting in a reduction in many inflammatory cytokines and oxidant compounds, and can decrease the congested blood vessels in the cerebral cortex as well [59]. Since oxidative stress ended by cellular apoptosis and epileptic seizures enhance cerebral ischemia ended by neuronal death [26], the apoptotic biomarkers were evaluated in this study. Following the PTZ injection, there were elevations in pro-apoptotic proteins (Bax and caspase-3) concurrent with a decline in anti-apoptotic (Bcl-2) protein levels; however, these abnormalities were restored to normal levels after treatment with SAE-SeNPs. As noticed in this study, SAE-SeNPs had an antioxidant activity that was able to reduce the oxidative stress and the consequent mitochondrial stress via reducing Bax level and elevating Bcl-2 levels, thereby exerting its anti-apoptotic effect. The resulted anti-apoptotic effect might be related to the anti-inflammatory and anti-apoptotic properties of SAE [58]. Additionally, biosynthesized nano-selenium inhibited cadmium-induced neuronal loss via decreasing Bax and increasing Bcl-2 levels [60]. Moreover, SeNPs showed an anticonvulsant effect via their anti-apoptotic effect in the cerebral cortex tissues of rats [3].

It is well-known that NE as a neurotransmitter is playing a crucial role in preventing epilepsy-induced seizures and neuronal changes [61]. It was reported that a high NE concentration in the cerebral cortex tissues can prevent pilocarpine-induced seizures [62]. Thus, it was reasonable to measure the NE cortical levels in this study to know whether it affects the anti-epileptic effect of SAE-SeNPs or not. The obtained results showed a substantial decrease in NE cortical levels following the PTZ injection. The PTZ-induced NE reduction was treated with SAE-SeNPs, showing its significant anti-epileptic effect. These data demonstrate the resultant seizures because the decrease in neurotransmitters triggers seizures and develops many neuropathological disorders as reported previously [63,64].

Acetylcholine is an important neurotransmitter present in postsynaptic neuromuscular junctions. Meanwhile, AChE hydrolyzes acetylcholine. The disturbance in the AChE activity results in cholinergic dysfunction, which is closely related to the epileptic mechanism because of the imbalance between neuronal excitation and inhibition [65]. In this study, AChE activity increased significantly after PTZ injection, meanwhile SAE-SeNPs treatment significantly reduced AChE activity, demonstrating its antiseizure effect. Elevated AChE activity in epileptic rats was reported because of oxidative stress in the cortical tissues [3]. In the current study, SAE-SeNPs revealed an anti-epileptic effect via modulating the neurotransmitters and reducing the oxidative stress. Although the anti-epileptic activity of SAE has not been reported yet, the neuroprotective effect of SeNPs has been studied, revealing their potential protective effect against many neurological diseases through their antioxidant and anti-inflammatory activities [3,17,66].

In this study, histopathological examinations of PTZ-exposed rats and treated rats were in accordance with the biochemical changes, revealing the ability of SAE-SeNPs to ameliorate the PTZ-induced degenerated neurons in the cerebral cortex and changes in the cortical architecture. The SAE-SeNPs-treated cortical tissues are mostly related to their antioxidant, anti-inflammatory, and anti-apoptotic properties, which end in a significant and potential neuroprotective effect.

5. Conclusions

This study concluded that green synthetized selenium nanoparticles using *Syzygium aromaticum* extract exhibit potential neuroprotective and anticonvulsant activities against PTZ-induced epileptic seizures in an epileptic model of rats. This protective effect is related to their antioxidant, anti-inflammatory, and anti-apoptotic activities, along with the neurotransmitter's modulation. SAE-SeNPs are suggested as a plant-derived anticonvulsant agent to be used in epilepsy treatment protocols after further investigations. However, additional studies should be conducted in the future to further confirm the antiepileptic effect of SAE-SeNPs using pure isolated compounds from the clove.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13021050/s1, Figure S1.

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