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Original Article

Neuroprotective Effect of Ruscogenin against Cognitive Impairment on Pentylenetetrazole-induced Epileptic Seizures in Rats

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ABSTRACT

Background: Epilepsy, which affects about 1% of the world's population, is the third most common neurological disease after stroke and Alzheimer's disease. It is characterized by recurrent spontaneous seizures due to abnormal neuronal excitability and hypersynchrony. Objectives: The current work was aimed at discovering the neuroprotective properties of ruscogenin against Pentylenetetrazole (PTZ)-induced epilepsy in rats. **Materials and Methods:** The epileptic episode was induced in the rats by injecting 45 mg/kg of PTZ. Rats were pretreated with 10 mg/kg of ruscogenin 30 min before the PTZ challenge. The seizure severity and latency were assessed in the control and experimental rats. The status of oxidative markers, inflammatory cytokines, and neurotransmitters was examined using the corresponding assay kits. The histopathological analysis was done on the brain tissues of the experimental rats. The viability of control and treated neural SHSY-5Y cells was assessed with the MTT assay. **Results:** The ruscogenin treatment effectively reduced the seizure severity and augmented the latency in the epilepsy rats. The levels of Nitric Oxide (NO) and NO syntheses were effectively reduced in the brain tissues of epilepsy rats by the ruscogenin treatment. Ruscogenin decreased the oxidative index and increased the antioxidant index in the brain tissues of epilepsy rats. The levels of neurotransmitters were increased, and inflammatory cytokines were decreased in the epileptic rats by the ruscogenin. The results of histopathological analysis demonstrated the neuroprotective effects of ruscogenin. The viability of PTZ-challenged neural SHSY-5Y cells was increased by the ruscogenin treatment. **Conclusion:** In conclusion, the current results revealed that pretreatment with ruscogenin decreased the PTZ-induced epilepsy in rats by exerting its antioxidant and anti-inflammatory effects. Therefore, it may be concluded that ruscogenin may be a possible therapeutic option to treat epilepsy.

Keywords: Seizure, Gammaaminobutyric acid, Nitric oxide synthase, Ruscogenin, SHSY-5Y cells.

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INTRODUCTION

Epilepsy is a common neurological disease characterized by recurrent spontaneous seizures due to abnormal neuronal excitability and hypersynchrony.[1] Epilepsy affects roughly one percent of the global population, resulting in impaired cognitive and motor functioning and a poor quality of life for those affected. A several neurological problems, including recurrent seizures, cognitive abnormalities, behavioral issues, and alterations in the electroencephalogram, are the hallmarks of this syndrome. About 30% of the people suffers from psychiatric comorbidities such as memory and learning problems, which exacerbate epilepsy.[2] It is a condition of the Central Nervous System (CNS) that may have an inherent predisposition or be the result of a chronic medical condition. Seizures develop when cortical neurons activated excessively or in an abnormal pattern. A disruption in the equilibrium between excitation and inhibition in neural networks is thought to have a role in seizure pathogenesis. Several mechanisms contribute to the pathophysiology of epilepsy, including genetic defects, neuroinflammation, oxidative stress, ion channels, and neuronal damage.[3] Most people with epilepsy also struggle with conditions including depression, anxiety, psychosis, and poor memory. The most common causes of epilepsy are intracerebral hemorrhage or stroke, brain tumors, CNS infections, persistent febrile seizures, and other forms of status epilepticus.[4]

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In order to comprehend the underlying mechanisms of seizures, experimental animal seizure models have been developed. As a specific antagonist of the Gamma-Aminobutyric Acid (GABA) receptor, Pentylenetetrazole (PTZ) has detrimental effects on the neuronal membrane and is extensively used to stimulate seizures in rodents.[5] PTZ is a well-established and proven paradigm for generating epileptic seizures by inhibiting the GABA receptor, a primary inhibitory neurotransmitter. As with human absence seizures, PTZ can be used to induce convulsions in rodents to serve as a model for epilepsy research.[6]

Treatment for epilepsy typically involves Antiepileptic Drugs (AEDs), despite the fact that they can have serious negative effects, including ischemia, depression, and memory loss. The application of AEDs is also unsuccessful in preventing seizures in 20–30% of patients with drug-resistant epilepsy.[7] Around one in three epileptic patients is drug-resistant and continues to have refractory seizures even when using AEDs. Inadequately, existing AEDs are becoming increasingly unreliable due to the prevalence of unwanted side effects, the high expense of treatment, and drug resistance. Thus, there is a pressing demand for improved and more reliable AEDs.[8]

Ruscogenin, a bioactive steroid sapogenin extracted from the plant genus Ophiopogon, has been utilized widely in the management of chronic inflammation[9] Furthermore, its anti-diabetic nephropathy,[10] regulation of neutrophil activation,[11] neuroprotective,[12] and antiallergic activities have already been reported. Several recent studies discovered that ruscogenin has therapeutic effects in protecting against hepatic injury,[13] acute lung injury,[14] and anticancer[15] activity. Nonetheless, the beneficial effects of ruscogenin on epilepsy have not been studied yet. Thus, the current work was aimed at discovering the neuroprotective properties of ruscogenin against PTZ-induced epilepsy in rats.

MATERIALS AND METHODS

Chemicals

Ruscogenin, PTZ, and other chemicals used in this study were acquired from the Sigma-Aldrich, USA. The corresponding assay kits were obtained from the Thermofisher Scientific, USA, and MyBioSource, USA.

Experimental rats

For this experiment, 8-week-old Wistar rats weighing above 230g were used. All the rats were accommodated in sterile polyacrylic confines and kept in a standard laboratory environment with a constant 22±3°C temperature, humidity of 55–65%, and a 12 hr dark/light cycle. The rats had unrestricted access to the regular chow and purified water. This research was approved by General Hospital of Ningxia Medical University animal ethical committee, Approved No. GHNMU2021-0325.

Experimental design

The rats were distributed randomly into five groups of six rats each (n = 6). Rats in group I received a 0.9% NaCl solution as a placebo. To induce epileptic episodes in group II rats, 45 mg/kg of PTZ dissolved in 0.9% saline was administered intraperitoneally. Rats in group III received 2 mg/kg of the standard drug diazepam before 30 min of the PTZ challenge. Rats in group IV received 10 mg/kg of ruscogenin orally 30 min before receiving the PTZ treatment, and rats in group V received only ruscogenin (10 mg/kg) without the PTZ challenge. Following the PTZ challenge for 30 min, the rats were carefully observed to identify seizure symptoms.

The seizure severity was quantified using a modified Racine's Convulsion Scale (RCS). The following seizure patterns were identified in experimental rats with RCS: A score of 0 indicates no convulsion, 1 indicates slight twitching of the vibrissae and pinnae, 2 indicates motor arrest with twitching, 3 indicates Myoclonic Jerks (MJs), 4 indicates a tonic-clonic seizure while the rats continued to eat, 5 indicates a tonic-clonic seizure with loss of righting reflex, 6 indicates a tonic-clonic seizure with jumping and climbing, and 7 indicates death.[16] Followed by the 30 min of PTZ challenge, the rats were carefully observed to identify the behavioral scoring using RCS to determine the onset and duration of Generalized Tonic-Clonic Twitch Seizures (GTCS) and the onset of first MJs.

Passive Avoidance (PA) test

The PA learning test was conducted in accordance with the principle of negative reinforcement. The grid-floored instrument was split into two sections, one of which was black and had a tiny door with a light in it. Since it was known that rats innately preferred dark conditions, the study was conducted accordingly. Two days prior to the training session, the rats became used to the instrument (300 s per day). Later that day, rats were placed in the bright area, and their latency to the dark zone was noted. Rats were given an electric shock (1 mA, 5 s) when they entered the dark area of the training area and were instructed to return to the light area. After that, the rats were reverted to their respective enclosures. In the retention test procedure performed 1 hr following the training sessions, temporal latency was detected while the rats were in the light area.

Preparation of brain tissue homogenates

Cortex and hippocampus tissues were collected from the rats and homogenized using a mechanical homogenizer after being mixed with a cold PBS solution (SpeedMILL Plus, Analytik-Jena, Germany). The homogenates were then centrifuged at 4000 rpm for 10 min at 4°C. To conduct the biochemical analysis, the supernatants were collected and frozen.

Measurement of NO, nNOS, and iNOS levels

Using corresponding assay kits and following manufacturer instructions (Thermofisher Scientific, Waltham, MA, USA), the status of NO, nNOS, and iNOS in the brain tissues of the experimental rats was examined.

Measurement of Dopamine (DA), GABA, Na+K+ATPase, and Ca+ATPase in the brain tissues

Assay kits purchased from ThermoFisher Scientific, USA, were used to measure the levels of DA and GABA in both control rats and experimental rats, following the manufacturer's instructions. Using commercially available assay kits (MyBioSource, San Diego, USA), the activity of the Na+K+ATPase and Ca+ATPase enzymes was tested in both treated and untreated rats.

Measurement of inflammatory cytokine levels

To measure the status of inflammatory cytokines TNF-α and IL-1β in the control and experimental rats, commercial assay kits were used, and assays were done in triplicate in accordance with the instructions recommended by the manufacturer (Thermofisher Scientific, Waltham, MA, USA).

Detection of Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI)

The proportions of TAS in the brain tissues of control and treated rats were measured using commercial kits based on manufacturer instructions (Rel Assay Diagnostics, Antep, Turkey). The OSI was determined as the percentage difference between TOS and TAS.[17]

Histopathological analysis

Brain hippocampus tissues were removed from the control and experimental rats and fixed in formalin (10%) for 24 hr prior to histological analysis. Following dehydration, paraffinization, and subsequent slicing into 5 µm diameter, the brain tissues were stained using eosin and hematoxylin and then assessed under a microscope at 40× magnification to capture the microphotographs.

In vitro studies

Cell culture

The human neural SHSY-5Y cells were purchased from ATCC, USA, and grown in DMEM with 10% FBS, 1% l-glutamine, and 1% antibiotic cocktail. The cells were kept at 37°C with CO_2 (5%) atmosphere for additional assays.

Cell viability assay

The growth of SHSY-5Y cells was investigated using MTT assay. Briefly, the cells were grown on 96-well plates and incubated overnight. Then cells were rinsed with fresh medium without serum and exposed to diverse doses (1, 2.5, 5, 7.5, 10 µM) of ruscogenin for 1 hr and then cells were treated with 30 µM of PTZ for consequent 24 hr. After the 24 hr of incubation, MTT reagent (1 mg/mL) was added to the wells for 4 hr and then DMSO was loaded to dissolve formed formazan deposits. Finally, the absorbance was determined at 490 nm.

Statistical analysis

The values are provided as the mean±SD of three replicates. One-way ANOVA and DMRT were used in SPSS software to analyze the data sets. The data was considered as significant if $p<0.05$.

RESULTS

Effect of ruscogenin on the seizure severity, FMJ, PA learning test in the experimental rats

Figure 1 exhibits the findings of an impact of ruscogenin treatment on seizure severity, FMJ, and the PA learning test in epilepsy rats. The results of RCS demonstrated the increased seizure score in the epilepsy rats. The PTZ-induced rats also revealed a significant decrease in the FMJ and latency. Fascinatingly, the PTZ-induced epileptic rats treated with 10 mg/kg of ruscogenin revealed a significant reduction in seizure severity while increasing the latency and FMJ, which is comparable to the results of the standard drug diazepam (Figure 1). A ruscogenin alone treated rats did not show any significant differences from the control.

Effect of ruscogenin on the iNOS and nNOS levels in the brain tissues

The influence of ruscogenin treatment on the levels of iNOS and nNOS in both cortex and hippocampal tissues was assessed, and the results are depicted in Figure 2. The epileptic rats showed a significant upsurge in both iNOS and nNOS status in the cortex and hippocampal tissues when compared to control. However, in the PTZ-induced epileptic rats, the 10 mg/kg of ruscogenin significantly diminished the nNOS and iNOS levels, which is similar to the results of standard drug diazepam treatment. Furthermore, no significant changes were noted in the iNOS and nNOS status in the ruscogenin alone treated rats (Figure 2).

Effect of ruscogenin on the TAS, TOS, and OSI levels

The levels of TAS, TOS, and OSI in both control and ruscogenin-treated epileptic rats were assessed, and the results are illustrated in Figure 3. When compared to control rats, the TOS and OSI status were gradually elevated, while the TAS was reduced in the brain tissues of the epileptic rats. Nevertheless, the ruscogenin at a dose of 10 mg/kg remarkably decreased the TOS and OSI levels while increasing the TAS status in the epileptic rats (Figure 3). These outcomes were similar to the results of the standard drug diazepam-treated epileptic rats.

Effect of ruscogenin on the NO levels in the brain tissues of experimental rats

The changes in the NO levels in the brain tissues of the control and experimental rats were assessed, and findings were showed in Figure 4. The increased NO level was observed in both the cortex and hippocampus tissues of the PTZ-induced rats. However, the 10 mg/kg of ruscogenin considerably reduced the NO in the brain tissues of the epileptic rats. The standard drug diazepam treatment also decreased the NO level in the epileptic rats, which supports the activity of ruscigenin (Figure 4).

Effect of ruscogenin on the GABA, DA, Na+K+ATPase, and Ca+2ATPase levels in the experimental rats

Results from an analysis of the effect of ruscogenin treatment on GABA, DA, Na+K+ATPase, and Ca+2ATPase levels in the experimental rats are presented in Figure 5. PTZ-induced rats with epilepsy have decreased levels of GABA, DA, Ca+2 ATPase, and Na+K+ATPase levels. Interestingly, the 10 mg/kg ruscogenin-treated epileptic rats revealed a considerable increase in the GABA, DA, Na+K+ATPase, and Ca+2 ATPase levels. The results of ruscogenin treatment were comparable to those of standard drug treatment with diazepam in epileptic rats. Additionally, the ruscogenin alone treated rats without showing any significant changes in the GABA, DA, Na+K+ATPase, and Ca+2 ATPase and found similar to the control (Figure 5).

Effect of ruscogenin on the levels of inflammatory cytokines

The effect of ruscogenin on the IL-1β and TNF-α levels in PTZ-induced epileptic rats was assessed, and findings are displayed in Figure 6. PTZ-induced epileptic rats exhibited elevated IL-1β and TNF-α levels. Intriguingly, 10 mg/kg of

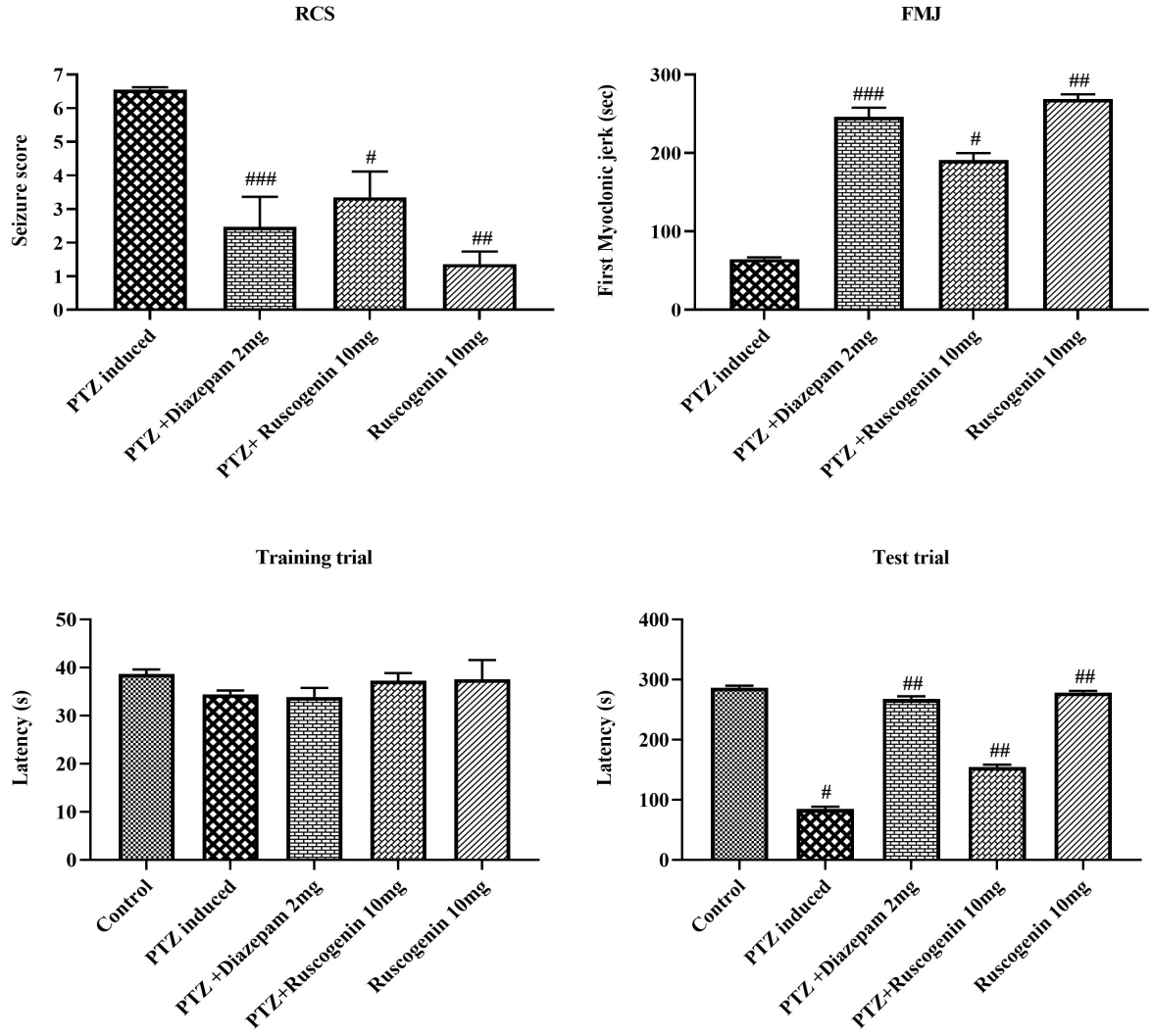

Figure 1: Effect of ruscogenin on the seizure severity, FMJ, PA learning test in the experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

ruscogenin substantially diminished the IL-1β and TNF-α levels in the epileptic rats (Figure 6). The results of ruscogenin treatment were supported by the standard drug diazepam, which also reduced the IL-1β and TNF-α in the epileptic rats.

Effect of ruscogenin on the brain histology of the experimental rats

Figure 7 demonstrates the findings of the histopathological analysis of the brain tissues of control and experimental rats. The untreated control and ruscogenin alone-treated rats revealed regular brain histological arrangements without any major changes. The PTZ-induced epileptic rats demonstrated several histological abnormalities in brain tissue, including pyknosis, congestion, neuronal necrosis, and increased infiltration of inflammatory cells. Contrastingly, epileptic rats given ruscigenin at a concentration of 10 mg/kg demonstrated remarkably decreased inflammation, pyknosis, and congestion in the brain tissues, which is comparable to the results of the standard drug diazepam treatment (Figure 7). Diazepam treatment also decreased brain histological damage in the epileptic rats.

Effect of ruscogenin on the viability of PTZ-induced neural SHSY-5Y cells

The influence of ruscogenin treatment on the growth of PTZ-induced neural SHSY-5Y cells was investigated by MTT assay, and the results were revealed in Figure 8. The 30 µM of PTZ treatment significantly decreased the growth of SHSY-5Y cells. However, the diverse doses (1, 2.5, 5, 7.5, and 10 µM) of ruscogenin treatment considerably increased the viability of PTZ-challenged SHSY-5Y cells. These findings proved the therapeutic effects of ruscogenin (Figure 8).

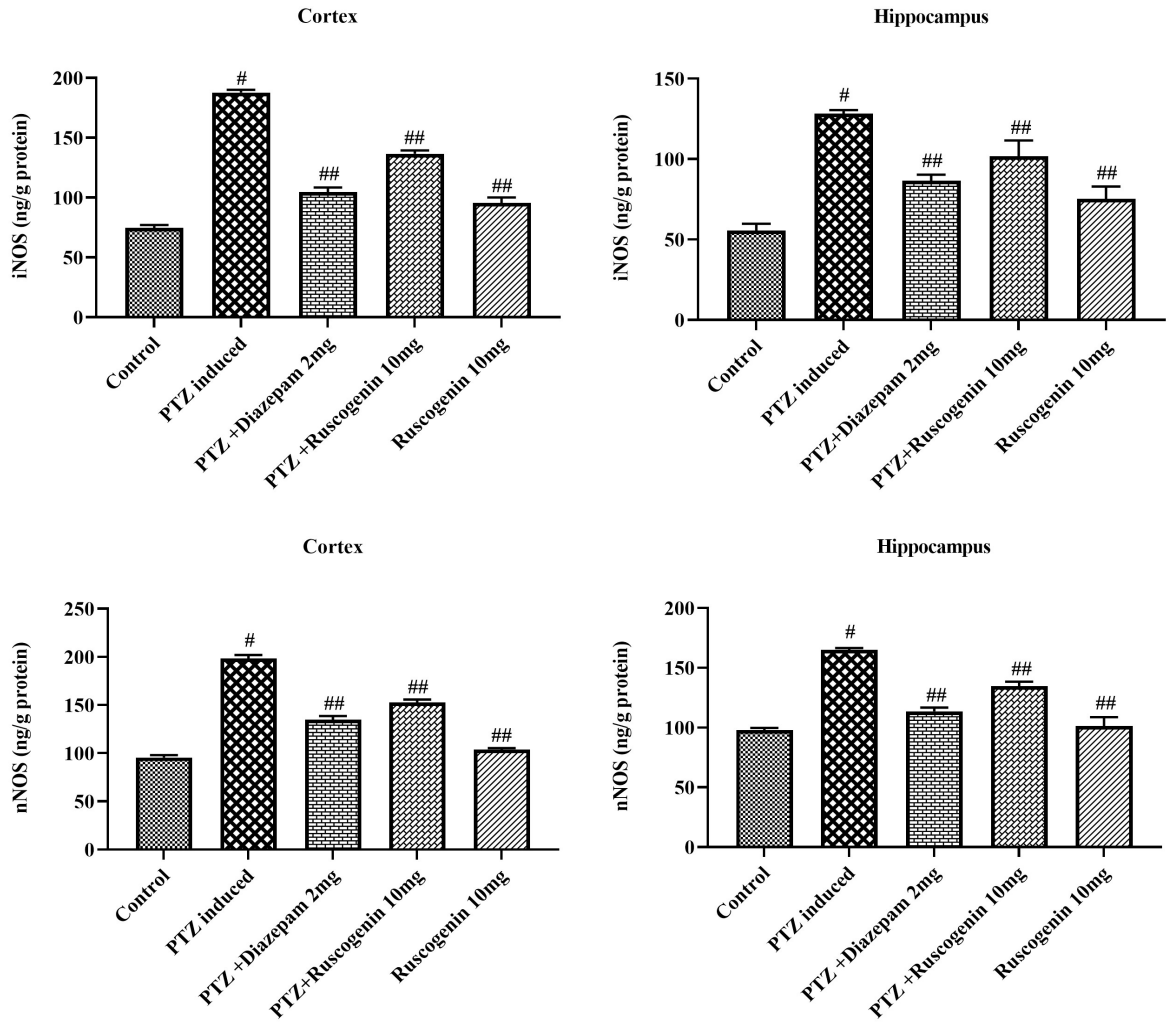

Figure 2: Effect of ruscogenin on the iNOS and nNOS levels in the brain tissues of experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

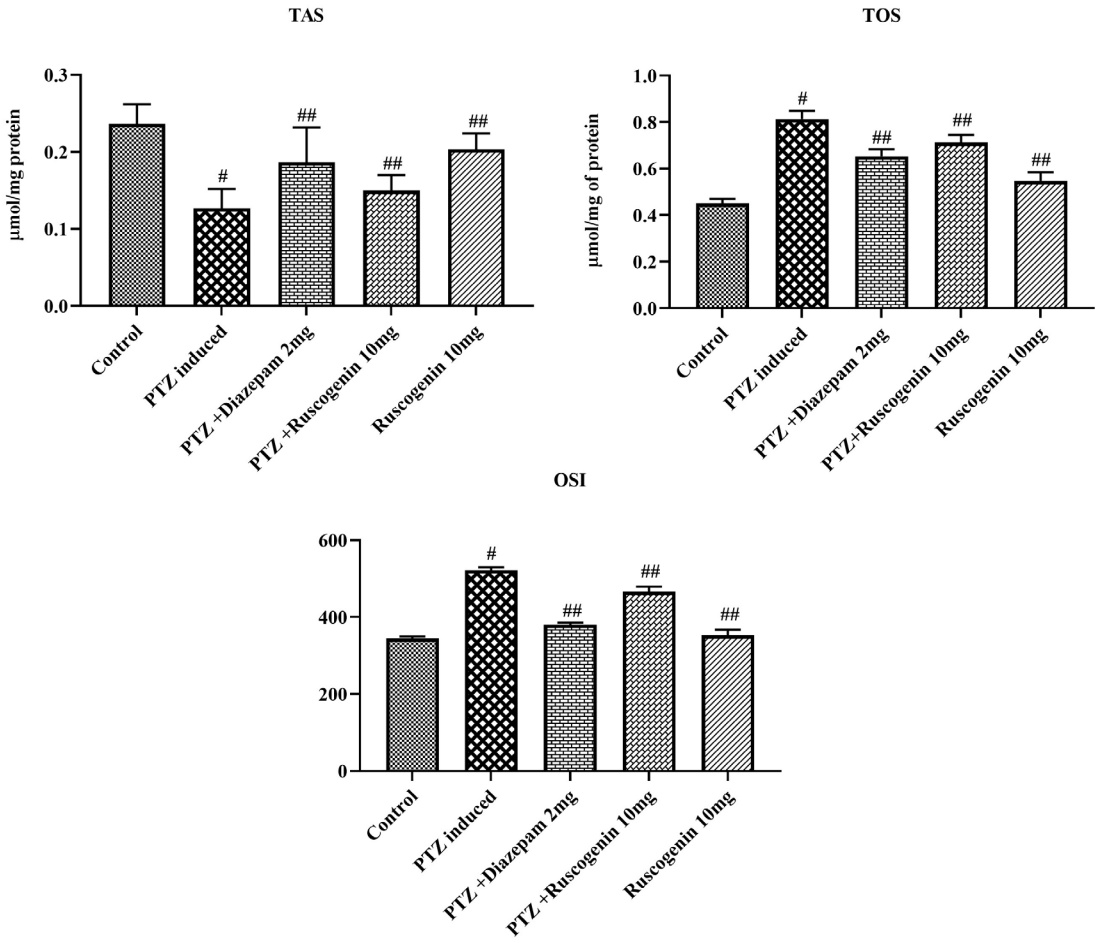

Figure 3: Effect of ruscogenin on the TAS, TOS, and OSI levels in the experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

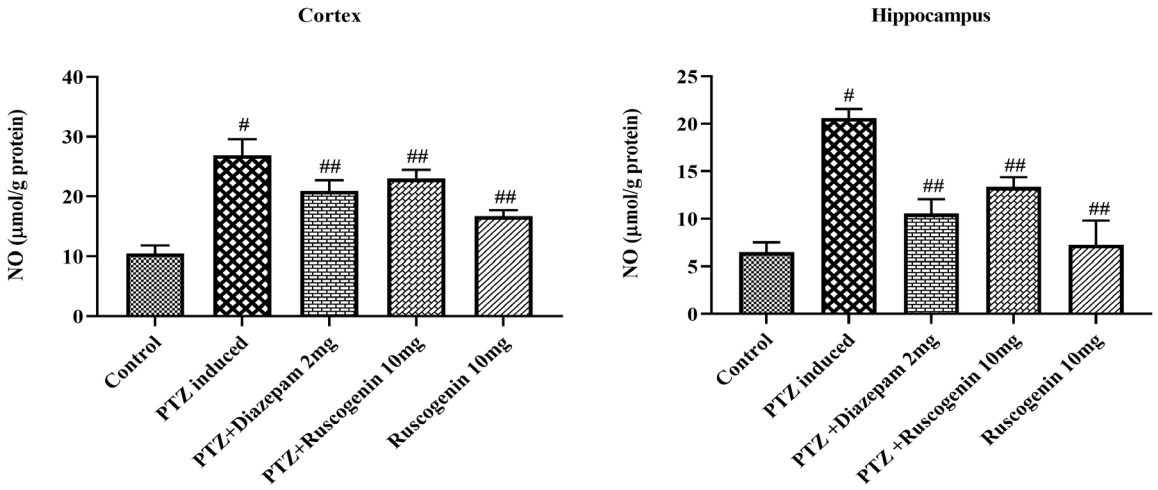

Figure 4: Effect of ruscogenin on the NO levels in the brain tissues of experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

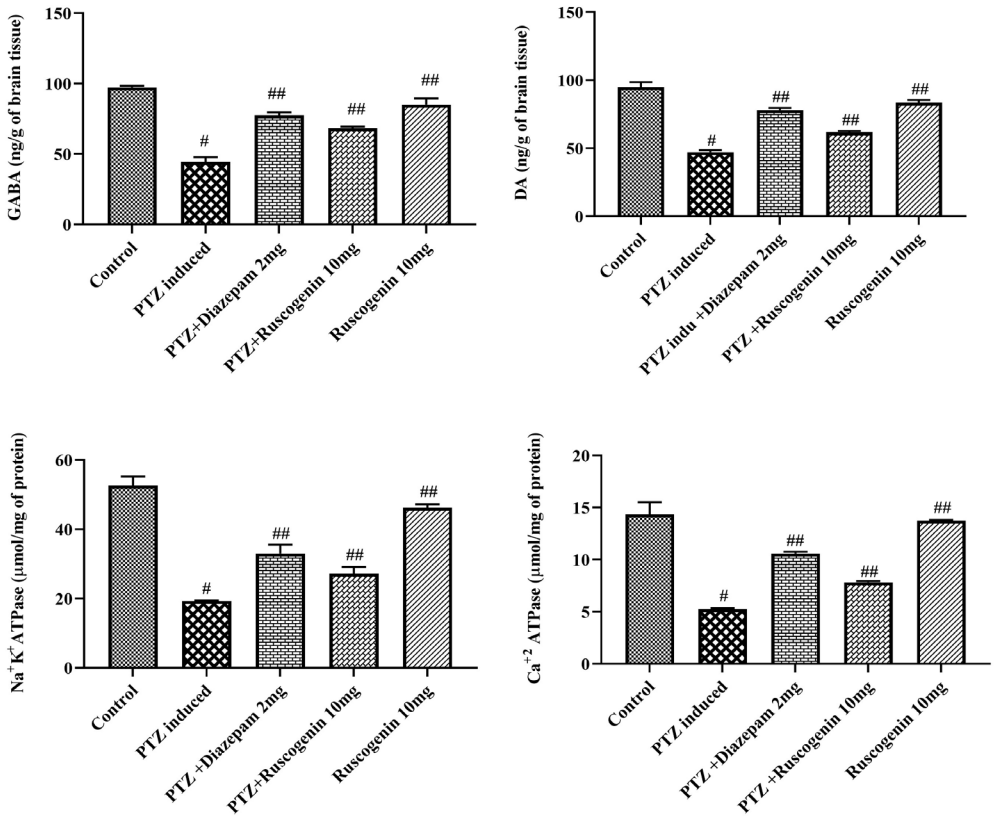

Figure 5: Effect of ruscogenin on the GABA, DA, Na+K+ATPase, and Ca+2 ATPase levels in the experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

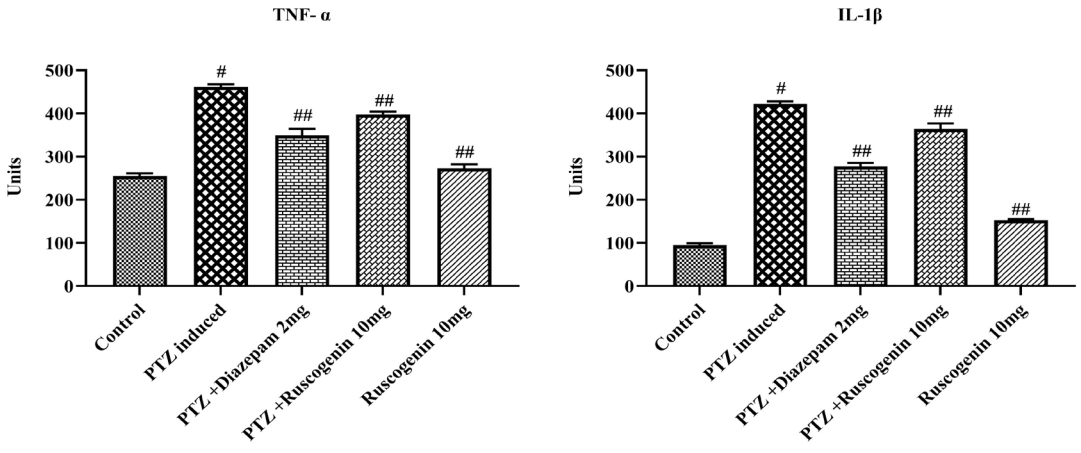

Figure 6: Effect of ruscogenin on the levels of inflammatory cytokines in the experimental rats. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Note: '*' $p<0.01$ indicates the significance when compared with control and '#' $p<0.05$ indicates the significance when compared with PTZ-induced epilepsy group.

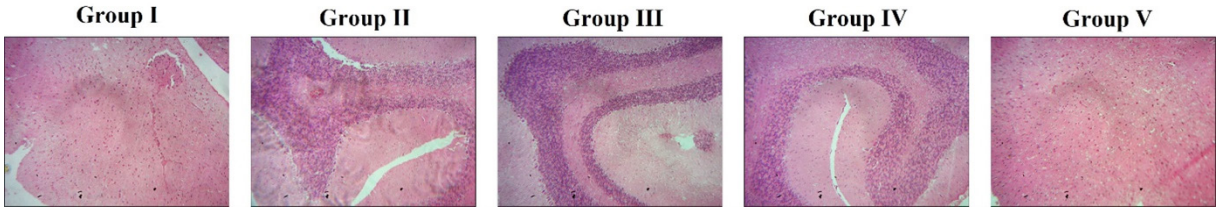

Figure 7: Effect of ruscogenin on the brain histology of the experimental rats. Control rats had normal histological structures in the brain (Group I). The PTZ-induced epileptic rats revealed several changes like congestion, pyknosis, neuronal necrosis, and infiltration of inflammatory cells (Group II). The treatment with 10 mg/kg of ruscogenin considerably reduced the inflammation, pyknosis, congestion, and neuronal necrosis (Group III). The histological changes were also reduced by the treatment with standard drug Diazepam (Group IV). The treatment with ruscogenin alone did not show any major histological changes in the brain tissues (Group V).

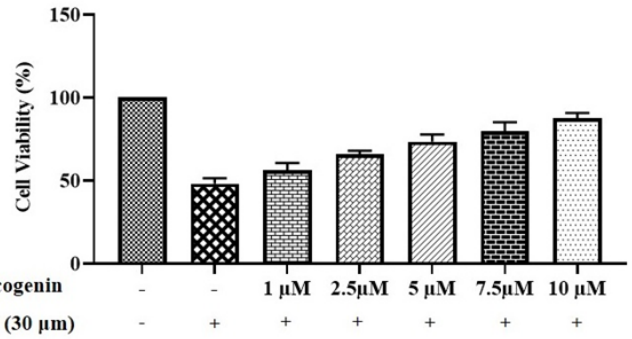

Figure 8: Effect of ruscogenin on the viability of PTZ-induced neural SHSY-5Y cells. Each bar illustrates the mean±SD of three replicates. The values are analyzed by one-way ANOVA and DMRT to measure the significance. Values not sharing common superscript and significantly vary at $p<0.05$ from control.

DISCUSSION

A seizure is a transient brain malfunction caused by an abnormal release of cortical neurons, resulting in a discrepancy of excitation and inhibition in cortical neuronal networks.[18] Seizures in epilepsy patients are complicated by excitatory glutamatergic pathways, inflammation, and oxidative stress. Seizures can be attributed in part to immune-inflammatory dysregulation and neuronal hyperexcitation driven by brain inflammation. An epileptic seizure occurs when a network of neurons in the brain becomes overexcited by a sudden influx of calcium ions, causing them to discharge rapidly.[19] The findings of the current work revealed that the ruscogenin treatment effectively diminished the seizure severity and augmented the latency in the epilepsy rats. The results of the ruscogenin treatment are supported by the results of the standard drug treatment with diazepam.

The brain tissues are more susceptible to oxidative damage than other tissues because of their higher oxygen consumption, higher lipid content, weaker antioxidant system, and higher metabolic requirements. By activating the glutamate receptor channel, oxidative stress facilitates cell death by disrupting ionic balance, disrupting neurotransmission, and allowing for the entry of increased Ca^{2+} ions.[20] Furthermore, numerous clinical and experimental studies point to the importance of oxidative stress in both the initiation and development of epileptic seizures and epileptogenesis. The brain has a limited ability to fight against free radicals; therefore, oxidative stress can also worsen epilepsy. Moreover, the level of oxidative injury is related to the incidence of epileptic events.[21] A link between PTZ-induced seizures and oxidative stress has been well established.[22] The results of this work demonstrated that epilepsy rats exhibited a considerable reduction in the TAS while increasing the TOS and OSI status. Intriguingly, the treatment with ruscogenin considerably increased the TAS while decreasing the TOS and OSI levels in the epilepsy rats. These findings proved that ruscogenin decreased the oxidative stress in the brains of epileptic rats.

The pathogenesis of epilepsy is tightly connected with the NO signaling system. NO is produced from the L-arginine amino acid oxidation by three different types of NOS, such as iNOS, endothelial NOS, and nNOS, and is a key neuromodulator. Studies have linked NO to both the excitatory and inhibitory neuronal systems, as well as the pathophysiology of seizure development.[23] In neurological diseases, nNOS has a significant role. Moreover, it has been discovered that nNOS has also been linked to seizures.[24] The findings of the current study found increased NO, iNOS, and nNOS status in the PTZ-induced epilepsy rats. Interestingly, the ruscogenin treatment substantially decreased the NO, iNOS, and nNOS levels in the epilepsy rats. These findings confirmed the antioxidant potentials of ruscogenin.

Neuroinflammation is thought to be the hallmark of neurodegeneration, despite the fact that it is a physiological response to maintain inherent homeostasis.[25] Inflammatory cytokines have an important role in the pathophysiology of seizures. These mediators can cause leukocyte infiltration, which in turn can lead to biochemical and functional abnormalities.[26] Additionally, activated glial cells cause the secretion of inflammatory cytokines, compromising the epilepsy prognosis.[27] It was well known that neuroinflammation has a link to the onset of seizures. Proinflammatory cytokines like TNF-α and IL-1β increase neuronal hyperexcitability and vulnerability by boosting glutamatergic transmission.[28] Also, they modulate neuronal transmission, which contributes to seizure activity and epileptogenesis. In addition, the prognosis of epilepsy is compromised by the rapid stimulation of local glial cells, which leads to the increased production of IL-1β and TNF-α.[29] A growing

body of evidence from clinical and experimental investigations suggests that inflammation plays a critical role in epilepsy, either as a cause or a result of the disease. The epileptic episodes can be avoided by blocking the production of specific inflammatory mediators. Many cytokines are released by the immune system as a response to injuries to the nervous system.[30] Increased levels of IL-1β and TNF-α are found in the brain tissues of epilepsy animals, leading to neuronal and cellular damage. Patients with epilepsy also have an elevated amount of these proinflammatory cytokines in their blood or Cerebral-Spinal Fluid (CSF).[31] In the present work, the results showed a substantial upsurge in the IL-1β and TNF-α levels in the PTZ-induced epilepsy rats. Fascinatingly, the treatment with the ruscogenin substantially decreased the IL-1β and TNF-α in the epileptic rats. These findings demonstrated that ruscogenin has strong anti-inflammatory activity.

GABA serves as the primary inhibitory neurotransmitter in the brain and CNS, which also plays a major role in controlling seizures by reducing the excitability of neurons. When released from presynaptic vesicles, GABA binds to the GABA-A receptor, activating chloride channels and leading to postsynaptic hyperpolarization.[32] It has been established that an ultimate purpose of AEDs is to increase GABA release, thus increasing GABA-mediated inhibitory activity.[33] Here, the current results exhibit a marked reduction in both GABA and DA in the brain tissues of the epilepsy rats. However, the ruscogenin treatment effectively boosted the GABA and DA levels in the brain tissues of the epilepsy rats. These outcomes confirmed that ruscogenin substantially modulated the neurotransmitter levels of epilepsy rats.

A decrease in the K+/Na+ ATPase enzymes cause the discharge of unregulated dendritic cells, which in turn leads to epileptogenesis. Previous research has shown that neuronal membrane-bound phosphatase enzymes such as Na+ K+ ATPase are crucial players in the regulation of membrane potential and transmembrane Ca2+ influx.[34] The activity of K+/Na+ ATPase enzymes was reduced in epileptic model brain tissues.[35] Similarly, we discovered that the Na+/K+ ATPase enzyme activities were lowered in epileptic rats. Meanwhile, ruscogenin treatment significantly enhanced the Na+/K+ ATPase enzymes in epileptic rats.

CONCLUSION

In conclusion, the present results showed that pretreatment with ruscogenin reduces the PTZ-induced epilepsy in rats by exerting its salutary effects. In epileptic rats, the ruscogenin treatment substantially modulated the neurotransmitter levels, decreased the oxidative stress and inflammation, and prevented the neuronal damage. The ruscogenin also increased the viability of the PTZ-induced neural SHSY-5Y cells. Therefore, it may be concluded that ruscogenin may be a talented therapeutic agent to treat epilepsy in the future. Though more studies are still required in the future to make a clear understanding of the underlying mechanisms of the therapeutic effects of ruscogenin against epilepsy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GABA: Gamma-aminobutyric acid receptor; **PTZ:** Pentylenetetrazole; **NO:** Nitric oxide; **CNS:** Central nervous system; **AEDs:** Antiepileptic drugs; **PA:** Passive avoidance; **TAS:** Total antioxidant status; **TOS:** Total oxidant status; **OSI:** Oxidative stress index.

SUMMARY

Seizures in epilepsy patients are complicated by excitatory glutamatergic pathways, inflammation, and oxidative stress. Ruscogenin treatment substantially decreased the NO, iNOS, and nNOS levels in the epilepsy rats. Ruscogenin decreased the PTZ-induced epilepsy in rats by exerting its antioxidant and anti-inflammatory effects.

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