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Pharmacological Potential Of Pinosylvin In Ptz Induced Epilipsy

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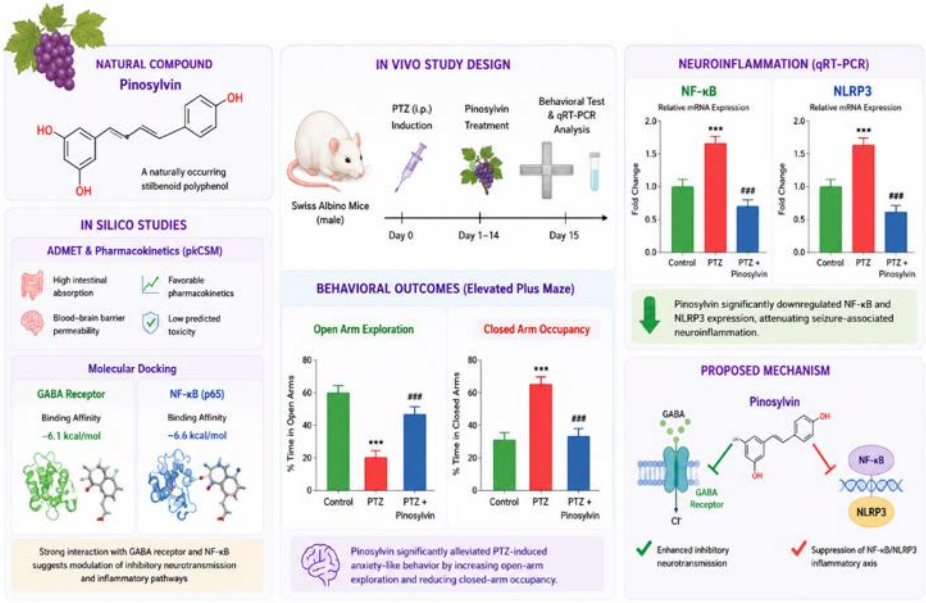
Abstract

Epilepsy is a chronic neurological disorder characterized by recurrent seizures and is frequently associated with neuroinflammation, oxidative stress, and behavioral impairments. Despite the availability of several antiepileptic drugs, treatment resistance and adverse effects remain major clinical challenges, highlighting the need for safer and more effective therapeutic alternatives. The present study investigated the anticonvulsant and neuroprotective potential of pinosylvin, a naturally occurring stilbenoid polyphenol, in a pentylenetetrazole (PTZ)-induced epilepsy model using integrated in silico and in vivo approaches. Pharmacokinetic profiling was performed using the pkCSM platform, while molecular docking analysis evaluated interactions with epilepsy-related targets, including γ-aminobutyric acid (GABA) receptors and nuclear factor-kappa B (NF-κB). In vivo experiments were conducted in PTZ-treated mice, followed by behavioral assessment using the Elevated Plus Maze (EPM) and evaluation of neuroinflammatory gene expression through quantitative real-time polymerase chain reaction (qRT-PCR). ADMET analysis demonstrated favorable pharmacokinetic properties, including high intestinal absorption, satisfactory blood-brain barrier permeability, and low predicted toxicity. Molecular docking revealed strong binding affinities of pinosylvin toward

GABA receptors (−6.1 kcal/mol) and NF-κB (−6.6 kcal/mol), suggesting potential modulation of inhibitory neurotransmission and inflammatory pathways. Behavioral findings showed that pinosylvin significantly alleviated PTZ-induced anxiety-like behavior by increasing open-arm exploration and reducing closed-arm occupancy. Furthermore, qRT-PCR analysis demonstrated significant downregulation of NF-κB and NLRP3 gene expression compared with the PTZ-treated group, indicating attenuation of seizure-associated neuroinflammation. Collectively, these findings suggest that pinosylvin exerts significant anticonvulsant, anxiolytic, and anti-inflammatory effects, potentially through modulation of GABAergic signaling and suppression of the NF-κB/NLRP3 inflammatory axis. Therefore, pinosylvin may represent a promising natural therapeutic candidate for epilepsy management and warrants further preclinical and clinical investigation.



Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent and unprovoked seizures resulting from abnormal, excessive, and synchronous neuronal discharges in the brain (World Health Organization [WHO], 2024). It is one of the most common and debilitating disorders of the central nervous system, affecting cognitive, psychological, and social well-being and contributing substantially to global morbidity and mortality (Feigin et al., 2025). The etiology of epilepsy is highly heterogeneous and includes genetic predisposition, traumatic brain injury, cerebrovascular diseases, brain tumors, neurodegenerative disorders, central nervous system infections, metabolic abnormalities, developmental defects, and autoimmune conditions (WHO, 2024). At the molecular level, epileptogenesis is associated with an imbalance between excitatory and inhibitory neurotransmission, particularly excessive glutamatergic signaling and impaired γ-aminobutyric acid (GABA)-mediated inhibition. Moreover, oxidative stress, mitochondrial dysfunction, neuronal apoptosis, and neuroinflammation are increasingly recognized as key contributors to seizure generation and progression. Several inflammatory mediators, including nuclear factor-kappa B (NF-κB), nucleotide-binding oligomerization domain-like receptor pyrin domain-containing protein 3 (NLRP3), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β), have been implicated in the pathogenesis of epilepsy and seizure-induced neuronal damage (Zou et al., 2024). Epilepsy represents a major global public health challenge. According to the World Health Organization (2024), approximately 50 million people worldwide are living with epilepsy, making it one of the most prevalent neurological disorders. The Global Burden of Disease Study further estimated that approximately 51.7 million individuals were affected by epilepsy in 2021, with an age-standardized prevalence of 658 cases per 100,000 population and an

increasing disease burden over the last three decades (Feigin et al., 2025). The disorder is associated with substantial socioeconomic consequences, increased risk of premature death, psychiatric comorbidities, and reduced quality of life, particularly in low- and middle-income countries where treatment gaps remain significant (WHO, 2024). Experimental animal models are essential for understanding the pathophysiology of epilepsy and for evaluating potential therapeutic agents. Among these models, pentylenetetrazole (PTZ)-induced seizures are extensively used because of their reliability and close resemblance to human generalized epilepsy (Shimada et al., 2018). PTZ is a chemoconvulsant that acts primarily as a non-competitive antagonist of the GABA_A receptor complex, thereby suppressing inhibitory neurotransmission and inducing neuronal hyperexcitability (Yuskaitis et al., 2021). Repeated administration of subconvulsive doses of PTZ produces a kindling phenomenon characterized by progressive seizure severity, oxidative stress, neuroinflammation, neuronal degeneration, and cognitive impairment (Zou et al., 2024). Consequently, the PTZ model has become a valuable experimental paradigm for investigating the anticonvulsant and neuroprotective properties of novel compounds. Despite remarkable progress in antiepileptic drug development, approximately one-third of patients continue to experience drug-resistant epilepsy (WHO, 2024). Currently available antiepileptic drugs include sodium channel blockers such as carbamazepine, phenytoin, lamotrigine, and lacosamide; GABAergic agents such as diazepam, clonazepam, phenobarbital, and vigabatrin; calcium channel modulators including gabapentin and pregabalin; and broad-spectrum agents such as valproic acid, levetiracetam, topiramate, and perampanel. However, these therapies are frequently associated with adverse effects, including sedation, cognitive impairment, hepatotoxicity, teratogenicity, and behavioral disturbances, necessitating the search for safer and more effective therapeutic alternatives (Feigin et al., 2025).

Pinosylvin (Figure 1) is a naturally occurring stilbenoid polyphenol predominantly isolated from the heartwood of pine species belonging to the genus *Pinus*. Structurally, it is an analog of resveratrol and has attracted considerable attention because of its diverse pharmacological activities. Previous studies have demonstrated that pinosylvin possesses potent antioxidant, anti-inflammatory, antimicrobial (Periferakis et al.,2025) anticancer, cardioprotective, and neuroprotective properties (Xu et al., 2020; Lee et al., 2020; Yun et al., 2021). Mechanistically, pinosylvin has been shown to attenuate oxidative stress by enhancing endogenous antioxidant defenses and to suppress inflammatory signaling pathways, including NF-κB, mitogen-activated protein kinase (MAPK), and NLRP3 inflammasome activation (Yun et al., 2021). Furthermore, pinosylvin has been reported to reduce microglial activation, preserve mitochondrial function, and protect against neuronal injury, suggesting its potential therapeutic utility in neurodegenerative disorders (Lee et al., 2020). However, despite its well-documented antioxidant and neuroprotective effects, the potential anticonvulsant and antiepileptic activities of pinosylvin have not yet been systematically investigated in experimental epilepsy models. To the best of our knowledge, no published study has comprehensively evaluated the effects of pinosylvin against PTZ-induced epilepsy or explored its influence on seizure-associated neuroinflammatory pathways. Therefore, the present study was designed to investigate the anticonvulsant and neuroprotective potential of pinosylvin in a PTZ-induced epileptic mouse model and to elucidate its underlying mechanisms with particular emphasis on oxidative stress and neuroinflammation.

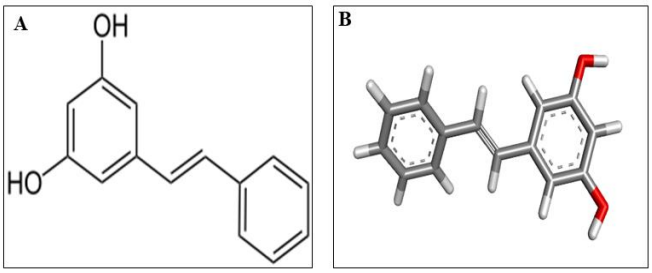


Figure 1: A and B represent 2D and 3D structures of pinosylvin.

Materials and Methods

Chemicals

Chemicals such as pinosylvin and pentylenetetrazole (PTZ) were purchased from Shanghai, China, and were of high analytical purity suitable for experimental use. Other reagents, including dimethyl sulfoxide (DMSO) and chloroform, were acquired from a certified local pharmaceutical supplier and were used directly without any additional purification. All chemicals and reagents utilized in the present investigation were of analytical grade to ensure experimental accuracy, consistency, and reproducibility of results.

Animals

Healthy adult mice (25–30 g body weight) were procured from the National Institutes of Health (NIH), Islamabad. Prior to experimentation, animals were acclimatized to laboratory conditions and housed in standard polypropylene cages, with five animals per cage to maintain appropriate social and environmental conditions. The animals were kept under controlled environmental settings, including a temperature of 25 ± 1 °C, relative humidity of 50 ± 10%, and a 12-hour light–dark cycle. Throughout the study period, standard laboratory food and clean drinking water were provided ad libitum.

PkCSM Analysis

The pharmacokinetic and toxicity profiles of the selected compounds were predicted using the pkCSM online server, which employs graph-based signatures to estimate absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. The canonical SMILES of each compound were retrieved from the chemical database and uploaded to the pkCSM web server for analysis. Parameters including intestinal absorption, blood-brain barrier permeability were evaluated. The predicted ADMET properties were compared to assess the drug-likeness and pharmacokinetic suitability of the compounds. The generated data was used to identify compounds with favorable pharmacokinetic characteristics and lower toxicity risks for further investigation.

In silico Study

The three-dimensional structure of the selected ligand was retrieved and visualized using BIOVIA Discovery Studio Visualizer for structural assessment. Protein targets relevant to epilepsy were chosen for molecular docking analysis, and their crystallographic structures were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The selected targets included the GABA receptor (PDB ID: 1KOT) and NF-κB (PDB ID: 4Q3J). Prior to docking, all protein structures were pre-processed to ensure suitability for simulation studies. This included removal of co-crystallized ligands and water molecules, followed by the

addition of polar hydrogen atoms to stabilize the protein structures and optimize hydrogen bonding interactions. The prepared protein files were then saved in PDB format for downstream computational analysis. Molecular docking studies were performed using AutoDock and PyRx software to evaluate the binding affinity and interaction patterns between the ligand and selected protein targets. The strength of binding was assessed on the basis of atomic contact energy (ACE), expressed in kcal/mol. For each protein–ligand complex, the most stable binding pose corresponding to the lowest ACE value was selected for detailed interaction and structural analysis (Morris & Lim., 2008).

In Vivo Experimental Design

A total of experimental mice were randomly divided into four groups, with each group consisting of an equal number of animals (n = 5 per group). Group I served as the normal control and received only physiological saline. Group II was designated as the disease control group and was administered PTZ at a dose of 35 mg/kg intraperitoneally, to induce epileptic seizures. Group III received pinosylvin at a dose of 40 mg/kg administered intraperitoneally, and served as the treatment group to evaluate its potential anticonvulsant and neuroprotective effects against PTZ-induced seizures. Group IV received diazepam as the standard reference drug at an established anticonvulsant dose administered intraperitoneally and served as the positive control group. All treatments were administered prior to PTZ, with a fixed pretreatment interval to ensure optimal pharmacological activity. PTZ was used to induce acute seizure activity in all groups except normal control. Drug solutions were freshly prepared throughout the experimental period to maintain stability and accuracy of dosing. The study was conducted under consistent experimental conditions to ensure reproducibility and reliability of results (Tawfik et al., 2018).

Behavioral Investigations

Mice were individually placed at the center of an elevated plus maze apparatus consisting of two open and two closed arms elevated 50 cm above the floor and allowed to explore freely for 5 minutes under controlled ambient conditions. The test was conducted in a quiet, dimly lit room to minimize external stress and variability in behavior. Each animal was positioned facing an open arm at the start of the trial, and behavior was recorded using a video tracking system. Parameters including time spent in open and closed arms and number of entries into each arm were measured as indices of anxiety-like behavior (Walf & Frye 2007).

Real Time Polymerase Chain Reaction

Total RNA was isolated from brain tissue samples using a commercially available extraction kit in accordance with the manufacturer's instructions. The purified RNA was subsequently reverse transcribed to generate complementary DNA (cDNA). Quantitative real-time PCR (qRT-PCR) was performed using gene-specific primers in combination with SYBR Green master mix on a real-time PCR system. Gene expression levels were normalized to the housekeeping gene β-actin. Relative quantification of target gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Amada et al., 2013).

Statistical Analysis

Cognitive data were presented as the mean ± standard deviation (SD). Statistical analyses were conducted using SPSS software version 25, while graphs were generated using GraphPad Prism version 9.5.0. Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) post hoc test for multiple comparisons. A p-value < 0.05 was considered indicative of statistical significance.

Results

ADMET Profiling

pkCSM prediction results showed that the pinosylvin demonstrated good intestinal absorption (89.223%) and high Caco-2 permeability (1.057 log Papp),

indicating favorable oral bioavailability. The compound exhibited moderate water solubility (-2.892 log mol/L) and low skin permeability (-2.735 log Kp). It was predicted to be neither a substrate nor an inhibitor of P-glycoprotein, suggesting a lower risk of transporter-mediated drug interactions. Distribution studies showed moderate blood-brain barrier penetration (log BB = -0.423). Furthermore, the compound was predicted to lack significant interactions with major CYP450 enzymes, indicating a potentially low risk of metabolism-related drug-drug interactions (Figure 2).

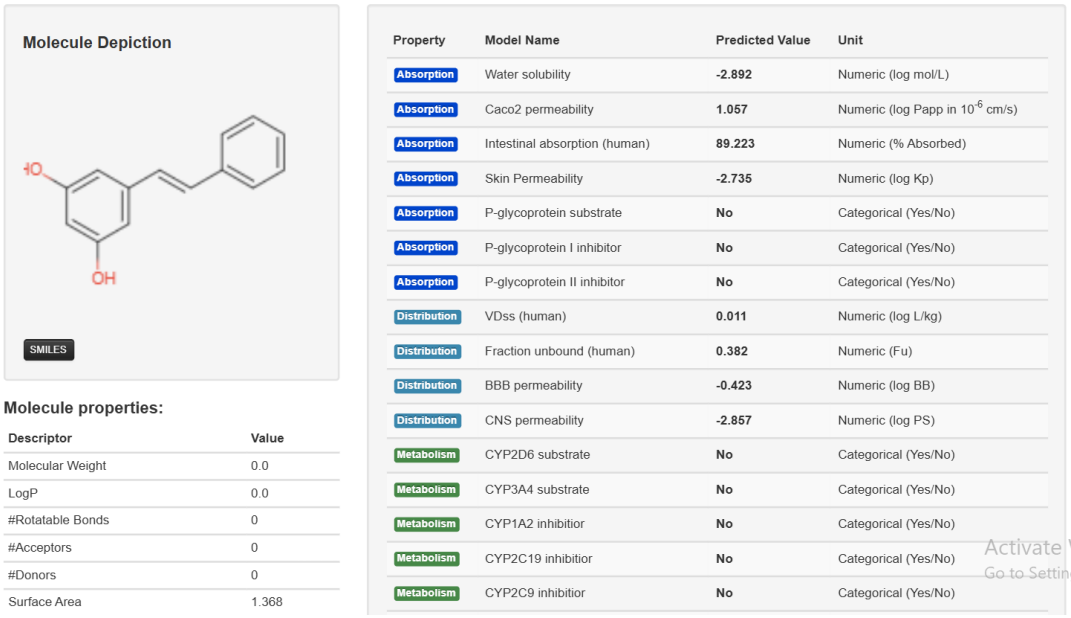


Figure 2: pkCSM Analysis of pinosylvin.

Docking Evaluation

Molecular docking studies demonstrated that pinosylvin possesses a strong binding affinity toward the selected neurobiological targets. The compound exhibited a binding energy of −6.1 kcal/mol against GABA receptors, indicating the formation of a stable ligand–protein complex. Likewise, pinosylvin showed a binding energy of −6.6 kcal/mol with NF-κB, suggesting a good biologically relevant interaction (Table 1). Detailed interaction analysis revealed favorable binding conformations stabilized by several key intermolecular interactions within the active binding pockets of the target proteins. Collectively, these findings suggest that pinosylvin may serve as a promising therapeutic candidate for epilepsy (Figure 3 and 4).

Receptor	PDB ID	Compound	Energy Values Kcal/mol
GABA	1KOT	Pinosylvin	-6.1
NFkB	4Q3J	Pinosylvin	-6.6

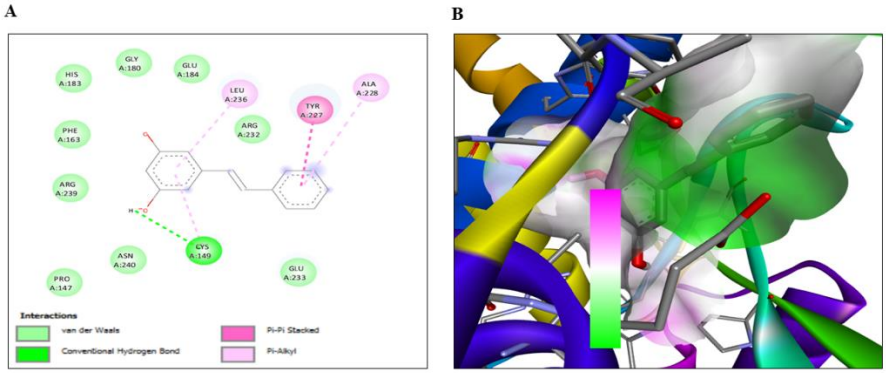


Figure 3: A and B represent 2D and 3D interaction of pinosylvin with NFkB receptor.

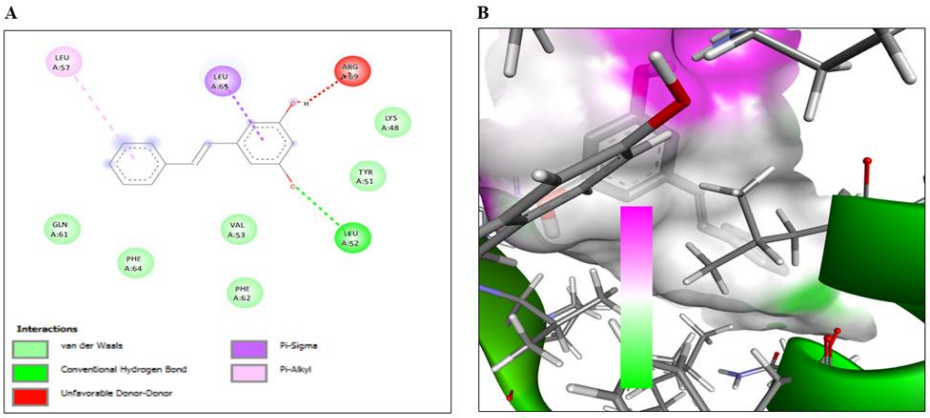


Figure 4: A and B represent 2D and 3D interaction of pinosylvin with GABA receptor.

Elevated Plus Maze

In the Elevated Plus Maze (EPM) test, the PTZ-treated group exhibited a significant reduction in time spent in the open arms and a corresponding increase in time spent in the closed arms compared with the saline-treated control group (###$p < 0.001$), indicating enhanced anxiety-like behavior. Administration of pinosylvin (40 mg/kg) significantly increased the time spent in the open arms (*$p < 0.05$) while reducing the duration in the closed arms (**$p < 0.01$) compared with the PTZ group. Similarly, diazepam treatment produced a more pronounced anxiolytic effect, significantly increasing open-arm exploration (**$p < 0.01$) and decreasing closed-arm occupancy (***$p < 0.001$) relative to PTZ-treated animals. These findings demonstrate that pinosylvin attenuated PTZ-induced anxiety-like behavior, with effects comparable

to the standard anxiolytic drug diazepam (Figure 5).

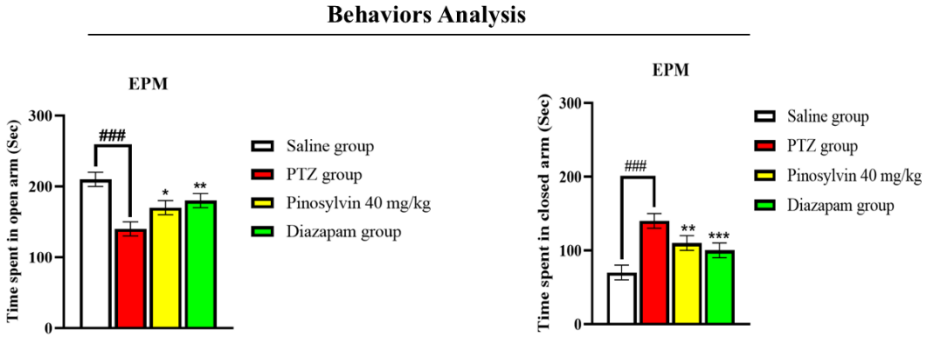


Figure 5: Elevated plus maze test

Gene Expression Analysis

RT-PCR analysis revealed a significant upregulation of NF-κB and NLRP3 mRNA expression in the PTZ-treated group compared with the saline control group (###$p < 0.001$). Treatment with pinosylvin (40 mg/kg) significantly reduced the expression levels of both NF-κB and NLRP3 compared with the PTZ group (**$p < 0.01$). Diazepam administration produced a more pronounced suppression of these inflammatory markers, resulting in significantly lower expression levels than those observed in the PTZ group (***$p < 0.001$). Despite the reduction, the expression of NF-κB and NLRP3 remained higher than that of the saline control group. These findings indicate that pinosylvin attenuates PTZ-induced neuroinflammation through the downregulation of NF-κB and NLRP3 signaling pathways (Figure 6).

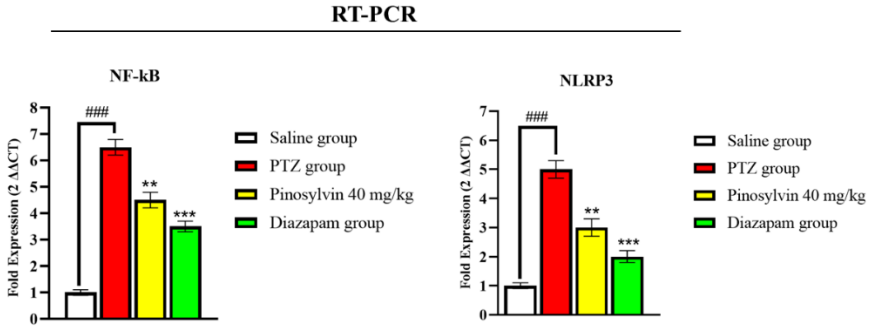


Figure 6: Real Time Polymerase Chain Reaction.

Discussion

Epilepsy is a chronic neurological disorder characterized by recurrent seizures resulting from abnormal neuronal hyperexcitability and synchronization within the central nervous system. Increasing evidence suggests that, beyond neurotransmitter imbalance, oxidative stress and neuroinflammation play pivotal roles in epileptogenesis

and seizure progression. The present study investigated the anticonvulsant and neuroprotective potential of pinosylvin in a PTZ-induced epilepsy model using integrated in silico and in vivo approaches. The findings demonstrated that pinosylvin possesses favorable pharmacokinetic characteristics, exhibits significant binding affinity toward epilepsy-related molecular targets, attenuates PTZ-induced anxiety-like behavior, and suppresses neuroinflammatory gene expression. Collectively, these results suggest that pinosylvin may represent a promising therapeutic candidate for the management of epilepsy and its associated neurological complications.

The initial phase of the study involved ADMET profiling of pinosylvin using the pkCSM platform. Drug-likeness and pharmacokinetic properties are critical determinants of the translational potential of neuroprotective compounds. The present findings revealed high intestinal absorption, good Caco-2 permeability, moderate blood-brain barrier penetration, and minimal interaction with major cytochrome P450 enzymes. These characteristics indicate that pinosylvin possesses favorable pharmacokinetic attributes necessary for central nervous system drug development. Blood-brain barrier permeability is particularly important in epilepsy therapeutics because effective antiepileptic agents must reach adequate concentrations within the brain to exert pharmacological effects. Similar pharmacokinetic characteristics have previously been reported for stilbene derivatives, including resveratrol and pterostilbene, which exhibit neuroprotective efficacy in experimental neurological disorders (Salehi et al., 2018). The observed BBB penetration of pinosylvin therefore supports its potential utility in targeting neuroinflammatory and excitotoxic pathways within epileptic brain tissue. Molecular docking analysis further strengthened the therapeutic rationale for pinosylvin. The compound demonstrated appreciable binding affinities toward both GABA receptors and NF-κB, two key molecular targets implicated in epilepsy pathogenesis. GABAergic dysfunction is considered one of the primary mechanisms underlying seizure generation. PTZ induces seizures primarily through antagonism of GABA_A receptors, resulting in reduced inhibitory neurotransmission and enhanced neuronal excitation (Yuskaitis et al., 2021). The favorable interaction of pinosylvin with GABA receptors observed in the current study suggests that it may partially restore inhibitory signaling disrupted by PTZ administration. Similar findings have been reported for several naturally occurring polyphenols that exert anticonvulsant effects through modulation of GABAergic neurotransmission (Khan et al., 2023). Furthermore, pinosylvin exhibited strong binding affinity toward NF-κB, a transcription factor that regulates the expression of numerous pro-inflammatory cytokines and inflammatory mediators. Activation of NF-κB has been consistently associated with seizure-induced neuroinflammation, neuronal damage, and disease progression in epilepsy (Vezzani et al., 2019). Therefore, inhibition of NF-κB signaling may represent a key mechanism through which pinosylvin exerts neuroprotective effects. Behavioral evaluation using the Elevated Plus Maze demonstrated significant anxiety-like behavior in PTZ-treated animals, as evidenced by reduced time spent in open arms and increased time spent in closed arms. Anxiety disorders frequently coexist with epilepsy and significantly impair quality of life in affected individuals. Experimental studies have shown that recurrent seizures induce alterations in limbic system structures, including the hippocampus and amygdala, which contribute to the development of anxiety and depressive behaviors (Mazarati et al., 2021). In the present study, treatment with pinosylvin significantly increased open-arm exploration while reducing closed-arm occupancy, indicating anxiolytic-like activity. These effects were comparable to those observed with diazepam, a well-established GABAergic anxiolytic agent. The behavioral improvements observed following pinosylvin administration may be attributed to its interaction with GABAergic pathways, antioxidant properties, and anti-inflammatory effects. Previous investigations have demonstrated that oxidative stress and neuroinflammation contribute substantially to anxiety-related behavioral deficits in

epilepsy models (Devinsky et al., 2018). By attenuating these pathological processes, pinosylvin may help preserve normal neuronal function and emotional regulation. One of the most significant findings of the present investigation was the suppression of NF-κB and NLRP3 gene expression following pinosylvin treatment. Neuroinflammation has emerged as a central mechanism contributing to epileptogenesis and seizure-induced neuronal injury. Among the inflammatory pathways implicated in epilepsy, the NF-κB/NLRP3 signaling axis has received considerable attention. Activation of NF-κB promotes transcription of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, while simultaneously facilitating activation of the NLRP3 inflammasome (Swamy et al., 2024). The NLRP3 inflammasome subsequently mediates maturation and release of IL-1β and IL-18, amplifying neuroinflammatory responses and exacerbating neuronal dysfunction. Elevated expression of NF-κB and NLRP3 has been documented in both experimental and clinical epilepsy, where their activation correlates with seizure severity and neuronal loss (Zou et al., 2024). The current study demonstrated significant upregulation of NF-κB and NLRP3 expression in PTZ-treated animals, confirming the involvement of inflammatory pathways in seizure-induced pathology. Importantly, pinosylvin treatment markedly reduced expression of both genes, indicating potent anti-inflammatory activity. These observations are consistent with previous studies demonstrating that pinosylvin suppresses NF-κB activation and inhibits NLRP3 inflammasome assembly in inflammatory and neurodegenerative disease models (Yun et al., 2021). The anti-inflammatory effects of pinosylvin may be particularly relevant in epilepsy because chronic neuroinflammation contributes not only to seizure generation but also to neuronal degeneration and cognitive decline. Therefore, attenuation of NF-κB/NLRP3 signaling may provide both anticonvulsant and neuroprotective benefits. The neuroprotective effects observed in the present study may also be linked to the well-established antioxidant properties of pinosylvin. Oxidative stress is a major contributor to seizure-induced neuronal damage and is characterized by excessive production of reactive oxygen species, lipid peroxidation, mitochondrial dysfunction, and depletion of endogenous antioxidant defenses. Previous investigations have demonstrated that pinosylvin enhances antioxidant enzyme activity, reduces reactive oxygen species generation, and preserves mitochondrial integrity under pathological conditions (Lee et al., 2020). Since oxidative stress and neuroinflammation are closely interconnected processes, the antioxidant activity of pinosylvin may indirectly suppress inflammatory signaling pathways and protect neurons from seizure-induced injury. This dual antioxidant and anti-inflammatory mechanism may explain the broad neuroprotective profile observed in the present investigation. The findings of this study are further supported by previous reports describing beneficial effects of structurally related stilbenoids in neurological disorders. Resveratrol, a close structural analog of pinosylvin, has demonstrated anticonvulsant, anti-inflammatory, and antioxidant effects in various epilepsy models through modulation of oxidative stress pathways and inflammatory mediators (Castino et al., 2019). Given the structural similarity between these compounds, it is plausible that pinosylvin exerts comparable biological activities. However, the present study provides novel evidence specifically demonstrating the efficacy of pinosylvin in PTZ-induced epilepsy and identifying NF-κB and NLRP3 as potential molecular targets. Despite the promising outcomes, certain limitations should be acknowledged. The study primarily focused on behavioral assessments and inflammatory gene expression without evaluating additional biochemical markers of oxidative stress, neurotransmitter alterations, or histopathological changes. Future investigations should explore the effects of pinosylvin on antioxidant enzymes, neuronal survival, synaptic plasticity, and cognitive performance. Moreover, detailed mechanistic studies involving protein expression analysis and signaling pathway characterization would provide further insight into the molecular basis of its anticonvulsant activity. Long-term studies and clinical investigations are also necessary to determine its safety, efficacy, and

translational potential in human epilepsy.

Conclusion

The present study demonstrates that pinosylvin possesses significant anticonvulsant, anxiolytic, and anti-inflammatory properties in a PTZ-induced epilepsy model. The compound exhibited favorable pharmacokinetic characteristics, strong interactions with GABA receptors and NF-κB, improved anxiety-related behavioral outcomes, and effectively suppressed NF-κB and NLRP3 gene expression. These findings suggest that modulation of neuroinflammatory signaling pathways, together with potential restoration of inhibitory neurotransmission, may underlie the neuroprotective effects of pinosylvin. Therefore, pinosylvin emerges as a promising natural therapeutic candidate for epilepsy and warrants further investigation for potential clinical application.

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