Research Article

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Rutin-loaded selenium nanoparticles modulated the redox status, inflammatory, and apoptotic pathways associated with pentylenetetrazole-induced epilepsy in mice

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Abstract: Worldwide, epilepsy is the second most prevalent neurological disorder. Disappointingly, various adverse effects are being observed with currently used antiepileptic drugs. Nanomedicine represents an effective strategy to overcome these limitations with a better central drug delivery. Hence, our work aimed to unravel the antiepileptic efficacy of rutin (Rut) loaded with selenium nanoparticles (SeNPs) against pentylenetetrazole (PTZ)-challenged mice. Ten days before PTZ (60 mg·kg^{-1}) intraperitoneal injection, mice were orally administered Rut (100 mg·kg^{-1}), sodium selenite (0.5 mg·kg^{-1}), SeNPs (100 mg·kg^{-1}), or sodium valproate (reference drug, 200 mg·kg^{-1}). Remarkably, administration of Rut-loaded SeNPs (Rut-SeNPs) to epileptic mice markedly increased the latency time and decreased the severity and duration of seizures. Remarkable increases were also noticed in acetylcholinesterase, brain-derived neurotrophic factor, dopamine, and norepinephrine levels in epileptic mice treated with Rut-SeNPs. Furthermore, Rut-SeNPs boosted the cellular antioxidant defense by increasing superoxide dismutase, catalase, GSH, Nrf2, and HO-1, along with decreased malondialdehyde and nitric oxide levels. In addition, the nanotherapy successfully mitigated the inflammatory mediators (tumor necrosis factor-α, interleukin-6, cyclooxygenase-2, and nuclear factor kappa B) in mice hippocampus. Rut-SeNPs antagonized neuronal apoptosis by decreasing Bax and caspase-3 and increasing the levels of Bcl-2. Conclusively, the present work suggests Rut-loaded SeNPs as an effective antiepileptic therapy through correction of disturbed neurotransmitters, oxidative status, neuroinflammation, and apoptosis.

Keywords: rutin, selenium nanoparticles, epilepsy, neuroinflammation, Nrf2/NF-κB pathways

1 Introduction

Epilepsy, an intricate neurological disorder, is characterized by abnormal electrical excitability of the cerebral neurons and manifested by the generation of spontaneous and recurrent seizures [1,2]. It is ranked as the fourth most prevalent brain disease that may affect 2.4 million patients per year, most of which belong to developing countries [3,4]. The main risk factors for this disease are head injury, tumors, prolonged fever, or infection [3,5]. Epilepsy is associated with major physical, psychological, and social sequences. Epileptic seizures may result in head trauma, fractures, soft tissue injury, and burns [3]. Former studies unveiled that patients with chronic epilepsy had cognitive deficits, depression, anxiety, and memory impairment [6]. The hippocampus is the most widely studied brain region in both human and experimental epilepsy. suggesting that epileptogenesis originates in the hippocampus [7,8]. Many scholars have documented that oxidative stress, neuroinflammation, and neurotransmission dysfunction are the basic pathological features in epileptogenesis [1,9].

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Undoubtedly, the brain is highly vulnerable to oxidative strain because of its lipid composition, low antioxidant enzymes, and high metabolic demands [10,11]. Oxidative stress has a direct effect on neuronal glutamate receptors and GABAergic signaling that result in brain excitotoxicity [12]. In epilepsy research, it has been demonstrated that nuclear factor-erythroid 2-related factor-2 (Nrf2) is strongly instigated by seizures, resulting in expressing of hippocampal antioxidant enzymes [2,13]. What is worse, these reactive species exaggerate devastating neuroinflammatory response in the epileptic brain, and activation of different downstream signaling pathways (cyclooxygenase-2 [COX II], nuclear factor kappa B [NF-κB], and tumor necrosis factor-α [TNF-α]) which in turn upsurge the likelihood of recurrent seizures [14]. Ultimately, the activation of these pathways causes activation of the apoptotic cascade with consequent neuronal cell death [13].

The first therapeutic management of epilepsy relies on using of antiseizure drugs as carbamazepine, valproic acid, rivotril, and so on. Despite the availability of antiepileptic drugs, nearly one-third of epileptic cases are not completely curable [15]. Besides, the use of antiseizure drugs is associated with adverse effects; sodium valproate therapy may cause gastrointestinal and central nervous systems disturbances as well as acute pancreatitis and neural tube defect [16]. The blood–brain barrier (BBB) represents a major obstacle for drug delivery to brain. The dysfunction of BBB was reported to be involved in the development of seizures and drug resistance mainly through limiting drugs' bioavailability [17]. Thus, nanoparticles offer more precise drug delivery for a wide range of therapeutic agents to the brain [1,3,16].

Rutin (Rut; quercetin-3-rutinoside), a flavonol, is vastly found in fruits, such as grapefruit, oranges, lemons, and cape gooseberries [18]. Also, it is abundant in other plants such as apples, berries, limes, and herbs. It has various biological properties and elicited notable antagonistic effects against oxidative stress, inflammation, tumorigenesis, hypertension, diabetes, ulcer formation, and allergy [19,20]. Former studies have verified the significant neuroprotective activities of Rut against various neurodegenerative diseases, such as Parkinson's disease [21], Alzheimer's disease [22], and Huntington's disease [18]. Several reports have also documented the neuroprotective effect of Rut on brain damage in different animal models [19,20,23,24]. Unfortunately, the water solubility of Rut is poor; therefore, its potential as a therapeutic agent is limited [25].

Selenium (Se), a fundamental microelement, is essential for proper brain function and possesses distinguished biological activities, including antioxidant and anti-inflammatory properties [26,27]. Recently, several reports have focused on the potency of Se nanoparticles (SeNPs) against various neurological disorders [1,2,28]. SeNPs exhibited better bioavailability and higher antioxidant capacity compared to Se compounds. Abouzaid et al. [29] found that supplementation with resveratrol-loaded SeNPs resulted in the mitigation of neural oxidative stress and inflammation in experimental rats with Alzheimer's disease. Furthermore, the combined transplantation of mesenchymal stem cells with SeNPs evoked better anti-Alzheimer effect than the sole treatment by each agent alone [26]. Also, glycine-coated SeNPs achieved a successful improvement of brain oxidative stress and neurobehavioral performance in parkinsonian rat models [30].

However, the therapeutic effect of Rut against pentylenetetrazole (PTZ)-induced epileptic seizures was reported in rats by Nassiri-Asl et al. [31], but the effect of its nanoformulation has not been studied. Hence, our study aimed to investigate the possible antiepileptic efficacy of Rut-loaded SeNPs against PTZ-induced epilepsy in mice. Sodium valproate was used as standard antiepileptic therapy. To achieve this, the possible modulating action of the tested non-formula on animal behavior, oxidant, inflammatory, and apoptotic stresses was investigated in mice hippocampus.

2 Materials and methods

2.1 Chemicals

PTZ and sodium selenite (Na_2SeO_3) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). All other applied chemicals used for the experiments were of analytical grade.

2.2 Plant material

Cape gooseberries (*Physalis peruviana*) were obtained from different Egyptian local markets in February 2021. Calyces of the fruits were collected, air-dried, and kept in plastic bag.

2.3 Extraction and isolation of Rut

Dried calyces of cape gooseberries (75.0 g) were grounded to a finely coarse powder and sonicated for 15 min with 300 mL of 80% methanol (v/v). The methanolic extract was centrifuged at 3,000 rpm for 10 min to remove the

residues. The solvent was then evaporated under a vacuum, and the aqueous layer was kept overnight at 4°C. A yellow precipitate was separated from the aqueous solution, and its chemical structure was confirmed by ^{1}H NMR and compared with the authentic sample (Figure A1 in Appendix) and HPLC analysis (Figure A2).

2.4 Preparation of Rut-loaded SeNPs (Rut-SeNPs)

Na_2SeO_3 (10 mL of 10 mM) was mixed with 10 mL of Rut (5 mg·mL^{-1}) under magnetic stirring for 24 h. The obtained solution of Rut-SeNPs was lyophilized by vacuum freeze dryer (Labconco Freezone 4.5L Freeze Dry System, Marshall Scientific, Hampton, NH, USA), and the developed solid material was utilized in the current study.

2.5 Characterization of Rut-SeNPs

The Zetasizer Nano ZS particle analyzer (Zetasizer Nano ZS90, Malvern Panalytical, UK) was employed to determine the average diameter, size distribution, and surface charges of the developed Rut-SeNPs, whereas Fourier transform infrared (FT-IR) spectroscopy (PerkinElmer, USA) was applied to estimate the molecular structure of the developed nanoformulation.

2.6 Experimental animals and ethics statement

Male Swiss mice weighing 25–30 g were housed in clean cages and supplied with standard mouse diet and water *ad libitum*. They were kept in hygienic conditions of 12 h dark/12 h light period in a temperature of 23–25°C and acclimatized for 10 days before the experimentation. The Zoology and Entomology Department, Faculty of Science, Helwan University (Cairo, Egypt; approval no. HU2021/Z/AEK0120-01), approved the experimental protocol.

2.7 Experimental protocol

Mice were randomly selected into six groups of 10 animals each.

Group I – control group (CONT): Mice received normal saline (0.9% NaCl) orally. On the 10th day, they received an intraperitoneal (i.p.) injection with saline 1 h after the administration of oral saline.

Group II – PTZ group (PTZ): In this group, animals were given normal saline for 10 days. On the 10th day, these animals received a single i.p. dose of PTZ (60 mg·kg^{-1}) 1 h after administering the oral saline [1,32].

Group III – PTZ + Rut group (PTZ-Rut): Mice were given Rut (100 mg·kg^{-1}) orally and injected with a single dose of PTZ (60 mg·kg^{-1}) on the 10th day [33].

Group IV – PTZ + sodium selenite (Na_2SeO_3-PTZ): Mice were received Na_2SeO_3 orally (0.5 mg·kg^{-1} [3]). On the 10th day, these animals received a single i.p. dose of PTZ (60 mg·kg^{-1}) 1 h after the administration of Na_2SeO_3.

Group V – PTZ + SeNPs (SeNPs-PTZ): Mice were received oral Rut-SeNPs (100 mg·kg^{-1}). On the 10th day, these animals received a single i.p. dose of PTZ (60 mg·kg^{-1}) 1 h after the administration of SeNPs.

Group VI – PTZ + VPA group (VPA-PTZ): Mice were given oral VPA (200 mg·kg^{-1}) [34] and i.p. injection with PTZ (60 mg·kg^{-1}) as a single dose on the 10th day.

2.8 Assessment of epileptogenesis

The seizure behavior was noticed and carefully scored for 20 min following the injection of PTZ according to the modified Racine scale [35]: 0 = no behavioral changes, 1 = ear and facial twitching, 2 = myoclonic jerks without rearing, 3 = myoclonic jerks, and rearing, 4 = turning over onto side position, tonic–clonic seizures, and 5 = turning over onto back position, generalized tonic–clonic seizures.

All animals were daily treated according to the above-mentioned regime and euthanized 24 h after the last treatment.

2.9 Hippocampal slices preparation

The brain hippocampus was immediately isolated and washed by isotonic saline. For biochemical evaluations, hippocampal tissue was homogenized in ice-cold 10 mM phosphate buffer (pH 7.4) to produce a 10% (w/v) homogenate. The protein level was measured in the hippocampal tissue using the method described by Lowry et al. [36].

2.10 Evaluation of hippocampal monoamines

HPLC report and chromatogram were obtained using the data acquisition program (ChemStation). Hippocampal samples were homogenized in 75% HPLC grade methanol

(10% w/v), centrifuged (4,000 rpm·10 min^{-1}), and subjected to a solid-phase extraction by CHROMABOND column (Cat. No. 730031) to remove trace elements and lipids. NH_2 phase was retrieved and injected into an AQUA column (150 mm; 5 µm; C18, Phenomenex, USA). After 12 min, dopamine (DA) and norepinephrine (NE) concentrations were identified by comparing the resulted chromatogram with that of the corresponding standard (Sigma Chemical Co., St. Louis, MO, USA). According to Pagel et al. [37], the concentration of each monoamine was relatively quantified to total brain tissue (µg·g^{-1}).

2.11 Neurochemical analysis of acetylcholinesterase (AChE) activity

The hippocampal AChE activity was measured based on the yellow color developed following the addition of thionitrobenzoic acid, measured at 412 nm according to the method described by Elman et al. [38].

2.12 Determination of brain-derived neurotrophic factor (BDNF) levels

BDNF was determined using enzyme-linked immunosorbent assay (ELISA) kits sourced from Abcam (Cat. No. ab213899) according to the manufacturer's instructions.

2.13 Assessment of hippocampal antioxidant enzymes

Hippocampal glutathione peroxidase (GPx) was assessed according to the procedures mentioned by Paglia and Valentine [39]. Superoxide dismutase (SOD) activity was determined at 480 nm using the technique described by Misra and Fridovich [40]. Catalase (CAT) activity was estimated according to the method described by Aebi [41].

2.14 Assessment of oxidative stress indices in mice hippocampus

Malondialdehyde (MDA) as an indicator for lipid peroxidation was estimated by the thiobarbituric acid method following Ohkawa et al. [42]. Griess reagent was utilized for measuring the nitric oxide (NO) levels in the hippocampus at 540 nm [43]. Moreover, GSH levels were estimated using Ellman's reagent, and the yellow chromogen was estimated at 412 nm [44].

2.15 Assessment of Nrf2 and HO-1 levels in mice hippocampus

To determine Nrf2 and HO-1 levels in hippocampal tissue, Nrf2 (Cat. No. MBS752046) and HO-1 (Cat. No. MBS9425834) were quantified using ELISA kits sourced from MyBioSource according to the manufacturer's instructions.

2.16 Evaluation of hippocampal inflammatory markers

Pro-inflammatory mediators were assessed in the hippocampal tissue of epileptic mice using commercial ELISA kits of Novus Biologicals (Centennial, CO, USA) as follows: COX II (Cat. No. NB600-971), TNF-α (Cat. No. NBP1-92681), interleukin-6 (IL-6; Cat. No. NBP1-92697), and NF-κB (Cat. No. NB100-2176) according to the manufacturer's instructions.

2.17 Evaluation of apoptotic markers in mice hippocampus

ELISA kits were used for the estimation of hippocampal apoptotic proteins; Bax (BioVision, Inc.; Cat. No. E4513) and Bcl-2 (Cat. No. CSB-E08854r) following the manufacturer's instructions. Caspase-3 activity was colorimetrically assessed by commercial kits (Sigma-Aldrich, St. Louis, MO, USA).

2.18 Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Measurements were examined by one-way analysis of variance, followed by Duncan's post hoc test, using the statistical package SPSS version 23.0. *P*-values <0.05 were considered statistically significant.

3 Results

3.1 Physical and chemical characterization of Rut-SeNPs

As illustrated in Figure 1, the Rut-SeNP nanoformulation is characterized by a mean zeta potential of −23.3 mV, which indicates the moderate stability of SeNPs biosynthesized with Rut. The −OH group is demonstrated

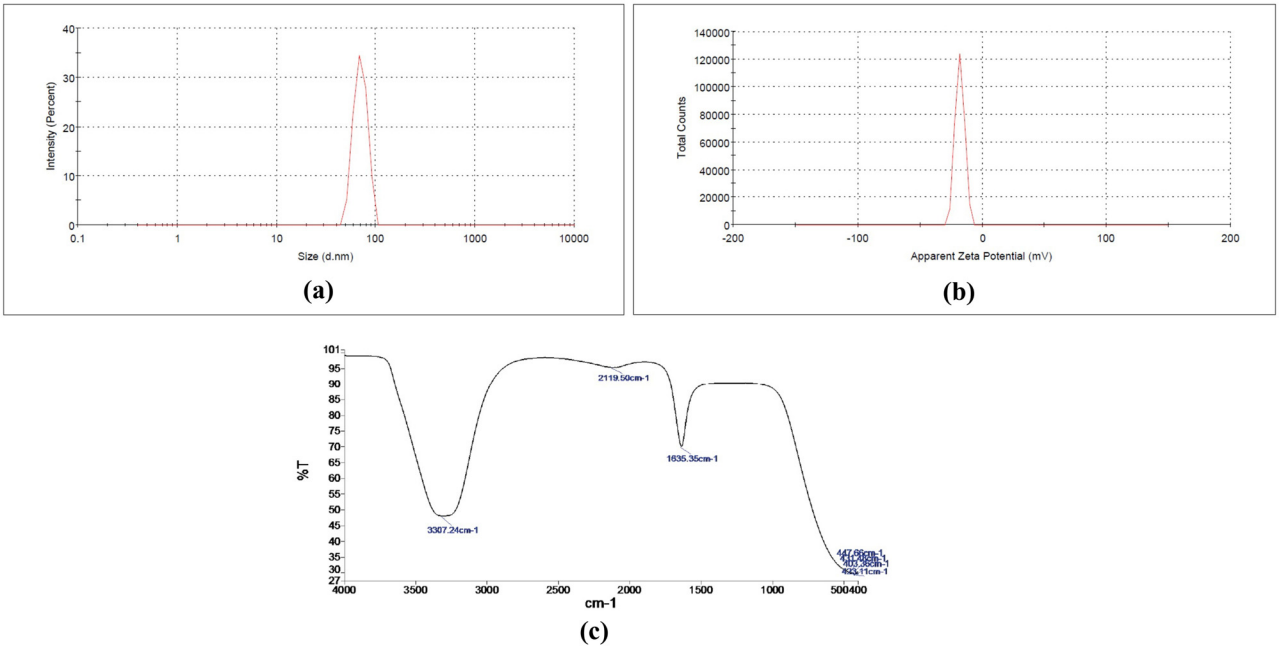

Figure 1: The characterization of Rut-loaded SeNPs: (a) size distribution by intesnsity, (b) zeta potential distribution, and (c) FT-IR.

by a broad peak at 3,307.24 cm^{-1}. C–H stretch alkynes are shown by the absorption peak at 2,119.50 cm^{-1}. The C–O asymmetric stretch carbon compounds are accountable for the band at 1,635.35 cm^{-1}. In alkyl halides, C–X stretching creates bands at 447.66, 431.48, 423.11, and 403.36 cm^{-1}. This study revealed the presence of many functional groups that may be required for Rut-SeNP reduction and stability.

3.2 Impact of Rut-SeNPs on the seizure intensity in PTZ-induced epileptic mice

The counteracting effect of Rut-SeNPs against epileptic seizures in mice was shown in Figure 2. The Racine scale indicated that prominent epileptic seizures ($P < 0.05$) and prolonged seizure duration ($P < 0.05$) had been developed in mice injected with PTZ in comparison with the control untreated mice. Epileptic mice received Rut did not display significant alterations in the seizure's severity or duration compared to the model group. However, pre-treatment with Rut-SeNPs or VPA resulted in noteworthy declines ($P < 0.05$) in the seizures score and duration compared to the PTZ group indicating the anticonvulsant effect of the formulated NPs in PTZ challenged mice.

In addition, a significant increase ($P < 0.05$) was observed in the latency period in the model group in comparison to the control mice. Remarkably, the groups administered Rut or Na_2SeO_3 displayed significantly longer latencies in seizure development relative to PTZ-challenged mice. Notably, the group that received Rut-SeNPs or VPA had distinctly longer seizure latency ($P < 0.05$) in relation to the other groups (Figure 2).

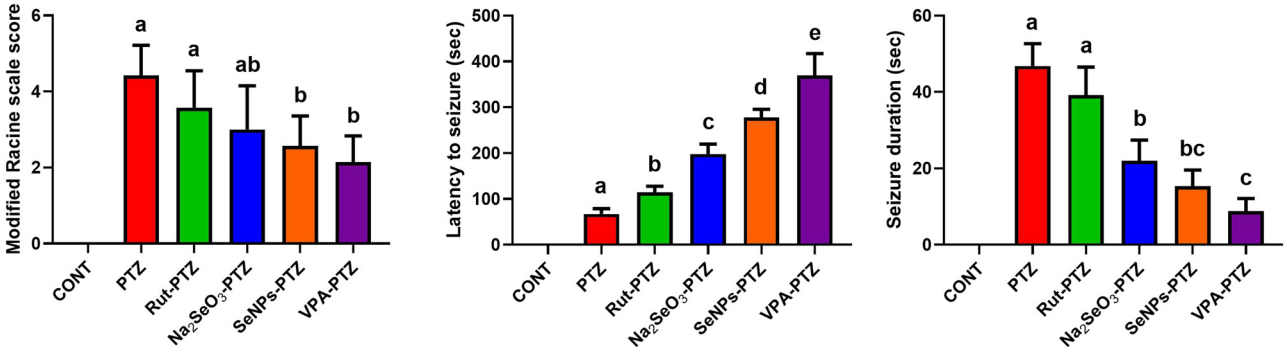

Figure 2: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on PTZ-induced behavioral changes, seizure latency, and duration. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

3.3 Effect of Rut-SeNPs on the levels of monoamines, BDNF, and AChE in the hippocampus of epileptic mice

Hippocampal AChE activity showed a significant fall ($P < 0.05$) in the PTZ-administered group when compared with the control one. However, administration of Rut (100 mg·kg^{-1}) or Na_2SeO_3 (0.5 mg·kg^{-1}) was not able to induce any substantial changes in AChE activities in comparison with the model group. It is noteworthy that SeNPs or VPA pre-treatment evoked notable increments ($P < 0.05$) in the hippocampal activity of AChE in PTZ-injected mice. These results indicated that Rut-SeNPs activated the cholinergic neurotransmission in the hippocampus of PTZ-treated mice (Figure 3).

The effect of SeNPs on the monoaminergic neurotransmission and BDNF was also investigated. Injection of mice with a single dose of PTZ evoked notable decreases ($P < 0.05$) in the levels of DA, NE, and BDNF in mice hippocampus relative to the control one. Adversely, the Na_2SeO_3, SeNPs, or VPA treatment to the PTZ-challenged mice substantially improved the hippocampal DA and NE levels ($P < 0.05$). The levels of BDNF showed notable increases ($P < 0.05$) in the hippocampus region of PTZ-challenged animals and pre-treated with Rut, Na_2SeO_3, SeNPs, or VPA. From these findings, it is remarkable that Rut-SeNP therapy did a better improvement ($P < 0.05$) in the hippocampal monoamines and BDNF in relation with the sole treatment with Rut (Figure 3).

3.4 Effect of Rut-SeNPs on the antioxidant capacity of the brain hippocampus of epileptic mice

The PTZ injection to mice was able to disturb the oxidant/antioxidant status in the hippocampus. PTZ challenge elicited notable suppression ($P < 0.05$) in the hippocampal activities of SOD and CAT enzymes compared to the control group. Nevertheless, administration of Rut, Na_2SeO_3, or SeNPs to epileptic mice caused noteworthy increments in their antioxidant activities compared to the epilepsy group. VPA treatment enhanced the activity of CAT with no observed effect on SOD activity in the hippocampus of PTZ group. In respect to the Rut-treated group, notable increases were detected in hippocampal CAT activity in the SeNP-administered group (Figure 4).

Moreover, the examination of the non-enzymatic parameters of oxidative insult in the hippocampus of epileptic

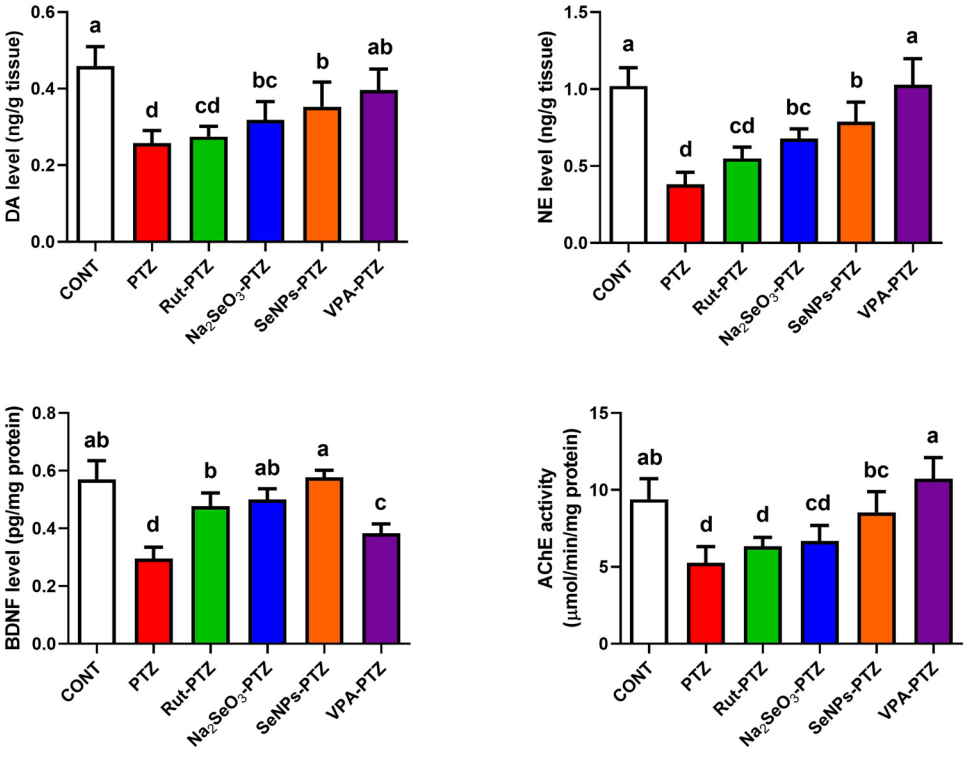

Figure 3: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on the levels of dopamine, NE, BDNF, and AChE activities in the hippocampus of PTZ-injected epileptic mice. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

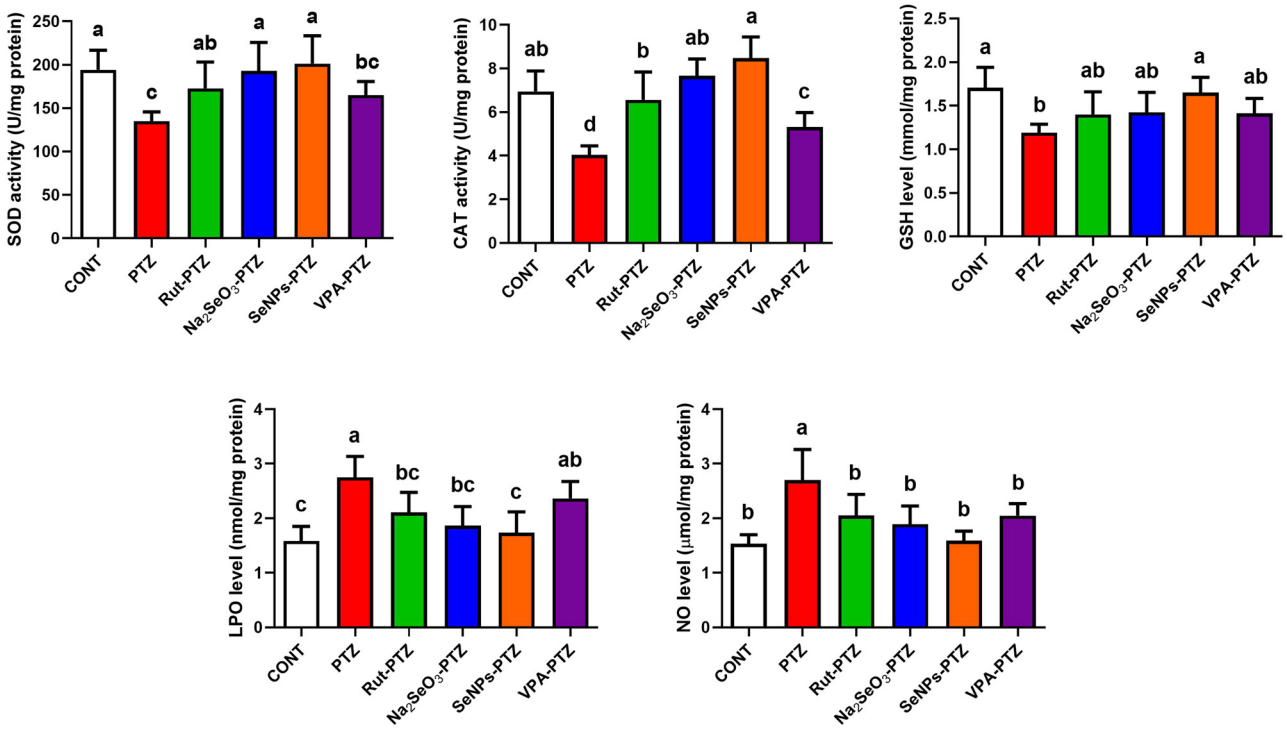

Figure 4: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on the levels of SOD, CAT, GSH, NO, and MDA in the hippocampus of PTZ-injected epileptic mice. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

animals revealed noticeable increments ($P < 0.05$) in MDA and NO levels in addition to rises in the levels of GSH in PTZ-injected mice. In contrast, Rut, Na_2SeO_3, or SeNP administration meaningfully diminished ($P < 0.05$) NO and MDA levels related to the epileptic group. The SeNP treatment only was able to increase ($P < 0.05$) the hippocampal GSH content in diseased mice. VPA treatment also evoked marked decreases ($P < 0.05$) in hippocampal NO levels without any detectable effect on either GSH or NO levels (Figure 4).

3.5 Effect of Rut-SeNPs on the Nrf2/HO-1 levels in the hippocampus of epileptic mice

Since epileptiform activity was associated with substantial modulation of Nrf2 pathway, we analyzed the hippocampal levels of the Nrf2 and HO-1 in treated mice. There were significant declines ($P < 0.05$) in the tissue levels of both antioxidant molecules in the PTZ-challenged group. Their levels in the hippocampus increased markedly ($P < 0.05$) in groups received Rut, Na_2SeO_3, or SeNPs. VPA therapy failed to induce significant increases in the levels of Nrf2 and HO-1 in the epilepsy group. No significant changes were also observed in their levels upon comparing Rut and Rut-SeNP treatment protocols. Hence, boosting the Nrf2 and HO-1 levels may explain the antioxidant defense of formulated NPs against epilepsy-related oxidative stress in the mice hippocampus (Figure 5).

3.6 Effect of Rut-SeNPs on the neuroinflammation in the hippocampus of epileptic mice

The inhibitory action of Rut-SeNPs on the inflammatory biomarkers in mice hippocampus is represented in Figure 6. Injection of PTZ at a dose of 60 $mg \cdot kg^{-1}$ to mice boosted ($P < 0.05$) all the inflammatory markers in the hippocampus analyzed in our study (NF-κB, TNF-α, IL-6, and COX II) in relation with the sham group. The groups received Rut, Na_2SeO_3, and SeNPs along with the standard VPA displayed significantly lower levels ($P < 0.05$) of all tested inflammatory mediators than those of the model group. Remarkably, Rut-SeNP therapy (100 $mg \cdot kg^{-1}$) provoked decreases ($P < 0.05$) in the levels of TNF-α, IL-6, and COX II in the mice hippocampus relative to the sole treatment with Rut. These

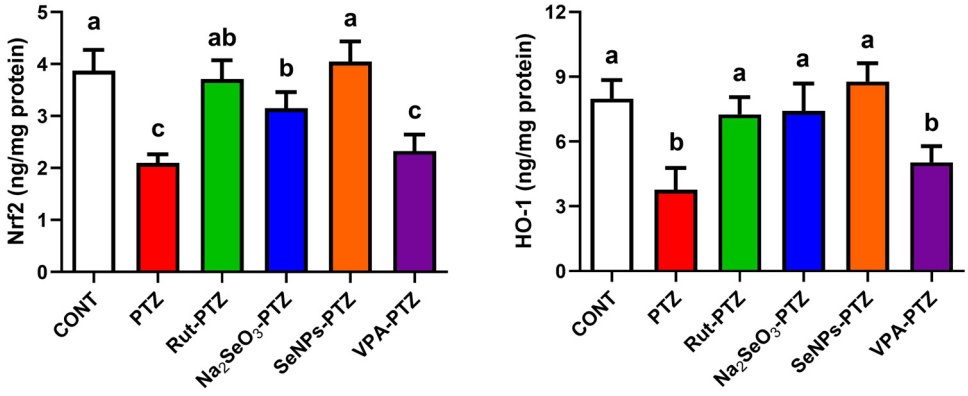

Figure 5: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on the levels of Nrf-2 and HO-1 in the hippocampus of PTZ-injected epileptic mice. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

findings concluded that Rut-SeNPs pointedly mitigated the hippocampal inflammation-mediated neuronal loss and recurrent seizures in epileptic mice.

3.7 Effect of Rut-SeNPs on the apoptotic events in the hippocampus of epileptic mice

Figure 7 depicts that PTZ injection provoked noteworthy apoptotic changes in the hippocampus of injected mice. Relative to the control mice, significant increases ($P < 0.05$) were noticed in the hippocampal levels of Bax and Cas-3 (pro-apoptotic mediators) along with declines ($P < 0.05$) in Bcl-2 (anti-apoptotic marker) in the model group. Nonetheless, the administration of Rut, Na_2SeO_3, or their nano-formulation was effective to decrease ($P < 0.05$) the level of Cas-3 in the hippocampus of epileptic mice. Only the administration of Rut-SeNPs induced significant declines ($P < 0.05$) in Bax levels in PTZ animals that indicated their antagonistic efficiency against epilepsy-associated-apoptotic damage. Marked increases ($P < 0.05$) in Bcl-2

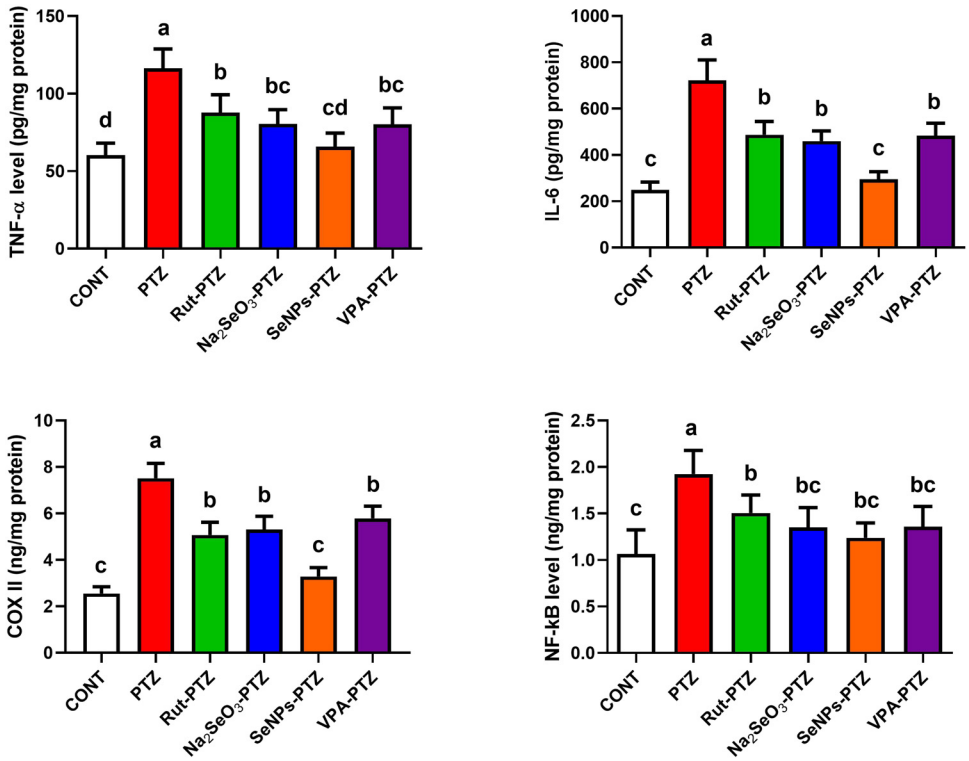

Figure 6: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on the levels of inflammatory biomarkers (IL-6, TNF-α, COX II, and NF-κB) in the hippocampus of PTZ-injected epileptic mice. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

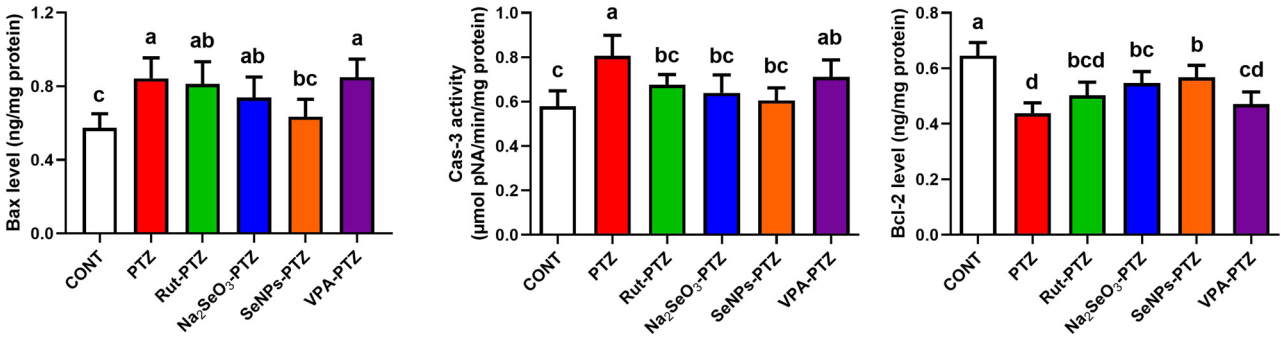

Figure 7: Effects of orally administered Rut, Na_2SeO_3, or Rut-SeNPs on the levels of pro-apoptotic markers (Cas-3 and Bax) and anti-apoptotic marker (Bcl-2) in the hippocampus of PTZ-injected epileptic mice. Different superscript letters indicate statistical significance at $P < 0.05$. All results are presented as the mean ± SD.

levels were noticed in Na_2SeO_3- or Rut-SeNPs-treated groups compared with the PTZ-treated group. Pretreatment with VPA at a dose of 200 mg·kg^{-1} did not induce any noticeable alterations in the apoptotic markers when compared to the PTZ group.

4 Discussion

Accumulating evidence reported that the NPs offered an excellent brain-targeted drug delivery system by facilitating the passage of drugs through blood brain barrier [1–3]. Despite the lipophilic nature of Rut, its low water solubility and rapid hepatic degradation hinder its bioavailability and access to the brain [45,46]. To overwhelm these obstacles, different drug carrier systems including metal NPs have been adopted for better outcomes. In this regard, this study illustrated the efficacy of Rut-conjugated NPs to counteract the epilepsy-related neural events in mice. In epilepsy studies, Racine's scale is usually utilized for the assessment of seizure intensity. Regarding the anxiety-like behavior, we observed that PTZ injection evoked pronounced epileptic seizures; however, Rut-SeNP administration exerted a significant anxiolytic effect in diseased mice. PTZ is able to provoke convulsions by blocking the gamma-aminobutyric acid ($GABA_A$) receptors, suppressing the chloride channels opening, and altering the levels of neurotransmitters [13]. Rut-SeNP administration to epileptic mice resulted in marked reductions in the score and duration of seizures as well as boosting the latency of seizures. Nassiri-Asl et al. [31] reported that pretreatment with Rut at 50 and 100 mg·kg^{-1} to PTZ-injected rats decreased the seizure severity and increased the memory retrieval in the passive avoidance task. Likewise, Rut therapy at a dose of 100 mg·kg^{-1} for 1 week relieved the manifestation of anxiety in PTZ-administered mice [47]. Former reports have demonstrated that flavonoids are ligands for $GABA_A$ receptors in the central nervous system and they have a benzodiazepine-like action [48]. In the same context, the intracerebroventricular injection of Rut dose-dependently minimized the clonic seizures in PTZ-treated rats that indicated the Rut modulating action on the $GABA_A$ receptor via the interaction at the benzodiazepine site [49]. Furthermore, the decreased serum levels of Se and zinc have been found to be the etiological basis for idiopathic intractable epilepsy in affected patients [50]. Remarkable decreases were observed in the number and intensity of epileptic spikes after Se supplementation [51–53]. In a former study, Yuan et al. [1] reported higher GABA hippocampal levels in epileptic mice received 0.5 mg·kg^{-1} SeNPs in respect to those injected with PTZ only. These findings indicate that the anticonvulsant activity of Rut-SeNPs was endorsed for the modulation of the GABAergic and opioid systems in epileptic mice.

In epileptic seizure activity, neurotransmitters are crucial players that control neuronal excitation, behavior, and cognitive function [14,54]. In accordance with former studies [2,4], injection of PTZ resulted in marked declines in the hippocampal levels of AChE, NE, and DA in treated mice. AChE is responsible for termination of the cholinergic neural transmission through rapid breakdown of acetylcholine. The accumulation of acetylcholine under epileptic conditions results in excess activation of muscarinic and nicotinic receptors [4]. Furthermore, the disturbance in brain monoamines was found to promote neuronal hyperexcitability in various animal models [1,9]. The decreases in monoamines levels in epilepsy may refer to the elevated monoamine oxidase activity with subsequent alteration in their synthesis, release, and reuptake [55]. Our results also revealed marked declines in the levels

of BDNF in mice hippocampus under PTZ challenge. This neurotrophic member has been reported to regulate neuronal survival, behavior, and synaptic plasticity in the process of epilepsy [56]. Localized delivery of BDNF to the lesioned hippocampus facilitated neurogenic process, reversed the neuronal damage, and attenuated the spontaneous seizures [57].

However, Rut-SeNP administration to the diseased mice reversed the PTZ-induced alterations in the hippocampal neurotransmission that indicated their noticeable anticonvulsant role in epilepsy seizures. Combined treatment with Rut and Se increased BDNF levels in the striatum in a mice model of Huntington's disease [18]. Similar results were reported in HT22 hippocampal neuronal cells exposed to ethanol and pre-treated with Rut [58]. These findings may referred to the increased synthesis and secretion of neurotrophic factors in the brain [59]. Rut was reported to increase the concentrations of DA, NE, and 5-HT in the brain of aged rats that greatly enhanced the spatial memory due to the suppression of monoamine oxidase activity [60]. In addition, SeNP administration was found to modulate the levels of AChE activity and monoaminergic transmission in the hippocampus of various animal models that is endorsed for their potent antioxidant activity [1,28]. Sadek et al. [61] also proved that SeNP administration to cadmium chloride-exposed rats restored the brain and serum AChE activities near to those values of the controls. Na_2SeO_3 counteracted the neurotoxic effect of mercuric chloride via activation of AChE and BDNF as well as increasing the brain levels of DA, NE, and 5-HT [62]. Collectively, these outcomes suggest that Rut-SeNP administration to mice was able to improve the disturbed neural transmission associated with epileptic conditions.

Oxidative stress is one of the fundamental cytotoxic mechanisms that are strongly implicated in the pathophysiology of epilepsy [1,5,63]. Because of the high oxygen consumption rate of the brain and its polyunsaturated fatty acids contents, it is prone to redox imbalance and oxidative damage. In consistence with previous studies [2,63], our results revealed that PTZ-injected animals displayed diminished levels of SOD, CAT, and GSH compared to the controls. PTZ treatment was reported to trigger NADPH oxidase complex with mitochondrial ultrastructural injury. This damage induces excess reactive oxygen species (ROS) and reactive nitrogen species production, peroxidation of lipids, suppression of antioxidant enzymes, and altered NO pathway that further worsens the neurodegeneration in brain hippocampus [64]. Lipid peroxidation was also observed in epileptic mice as indicated by high MDA contents as a result of the damaging effect of hydroxyl radicals on the unsaturated fatty acids [5]. Furthermore, PTZ injection resulted in increases in NO levels that may be endorsed for the activation of iNOS with the consequent formation of peroxynitrite radicals. In support, the hippocampal pathological screening validated these changes.

On contrary, Rut-SeNPs treatment counteracted the deleterious effect of ROS on hippocampal neurons in epileptic mice. Rut treatment enhanced the level of GSH and the activities of SOD and CAT in ethanol-mediated oxidative strain in HT22 hippocampal neuronal cells [58]. Moreover, oral Rut significantly augmented SOD, CAT, and GPx with concomitant declines in MDA in the hippocampus and striatum of manganese-intoxicated rats [24]. The free radical scavenging ability of Rut might be explained by inhibiting the Fenton reaction or inhibiting the activity of xanthine oxidase enzyme with subsequent decreases in production of hydrogen peroxide radicals. Furthermore, the existence of a phenolic group in its chemical structure can act as a hydrogen donor for scavenging the hazardous radicals [23]. Se is a crucial component of various antioxidant enzymes and extensively explored for its application in the recovery of antioxidant status in various animal models [1,27,61,62]. Based on these evidence, we investigated the possible involvement of Nrf2/HO-1 in the Rut-SeNP neuroprotection in the hippocampus of PTZ-treated animals. Nrf2 is a sensor that protects the cells against oxidative insults by binding to ARE and initiating the downstream activation of HO-1 and other cytoprotective enzymes [65,66]. Previous studies have demonstrated the contributing role of Nrf2 in the hippocampus under spontaneous seizures conditions [63,67,68]. Our results unveiled marked stimulation of Nrf-2 and HO-1 in the mice hippocampus after Rut-SeNPs therapy. Rut protected against acrylamide or gamma radiation-mediated neurotoxicity in rats via activation of NRF-2 signaling pathway [23]. Hence, the beneficial effect of Rut-SeNPs in the epileptic brain may be achieved via activation of Nrf2/ARE/HO-1 defense pathway.

The inflammatory reactions in the neurons play a vital role in epileptogenesis that lead to brain hyperexcitability through enhancement of the synaptic transmission [5,63]. Our results are supported by various studies on epilepsy that showed notable increases in the levels of pro-inflammatory mediators such as NF-κB, TNF-α, IL-6, and COX II indicating remarkable neural inflammation [2,14]. Mao et al. [69] found significant elevations in the serum levels of IL-1β, IL-6, IL-17A, and IFN-γ of epileptic patients when compared with healthy controls. ROS induced during the epileptic seizure is involved in the activation of inflammatory regulators as NF-κB and COX II, which further stimulates the release of other inflammatory mediators [2]. TNF-α displays pro-convulsive actions

through increasing the glutamate receptors and triggers the synaptic pruning that sequentially increases the neural excitation and epilepsy progression [14].

Rut-SeNP treatment was successful in mitigating the hippocampal inflammation induced by PTZ injection in mice. In accordance with our outcomes, Rut suppressed the NF-κB signaling pathways with resultant decrease in its downstream molecules as TNF-α, IL-1β, and IL-6 in the hippocampus and striatum of manganese-treated rats [24]. Abdelfattah et al. [18] reported notable declines in TNF-α, IL-1β, and MPO in the striatum of 3-nitropropionic acid-injected mice after Rut and Se co-administration in an experimental model of Huntington's disease. In addition, Rut decreased the brain levels of IL-1b and IL-6 in rats exposed to acrylamide or gamma-radiation [23]. SeNPs loaded with prodigiosin inhibited the levels of TNF-α and IL-1β and downregulated the gene expression of Nos2 and COX II and NF-κB in PTZ-induced epilepsy [28]. Yuan et al. [1] also found that SeNP administration suppressed the pro-inflammatory cytokines in the hippocampal tissue of epileptic mice. Therefore, the anti-inflammatory action of Se can be attributed to its antioxidant action and inhibition of the NF-κB pathway [62].

Neuronal apoptosis is closely linked to oxidative stress and neuroinflammation in the epileptogenesis process [1,4]. The current data showed significant increases in the levels of Bax and Cas-3 levels with decreases in Bcl2 levels in the hippocampal tissue after PTZ treatment. Similar findings were previously reported by other authors [1,68,70]. In response to ROS overproduction, destabilization of mitochondrial happens that allows the escape of proapoptotic proteins causing DNA damage and cell death. Li and collaborators investigated the mitochondrial ultrastructure of mice hippocampus following the injection of PTZ and they found marked swelling, vacuolations, and autophagy [67]. On the other side, our results indicated that Rut-SeNPs limited the apoptotic brain damage through increasing the Bcl-2 and decreasing the caspase-3 and Bax levels in hippocampal neurons. Rut treatment was efficient in mitigating the retinal apoptosis by decreasing both activity and the expression of Cas-3 as well as increased the expression of Bcl-2 in the diabetic retina [59]. Moreover, the anti-apoptotic effect of nano-sized Se has demonstrated noteworthy anti-apoptotic action in different experimental protocols [1,27,61]. Combined Rut and Se mitigated the neural apoptosis in the striatum of 3-nitropropionic acid intoxication in mice [18]. The anti-apoptotic effects of Rut and Se have been attributed to their ROS scavenging activity and conservation of mitochondrial membrane integrity and permeabilization with subsequent control of cytochrome *c* release [62,71]. Hence after, our study concluded that targeting oxidative stress, inflammation, and mitochondrial apoptosis by Rut-SeNP therapy could be a new approach for the control of epileptic seizures and excluding the neural death.

5 Conclusion

In summary, the formulated Rut-SeNPs exerted a powerful anti-epileptic impact against PTZ challenge in mice. The neuroprotection of Rut-SeNPs was achieved via modulation of various signaling pathways involved in epileptogenesis. Pretreated diseased mice with the prepared NPs displayed remarkable attenuation in seizure duration and latency with enhancement in hippocampal neurotransmitters. Besides, Rut-SeNPs treatment augmented hippocampal levels of antioxidant defense, decreased levels of pro-inflammatory cytokines and increased neural survival in epileptic mice. Furthermore, marked enhancement of Nrf2 and HO-1 was also involved in the neuroprotective action of Rut-SeNPs. Henceforth, our results decipher Rut-SeNPs as a promising antiepileptic agent with high efficacy, providing a novel valuable window to circumvent the epilepsy *in vivo*.

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Appendix

Rut (1): yellow solid, ^{1}H NMR (DMSO-d_6, 100 MHz) δ 12.61 (s, 1H), 7.56 (dd, J = 2.1 Hz, 7.5 Hz, 2H), 6.86 (d, J = 8.7 Hz, 1H), 6.40 (d, J = 2.1 Hz, 1H), 6.20 (d, J = 2.1 Hz, 1H), 5.35 (t, J = 7.2 Hz, 9.9 Hz, 2H), 5.10 (t, J = 7.8 Hz, 5.4 Hz, 2H), 4.39 (brs, 4H), 3.70 (d, J = 9.9 Hz, 1H), 3.30–3.06 (m, 6H), 0.99 (d, J = 6.3 Hz, 3H).

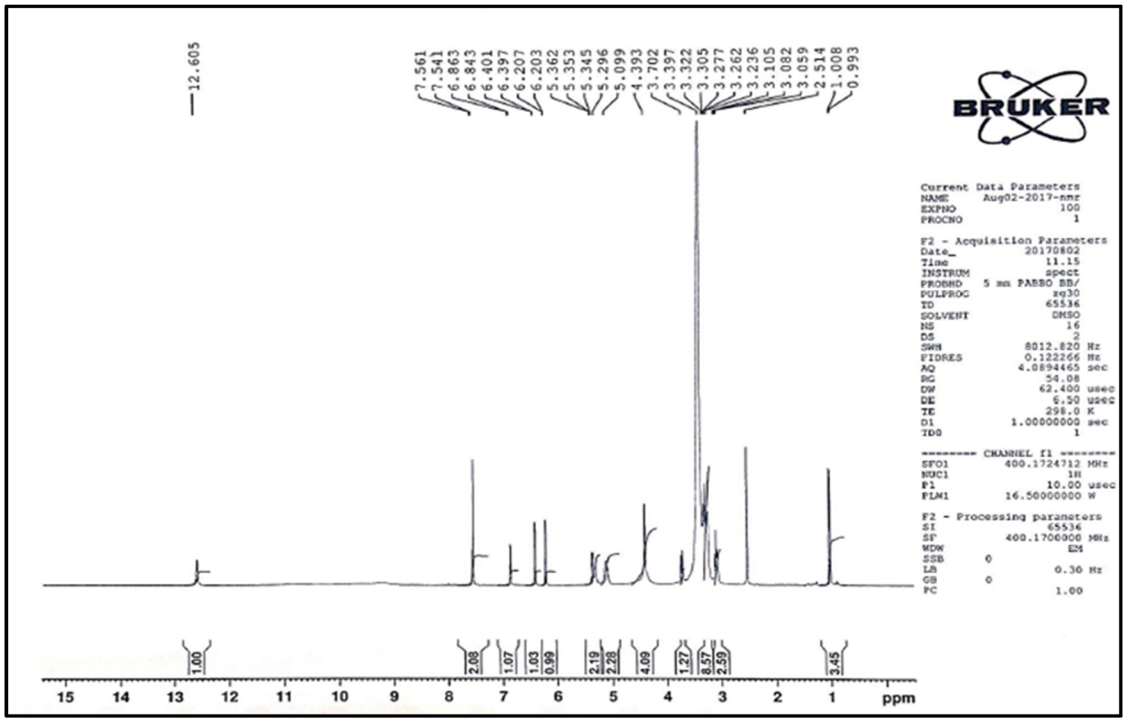

Figure A1: ^{1}H NMR spectrum of Rut (DMSO-d_6, 400 MHz).

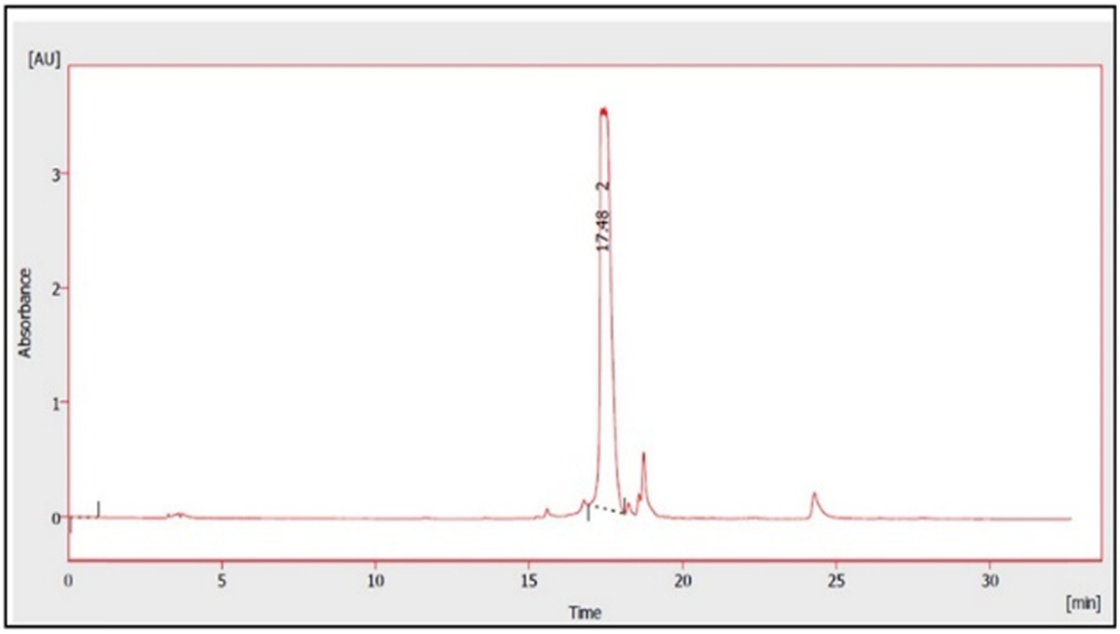

Figure A2: HPLC-PDA analysis of Rut.