

GABAergic Modulation and Antioxidant Shielding by Karanjin from *Pongamia pinnata*: Mechanistic Insights into the Attenuation of Seizure-Induced Hippocampal Damage

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Abstract

Epilepsy, being a complex neurological disorder, is considered to result from an imbalance between GABAergic inhibition and glutamatergic excitation, leading to recurrent seizures, oxidative stress, and hippocampal neurodegeneration. This study investigated the anticonvulsant efficacy of Karanjin (a lipophilic furanoflavonoid from *Pongamia pinnata*), its synergistic interactions with standard anti-epileptic drugs (AEDs), and its neuroprotective effects against oxidative damage and hippocampal cell death. Anticonvulsant activity of high-purity Karanjin (97.25% by HPLC) was evaluated in male Swiss albino mice using the Maximal Electroshock and Pentylenetetrazol models at oral doses of 25, 50, and 100 mg/kg. Synergy studies analyzed fixed-ratio co-administration arrays of Karanjin with standard AEDs, including Phenytoin, Carbamazepine, Phenobarbitone, and Sodium Valproate. A Flumazenil reversal assay to confirm GABAergic activity and brain biomarker assays (MDA, SOD, CAT, GABA) were used to assess post-seizure neuroprotection, alongside histopathological evaluation of hippocampal subfields. Karanjin significantly reduced the duration of Tonic Hindlimb Extension in the MES model ($p < 0.001$ at 100 mg/kg) and markedly delayed the onset latency of PTZ-induced clonic seizures ($p < 0.001$). It showed supra-additive synergy with standard AEDs (allowing lower AED doses) and positive GABA_A receptor modulation (reversed by Flumazenil). Additionally, Karanjin elevated brain GABA, suppressed MDA, restored SOD and CAT levels ($p < 0.01$), and preserved neuronal architecture in the CA1, CA3, and Dentate Gyrus hippocampal zones. Karanjin exerts robust anticonvulsant efficacy mediated via GABA_A receptor modulation, coupled with significant antioxidant shielding and structural neuroprotection within the hippocampus, presenting a viable multi-target natural candidate for epilepsy therapy.

Keywords: Karanjin, Anticonvulsant, GABA_A Receptor, Oxidative Stress, *Pongamia pinnata*

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Introduction

Epilepsy affects over 50 million people globally, imposing a substantial socioeconomic burden in low- and middle-income countries. The disorder is defined by a persistent susceptibility to spontaneous, recurring seizures triggered by synchronized, high-frequency neuronal firing. Ictogenesis is primarily driven by an imbalance between neural excitation and inhibition, typically involving reduced GABA-mediated inhibitory activity or heightened glutamatergic signaling. Although over 20 anti-seizure medications (ASMs) are currently available, roughly 30% of affected individuals experience drug-resistant epilepsy (DRE), remaining vulnerable to uncontrolled seizures and cumulative neuropathological damage. Additionally, long-term use of standard ASMs is frequently constrained by adverse side effects, including cognitive impairment, liver toxicity, and dose-dependent neurotoxicity, emphasizing the critical demand for novel therapeutic options with superior safety profiles^{1,2,3}.

Ethnopharmacological repositories offer valuable platforms for discovering safer neuroprotective compounds. *Pongamia pinnata* (L.) Pierre (Karanja), an established medicinal plant in Ayurvedic and Siddha traditions, is historically utilized for fevers, inflammatory conditions, and CNS disorders. Crude seed extracts of *P. pinnata* have demonstrated broad-spectrum anticonvulsant activity, an effect largely attributed to its lipophilic furanoflavonoids. The primary crystalline bioactive compound isolated from *P. pinnata* seed oil is Karanjin (4-methoxy-7-phenylfuro[3,2-g] chromen-5-one). While Karanjin has demonstrated clear anti-inflammatory, gastroprotective, and anti-proliferative activities, its precise therapeutic mechanisms against acute seizure activity require further empirical clarification. Early computational modeling and

neurobehavioral observations indicate that furanoflavonoid structures effectively cross the blood-brain barrier to modulate central neurotransmitter systems, specifically targeting the GABA_A receptor complex^{4,5,6}.

Severe seizure activity causes a surge in intracellular calcium, triggering mitochondrial impairment and an overproduction of reactive oxygen species (ROS). This induced oxidative stress depletes endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), leading to lipid peroxidation and apoptotic cell death within vulnerable hippocampal subregions, including CA1, CA3, and the Dentate Gyrus. Because post-seizure hippocampal degeneration further accelerates epileptogenesis and cognitive dysfunction, effective therapeutic candidates must combine ion channel modulation with antioxidant protection^{7,8}. To address these mechanisms, this study evaluated high-purity crystalline Karanjin isolated from *Pongamia pinnata* seeds. The research assessed Karanjin's efficacy in maximal electroshock (MES) and pentylenetetrazol (PTZ) seizure models and investigated its role as a positive modulator of the GABA_A receptor benzodiazepine site via a Flumazenil antagonism assay. Additionally, the study explored synergistic dose-reduction potential by testing fixed-ratio combinations with standard anti-seizure medications (Phenytoin, Carbamazepine, Phenobarbitone, and Sodium Valproate). Finally, central GABA replenishment, oxidative stress markers, structural protection across hippocampal zones (CA1, CA3, Dentate Gyrus), and baseline neurobehavioral safety were quantified⁹.

1.1. Materials and Methods

1.1.1. Isolation and Purification of Test Compound

Pulverized seeds (3250 g) of authenticated *Pongamia pinnata* (L.) were extracted using petroleum ether (60–80°C) in a Soxhlet apparatus to yield 447 g of oil (13.75% yield). Chilled methanolic partitioning of the oil, followed by overnight refrigeration at 4°C, facilitated the precipitation of crude Karanjin crystals^{10,11,12}. The crude product was purified via silica gel (60–120 mesh) column chromatography using a petroleum ether/ethyl acetate gradient elution. Monitoring via TLC (silica gel 60 plates, mobile phase: chloroform: methanol 9:1 v/v under UV light (254 nm). Target fractions with matching R_f values (R_f ≈ 0.60) were combined, concentrated, and recrystallized from methanol to yield pure, off-white Karanjin crystals (7.152 g, 1.6% yield based on seed powder)¹³.

1.1.2. Experimental Animals and Dosing Framework

Healthy male Swiss albino mice (25–30 g, 3–4 months old) were housed in polypropylene cages under controlled room conditions (22±2°C, 55±5% relative humidity, 12-hour light/dark cycle) with ad libitum food and water. All procedures were executed in accordance with CPCSEA rules and Institutional Animal Ethics Committee (IAEC) guidelines¹⁴. Fasted female Swiss albino mice received stepwise single oral doses of Karanjin (5, 50, 300, and 2000 mg/kg) suspended in 0.5% w/v sodium carboxymethyl cellulose (CMC) via gastric gavage (10 mL/kg). Animals were evaluated for behavioral, autonomic, and neurological toxicity over 14 days, with body weights and food intake tracked on days 0, 7, and 14¹⁵. Animals were randomized into treatment groups (*n* = 6 per group) for behavioral and seizure evaluations. Test treatments were suspended in 0.5% w/v CMC and delivered orally (p.o.) at 10 mL/kg, whereas reference standard drugs were delivered intraperitoneally (i.p.)¹⁶. Total animals were divided into five experimental groups, group I was treated with 0.5% w/v CMC (10 mL/kg, p.o.), group II was treated with phenytoin (25 mg/kg, i.p. for MES) / diazepam (4 mg/kg, i.p. for PTZ), group III received Karanjin (25 mg/kg, p.o.), group IV received Karanjin (50 mg/kg, p.o.) and finally group V received Karanjin (100 mg/kg, p.o.).

1.1.3. Acute Seizure Models

1.2.3.1. Maximal Electroshock (MES) Model: Evaluated efficacy against generalized tonic-clonic seizures and seizure propagation. Auricular/corneal electrodes moistened with 0.9% saline delivered an electrical pulse (150 mA/50–60 Hz for 0.2 seconds) 45–60 minutes after oral dosing or 30 minutes post-i.p. injection. The duration (seconds) of Tonic Hindlimb Extension (THLE, limbs extended parallel or >90° relative to the body axis) was recorded. Complete abolition of THLE was defined as protection, and percentage protection per group was calculated^{17,18}.

1.2.3.2. Pentylenetetrazol (PTZ)-Induced Seizure Model: Evaluated protection against chemical excitation, as well as myoclonic and absence seizures. Forty-five minutes after oral Karanjin or 30 minutes after i.p. Diazepam, mice received a convulsive dose of PTZ (80 mg/kg, s.c. or i.p.). Parameters monitored during a 30-minute observation window included clonic seizure latency (time to the first myoclonic jerk) and the total duration of continuous clonic-tonic convulsions^{19,20}.

1.1.4. Combination, Synergy, and Antagonism Assessments

1.2.4.1. Fixed-Ratio Synergy Arrays: Pharmacodynamic interaction was executed using fixed-ratio combinations (1:3, 1:1, and 3:1) of Karanjin paired with standard anti-epileptic drugs (phenytoin, phenobarbitone, carbamazepine for MES; sodium valproate for PTZ) across groups (*n* = 6). Experimental duration of THLE (HLTE_{EXP}) or latency to clonic onset (Onset_{EXP}) was compared directly against mathematically simulated additive baselines (HLTE_{ADD} or Onset_{ADD}) to establish synergistic, additive, or antagonistic profiles via isobolographic and dose-response curve mapping^{21,22}.

1.2.4.2. Flumazenil Antagonism Assay: To confirm GABA_A receptor mediation, mice received the selective GABA_A antagonist Flumazenil (5–10 mg/kg, i.p.) 15 minutes before oral Karanjin (50–100 mg/kg, p.o.) or Diazepam (1 mg/kg, i.p.). Following a PTZ challenge (80 mg/kg) 45 minutes later, clonic seizure latency, convulsion duration, and survival rates were quantified to evaluate receptor-level blockade^{23,24}.

1.1.5. Neuro-Behavioral and Motor Coordination Evaluation

1.2.5.1. Motor Coordination (Rota-Rod Test): Mice pre-trained to maintain balance on a rotating cylinder (20 rpm, 180 seconds baseline cutoff) were evaluated every 15 minutes up to 90 minutes post-treatment with vehicle, Diazepam (1 mg/kg), or Karanjin (25, 50, 100 mg/kg). Fall latencies were recorded to assess skeletal muscle relaxation, ataxia, and motor impairment²⁵.

1.2.5.2. Locomotor and Exploratory Activity (Open Field Test): Sedative effects were assessed 60 minutes post-treatment during a 5-minute session in a 16-square grid acrylic apparatus (72 x 72 x 72 cm). Parameters measured included line crossings (horizontal ambulation), rearing frequency (vertical exploration), and grooming duration²⁶.

1.2.5.3. Anxiolytic Activity (Elevated Plus-Maze Test): Evaluated 60 minutes post-dosing using an elevated maze featuring two open (50 x 10 cm) and two closed arms (50 x 10 x 40 cm). Open/closed arm entry counts and total duration spent in each arm during a 5-minute exploration window were quantified²⁷.

1.2.5.4. Protective Index (PI) Determination: The therapeutic safety window of Karanjin relative to classic anti-epileptic drugs was quantified using the formula:

$$\text{Protective Index (PI)} = \frac{\text{TD50 (Median Toxic Dose via Rota-Rod)}}{\text{ED50 (Median Effective Dose via MES/PTZ)}}$$

1.1.6. Biochemical Neurotransmitter and Oxidative Stress Profiling

1.2.6.1. Tissue Processing and Preparation: Following behavioral testing, animals were euthanized, and brain tissues (whole brain or isolated hippocampus/cerebral cortex) were harvested, weighed, and homogenized (10% w/v) in ice-cold 0.1 M phosphate-buffered saline (pH 7.4). Homogenates were centrifuged at 10,000 x g for 15 minutes at 4°C to harvest the supernatant, and total protein concentrations were quantified using a Bicinchoninic Acid (BCA) assay against a bovine serum albumin (BSA) standard curve²⁸.

1.2.6.2. GABA Quantification: Synaptic membranes were isolated through differential centrifugation (1,000 x g for 10 minutes followed by 20,000 x g for 20 minutes for 20 minutes

in 0.32 M sucrose), and central GABA levels were measured using a commercial mouse GABA ELISA kit ²⁹.

1.2.6.3. Lipid Peroxidation (MDA) Assay: Malondialdehyde (MDA) content was determined using the TBARS assay by heating supernatant with 25% trichloroacetic acid (TCA) and 0.67% TBA in a boiling water bath for 20 minutes. Absorbance of the pink chromogen was measured at 532 nm and calculated using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ (expressed as nmol MDA/mg protein) ³⁰.

1.2.6.4. Superoxide Dismutase (SOD) Assay: Enzyme activity was evaluated by measuring the inhibition of nitroblue tetrazolium (NBT) reduction induced by phenazine methosulfate (PMS) and NADH. Reactions were quenched after 1 minute using glacial acetic acid, absorbance was read at 560 nm, and activity was expressed as units of SOD/mg protein ³¹.

1.2.6.5. Catalase (CAT) Assay: Activity was evaluated by tracking the decomposition rate of 0.03 M H_2O_2 in phosphate buffer (pH 7.0) at 240 nm over 1 minute. Activity was calculated using an extinction coefficient of $43.6 \text{ M}^{-1}\text{cm}^{-1}$ and expressed as units of CAT/mg protein ³².

1.1.7. Histopathological Mapping of Hippocampal Subfields

Excised brain specimens were fixed in 10% neutral buffered formalin (NBF) for 24-48 hours, dehydrated through a graded ethanol series (70–100%), cleared in xylene, and embedded in paraffin. Coronal hippocampal sections (5-7 μm) were cut with a rotary microtome, mounted, stained with Hematoxylin and Eosin (H&E), and examined under light microscopy (100x and 400 x magnifications). Morphological features, including intact neuronal counts, pyknosis, cytoplasmic vacuolation, and leukocyte infiltration, were semi-quantitatively scored on a 0-4 severity scale across the CA1, CA3, and Dentate Gyrus (DG) subfields ³³.

1.2.8. Statistical Analysis

Data were expressed as Mean $\pm$ Standard Error of the Mean (SEM) ($n = 3$ for analytical procedures; $n = 6$ for animal protocols). Parametric statistical evaluations were conducted using One-Way Analysis of Variance (ANOVA) followed by Dunnett's post hoc multiple comparison tests. Thresholds of $p < 0.05$, $p < 0.01$, and $p < 0.001$ were defined as statistically significant. All calculations and graphical plots were generated using GraphPad Prism software.

1.2. Results and Discussion

1.2.1. Isolation, Characterization, Purity Assessment

Extraction of coarse *Pongamia pinnata* seed powder yielded 447 g of seed oil (13.75% w/w). Cold methanolic partitioning followed by low-pressure silica gel column chromatography successfully yielded 7.152 g of pure Karanjin (1.6% w/w based on dry seed mass). The isolated off-white crystalline compound exhibited a sharp melting point of $151.6 \pm 0.6^\circ\text{C}$, aligning closely with literature reference values for pure Karanjin.

1.2.2. Acute Oral Toxicity and Dose Selection

In the OECD 423 acute oral toxicity protocol, administration of Karanjin at doses up to 2000 mg/kg produced no mortality, signs of autonomic instability, or behavioral toxicity in Swiss albino mice over 14 days. Body weights and feed consumption remained comparable to control animals. Consequently, the median lethal dose (LD_{50}) was categorized as $> 2000 \text{ mg/kg}$

(GHS Category 5/unclassified). Based on these safety data, sub-toxic working doses of 25, 50, and 100 mg/kg (p.o.) were chosen for subsequent in vivo evaluations.

1.2.3. Evaluation of Anticonvulsant Efficacy in Acute Seizure Models

1.3.3.1. Maximal Electroshock (MES) Model: Pretreatment with Karanjin significantly suppressed transcranial shock-induced seizures in a dose-dependent manner (Figure 1).

Table 1. Quantitative Assessment of Karanjin and Phenytoin on MES-induced seizures

Treatm ent Group	Dose (mg/k g)	MES: THLE Duration (s)	Animals Protected (Protected/T otal)	% Protecti on
Vehicle Control	0.5% CMC (p.o.)	12.20 ± 0.72	0/6	0%
Phenyt oin (MES)	25 (i.p)	0.00 ± 0.00 ***	6/6	100%
Karanji n (Low)	25 (p.o.)	9.80 ± 0.55 *	2/6	33.3%
Karanji n (Mid)	50 (p.o.)	7.15 ± 0.38 **	4/6	66.7%
Karanji n (High)	100 (p.o.)	4.25 ± 0.32 ***	5/6	83.3%

*Values are Mean $\pm$ SEM ($n=6$ per group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to Vehicle Control.

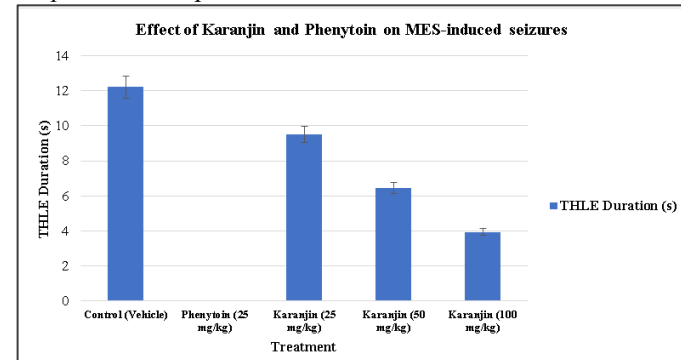


Figure 1. Dose-dependent effects of Karanjin and Phenytoin on MES-induced seizure models

Vehicle-treated mice exhibited prolonged tonic hindlimb extension (THLE = $12.20 \pm 0.72 \text{ s}$). Oral administration of Karanjin at 50mg/kg and 100 mg/kg significantly reduced THLE duration to $7.15 \pm 0.38 \text{ s}$ ($p < 0.01$) and $4.25 \pm 0.32 \text{ s}$ ($p < 0.001$), conferring 66.7% and 83.3% group protection, respectively. Complete abolition of THLE (100% protection) was achieved by the positive reference standard Phenytoin (25 mg/kg, i.p.). The dose-dependent reduction and suppression of hindlimb extension indicate that Karanjin effectively inhibits the propagation of seizure activity across cortical and subcortical neuronal networks ³⁴.

1.3.3.2. Pentyleneetetrazol (PTZ) Model: In the PTZ-induced chemoconvulsant model, Karanjin produced a significant, dose-dependent delay in the onset latency of myoclonic jerks and clonic seizures.

Table 2. Quantitative Assessment of Karanjin and Diazepam on PTZ-induced seizures

Treatm ent Group	Dose (mg/k g)	PTZ: Latency to Jerk/Clo nus (s)	Animals Protected (Protected/T otal)	% Protecti on
Vehicle Control	0.5% CMC (p.o.)	99.15 ± 4.35	0/6	0%
Diazepa m (Std)	4 (i.p)	338.15 ± 11.45** *	6/6	100%
Karanjin (Low)	25 (p.o.)	135.35 ± 6.30*	2/6	33.3%
Karanjin (Mid)	50 (p.o.)	185.55 ± 7.85**	4/6	66.7%
Karanjin (High)	100 (p.o.)	229.15 ± 9.80***	5/6	83.3%

*Values are Mean ± SEM (n=6 per group). **p*<0.05, ***p*<0.01, ****p*<0.001 compared to Vehicle Control.

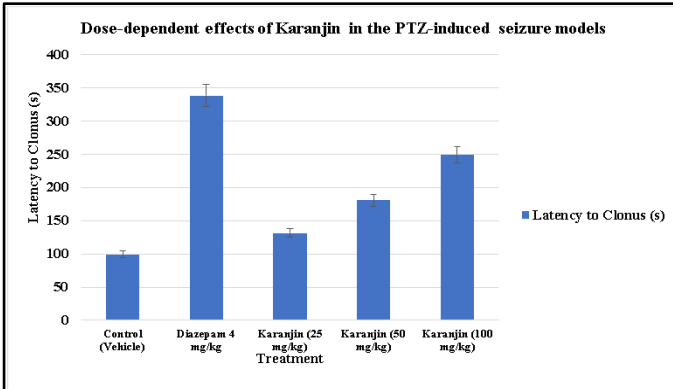


Figure 2. Dose-dependent effects of Karanjin in the PTZ-induced seizure models

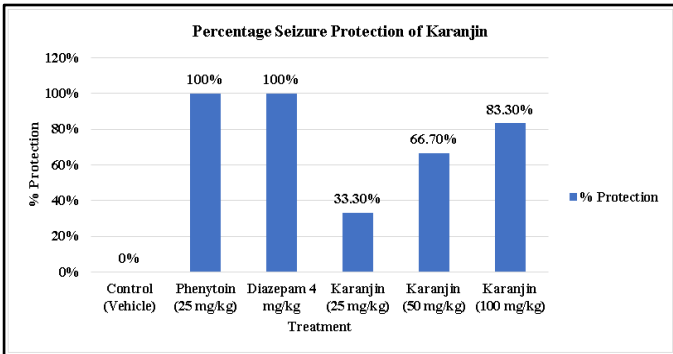


Figure 3. Percentage seizure protection by Karanjin
Mice pretreated with high-dose Karanjin (100 mg/kg) exhibited a marked prolongation of seizure onset latency (229.15±9.80 s vs 99.15±4.35 s in vehicle control, *p* < 0.001) along with a substantial reduction in the total duration of continuous clonic-tonic convulsions, yielding 83.3% protection. Full protection (100%) and complete abolition of PTZ-induced mortality were achieved by the positive control Diazepam (4 mg/kg, i.p.). Because PTZ acts primarily as a competitive antagonist at the GABA_A receptor complex, the significant protection against PTZ-induced convulsions indicates that Karanjin directly or

indirectly enhances central GABAergic inhibitory neurotransmission, or blocks T-type calcium currents ³⁵.

1.3.4. Synergy Profiling and Receptor Mechanism
1.3.4.1. Fixed-Ratio Synergistic Interactions with Standard ASMs

To explore the adjuvant potential of Karanjin, fixed-ratio co-administration arrays (1:3, 1:1, and 3:1) were evaluated with standard anti-epileptic drugs (Phenytoin, Phenobarbitone, Carbamazepine, and Sodium Valproate). Co-administration of sub-threshold doses of Karanjin with Sodium Valproate produced marked supra-additive protection against PTZ-induced seizures (Table 3).

Table 3. Interaction profile of Karanjin and Sodium Valproate combinations on PTZ-induced seizure latency and protection metrics

Treatmen t Group	Fixe d Rati o	Experimen tal Latency (Latency _{EXP} , s)	Calculate d Additive Latency (Latency _{ADD} , s)	% Seizure Protecti on
Vehicle Control	-	82 ± 9	-	0
Karanjin monothera py	-	118 ± 11	-	16.7
Sodium Valproate monothera py	-	248 ± 18	-	66.7
Karanjin + Valproate	1:3	270 ± 20*	215.5	83.3
Karanjin + Valproate	1:1	312 ± 22***	183.0	100
Karanjin + Valproate	3:1	285 ± 19**	150.5	83.3

Values are expressed as Mean ± SD (n=6). *p* < 0.05**, *p* < 0.01***, *p* < 0.001 comparing Latency_{EXP} against Latency_{ADD} using an unpaired t-test*

Across all evaluated combination proportions, experimental clonic onset latencies (Latency_{EXP}, s) significantly exceeded the calculated theoretical additive baselines (Latency_{ADD}, s). The optimal 1:1 fixed-ratio combination achieved 100% protection and extended onset latency to 312 ± 22s (*p* < 0.001 relative to additive baseline). This potent supra-additive interaction indicates that Karanjin could serve as an effective clinical adjuvant, facilitating substantial dose reductions of conventional anti-seizure medications while maintaining or improving overall therapeutic efficacy and mitigating dose-dependent toxicities ²².

1.3.4.2. Flumazenil Antagonism and GABA_A Receptor Mechanism:

To confirm whether the anticonvulsant activity of Karanjin is directly mediated via the GABA_A-benzodiazepine receptor complex, a competitive reversal assay was performed using the selective antagonist Flumazenil.

Table 4. Effect of Flumazenil on Karanjin- and Diazepam-mediated seizure protection in the PTZ chemoconvulsant model

Group	Seizure Latency (sec)	Seizure Duration (sec)	Quantified Protection (%)	Functional Outcome
Vehicle control	62.0 ± 8.0	198.0 ± 21.0	0	Baseline Seizure Activity
Diazepam (1 mg/kg)	176.0 ± 16.0***	85.0 ± 10.0***	83	Potent GABA _A Modulation
Flumazenil (10mg/kg) + Diazepam	70.0 ± 8.0###	182.0 ± 20.0###	8	Complete Blockade of Standard
Karanjin (100 mg/kg)	265.4±13.6***	18.2±2.4***	83.3	Marked Anticonvulsant Activity
Flumazenil + Karanj	120.5±9.8##	45.6±4.2###	16.7	Significant Reversal of Protection

Values are expressed as Mean ± SEM (n=6). **p* < 0.001 compared directly to the Vehicle Control group; ###*p* < 0.001 compared directly to the respective non-antagonized treatment group (Group II or Group IV).

Pretreatment with Flumazenil (10 mg/kg, i.p.) significantly reversed the neuroprotective effects of Karanj (100 mg/kg, p.o.), reducing seizure latency from 265.4±13.6s to 120.5±9.8s (*p* < 0.001) and lowering total protection from 83.3% to 16.7%. A parallel blockade was observed in the Diazepam reference group (83% protection reduced to 8%). These findings confirm that the anticonvulsant action of Karanj is driven, at least in part, through positive allosteric modulation at the benzodiazepine binding site of the central GABA_A receptor complex²⁴.

1.3.4.3. Neuro-Behavioral Dynamics and Motor Safety Evaluation

1.3.4.3.1. Motor Coordination and Muscle Relaxation (Rota-Rod Test): In the Rota-Rod assay, Karanj showed negligible motor impairment across all tested therapeutic doses. **Table 5. Effect of Karanj and Diazepam on Motor Coordination in the Rota-Rod Test**

Treatment Group	Dose (mg/kg)	Time Spent on Rod (s)	Clinical Observation
Control	0.5% CMC (p.o.)	180.0 ± 0.00	Normal motor coordination
Diazepam (Standard)	1 (i.p)	65.4 ± 8.2***	Significant motor impairment
Karanjin (Low)	25 (p.o.)	180.0 ± 0.0 ^{ns}	No motor impairment
Karanjin (Mid)	50 (p.o.)	180.0 ± 0.0 ^{ns}	No motor impairment

Karanjin (High)	100 (p.o.)	178.5 ± 1.2 ^{ns}	Preserved motor coordination
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Values are Mean ± SEM (n=6). ****p* < 0.001 compared to Control; ^{ns} = non-significant compared to the vehicle control group.

Mice treated with Karanj (25-100mg/kg) maintained full endurance (178.5-180.0 s) without significant fall-off events. In contrast, the standard reference Diazepam (1mg/kg) caused acute motor ataxia, significantly decreasing fall latency to 65.4±8.2 s (*p* < 0.001)³⁵.

1.3.4.3.2. Spontaneous Locomotor Activity (Open Field Test): In the Open Field Test, Karanj showed no significant reduction in horizontal ambulation (grid crossings) or vertical rearing frequency relative to control mice.

Table 6. Effect of Karanj and Diazepam on exploratory and locomotor behavior in the Open Field Test

Treatment Group	Dose (mg/kg)	Ambulation (Grid Crossed)	Rearing (Frequency)	Observation
Control (Saline)	Saline (p.o.)	88.5 ± 4.2	18.4 ± 1.5	Normal Exploratory Activity
Diazepam (Std)	1 (i.p)	32.1 ± 3.4***	5.2 ± 0.8***	Significant central Sedation
Karanjin (Low)	25 (p.o.)	85.2 ± 5.1 ^{ns}	17.1 ± 1.2 ^{ns}	No Sedation
Karanjin (Mid)	50 (p.o.)	82.4 ± 4.8 ^{ns}	16.5 ± 1.4 ^{ns}	No Sedation
Karanjin (High)	100 (p.o.)	79.8 ± 5.5 ^{ns}	15.8 ± 1.1 ^{ns}	No Sedation

Values are mean ± SEM (n=6); **p* < 0.001 compared to the saline control group; ^{ns} = non-significant compared to the saline control group.

While Diazepam (1 mg/kg) induced marked central sedation (reducing grid crossings to 32.1±3.4, *p* < 0.001), Karanj maintained baseline exploratory behavior even at the maximum effective anticonvulsant dose (100 mg/kg).

1.3.4.3.3. Anxiolytic Evaluation (Elevated Plus-Maze Test)

Karanjin exhibited a dose-dependent, anxiety-attenuating effect in the Elevated Plus-Maze Table 7.

Table 7. Anxiety-alleviating potential of Karanj and Diazepam in the Elevated Plus Maze (EPM)

Treatment Group	Dose (mg/kg)	Time Spent in Open Arms (s)	Entries into Open Arms	Behavior Observation
Control	Saline (p.o.)	42.5 ± 3.1	5.2 ± 0.8	Baseline Anxiety
Diazepam	1 (i.p)	115.4 ± 7.5***	12.8 ± 1.2***	Potent Anxiolytic
Karanjin (Low)	25 (p.o.)	45.8 ± 4.2 ^{ns}	5.5 ± 0.9 ^{ns}	No Significant Effect

Karanjin (Mid)	50 (p.o.)	68.2 ±5.4*	7.4 ±1.1*	Mild Anxiolytic
Karanjin (High)	100 (p.o.)	82.6 ±6.2**	9.1 ±1.3**	Moderate Anxiolytic

Values are mean ± SEM (n=6). *p < 0.05, p** <0.01, p*** <0.001 vs Control; ns= Non-significant.

Administration of Karanjin at 50 mg/kg and 100 mg/kg significantly increased both open-arm occupancy (68.2 ± 5.4 s and 82.6 ± 6.2 s, respectively) and entry frequencies (p < 0.05 and p < 0.01), reflecting a moderate, non-sedating anxiolytic profile.

1.3.4.3.4. Therapeutic Safety Window: Protective Index (PI)

The therapeutic window of Karanjin was compared directly to benchmark anti-seizure medications by calculating the Protective Index (PI = TD₅₀ / ED₅₀) (Table 8).

Table 8. Comparative Protective Index (PI) and neurobehavioral safety profile of Karanjin vs. Standard AEDs

Compound / Test Group	Median Effective Dose ED ₅₀ (mg/kg)	Median Toxic Dose TD ₅₀ (mg/kg)	Protective Index (PI)	Neurological Safety Profile
Phenytoin (vs. MES)	12.0	35.0	2.9	Narrow therapeutic index; rapid onset of ataxia
Diazepam (vs. PTZ)	1.3	4.5	3.46	Low safety margin; dose-dependent heavy sedation
Karanjin (vs. MES/PTZ)	42.0	>400.0	> 9.52	Expanded therapeutic window; highly selective

*Note: ED₅₀ and TD₅₀ values for reference standards represent established literature thresholds for neurobehavioral impairment and seizure protection in matching rodent models.

Karanjin demonstrated a Protective Index (PI > 9.52) substantially higher than both Phenytoin (PI = 2.91) and Diazepam (PI = 3.46). This wider safety margin highlights a superior therapeutic index, confirming that Karanjin achieves robust seizure control at doses far below those that induce motor deficits, ataxia, or central nervous system depression³⁴.

1.3.5. Biochemical Neurotransmitter and Anti-Oxidative Responses

1.3.5.1. Brain GABA Modulation: To evaluate the impact of Karanjin on neurochemical dynamics, whole-brain GABA concentrations were quantified following seizure induction.

Table 9. Effect of Karanjin and Diazepam on whole-brain GABA levels in seizure-induced rodent models

Group/Treatment	GABA Concentration (µg/g of wet tissue)	% Change vs. Control	Biochemical Profile
Normal Control	2.42 ±0.18	-	Baseline neurotransmitter tone
Disease Control (Seizure-Induced)	1.15 ±0.11 ^{##}	-52.48% decrease vs Normal control	Severe GABA depletion
Diazepam (4 mg/kg)	2.13 ± 0.15**	+100.87% increase vs Disease Control	Marked GABA restoration
Karanjin (100 mg/kg)	1.98 ±0.14*	+72.17% increase vs Disease Control	Enhanced GABAergic tone

Values are expressed as Mean ± SEM. ^{##}p < 0.01 vs. Normal Control; *p< 0.05, **p < 0.01 vs. Disease Control.

Seizure challenge induced a marked depletion of endogenous brain GABA in the Disease Control group (1.15 ± 0.11µg/g), representing a 52.48% reduction compared to Normal Control levels (2.42 ±0.18µg/g, p < 0.01). Oral administration of Karanjin (100 mg/kg) significantly attenuated this neurochemical deficit, restoring brain GABA levels to 1.98 ±0.14µg/g (+72.17% increase relative to Disease Control, p < 0.05). This elevation aligns with the behavioral protection observed in the PTZ model, confirming that Karanjin enhances central GABAergic inhibitory tone²⁴.

1.3.5.2. Attenuation of Seizure-Induced Oxidative Stress:

Chemoconvulsant and electrical seizure challenges induced severe oxidative stress in brain homogenates, evidenced by elevated lipid peroxidation and significant depletion of endogenous enzymatic defenses (Table 10).

Table 10. Effect of Karanjin and standard AEDs on whole-brain oxidative stress markers (MDA, SOD, and Catalase)

Group	Treatment	MDA Levels (nmol/mg protein)	SOD Activity (U/mg protein)	Catalase Activity (U/mg protein)
Normal control	Saline Baseline	2.18 ± 0.16	9.62 ± 0.54	52.4 ± 2.8
Vehicle control	PTZ/MES Challenge	6.74 ± 0.42 ^{###}	4.18 ± 0.37 ^{###}	23.7 ± 1.9 ^{###}

Standard drug	Phenytoin/Diazepam	2.86 ± 0.21***	8.71 ± 0.49***	47.9 ± 2.5***
Karanjin (100 mg/kg)	Isolated Furanoflavonoid	3.24 ± 0.27*	7.86 ± 0.46*	43.6 ± 2.2*

Values represent Mean ±SEM (n=6). ###p < 0.001 compared directly to the healthy Normal Control group; *p < 0.001 compared directly to the untreated, seizure-induced Vehicle Control group.

Lipid Peroxidation (MDA): Seizure induction caused a 3.1-fold spike in brain malondialdehyde levels (6.74 ± 0.42 nmol/mg) compared to Normal Control (2.18 ± 0.16 nmol/mg, p < 0.001). Treatment with Karanjin (100 mg/kg) suppressed lipid peroxidation by 51.9%, reducing MDA accumulation to 3.24 ± 0.27 nmol/mg (p < 0.001).

Superoxide Dismutase (SOD): Endogenous SOD activity dropped significantly in non-treated seizure controls (4.18 ± 0.37 U/mg) relative to intact controls (9.62 ± 0.54 U/mg). Karanjin administration substantially preserved SOD activity (7.86 ± 0.46 U/mg, p < 0.001).

Catalase (CAT): Catalase activity experienced a 54.8% reduction following seizure challenge (23.7 ± 1.9 U/mg). Karanjin treatment effectively restored CAT activity to 43.6 ± 2.2 U/mg (p < 0.001), approaching the efficacy of standard therapy (47.9 ± 2.5 U/mg).

The anti-oxidative capability of Karanjin is attributed to the electron-donating radical-scavenging dynamics of its furanoflavonoid backbone, mitigating seizure-induced secondary excitotoxic neuronal injury³¹.

1.3.6. Histopathological Evaluation of Hippocampal Architecture

Histopathological examination of Hematoxylin and Eosin (H&E)- stained coronal brain sections from untreated, seizure-challenged control mice revealed extensive neurodegenerative damage across the vulnerable hippocampal subfields, specifically the CA1, CA3, and Dentate Gyrus (DG) regions (Figure 4). Key histopathological hallmarks in vehicle-treated, seizure-challenged brains included widespread pyknotic nuclei (shrunken, hyperchromatic neuronal nuclei), severe cytoplasmic vacuolation, perineuronal edema, microglial activation, and marked loss of structural pyramidal cell layer integrity. Conversely, pretreatment with isolated Karanjin (50-100 mg/kg, p.o.) produced substantial, dose-dependent structural preservation across all evaluated hippocampal subfields. Brain tissue from Karanjin-treated cohorts exhibited preserved neuronal morphology, defined nuclear boundaries, intact cellular alignment, and a marked reduction in apoptotic/pyknotic cell counts.

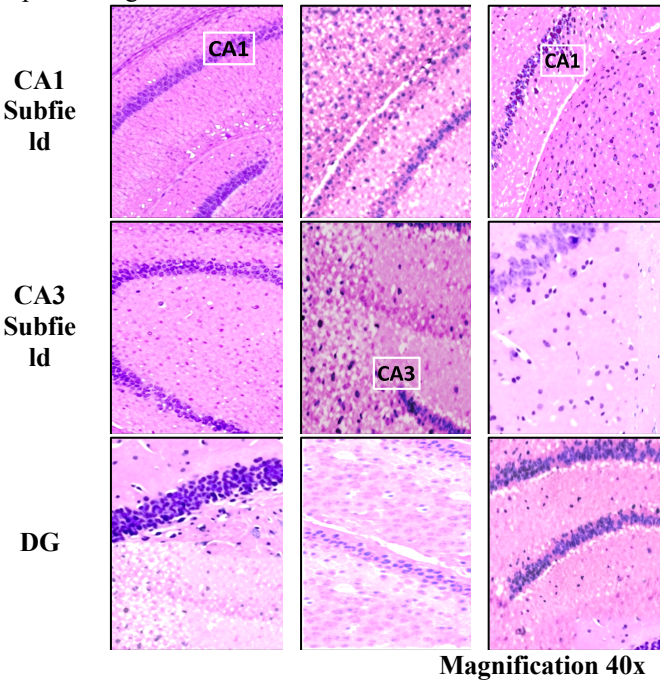
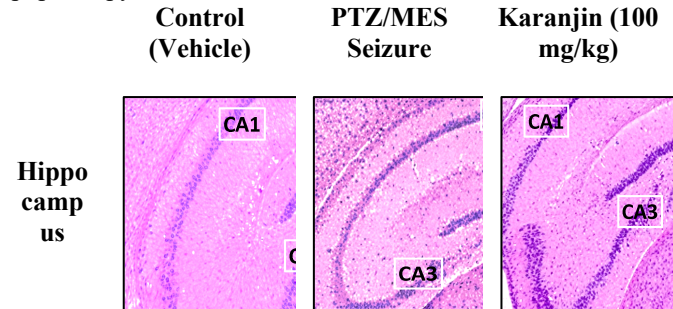


Figure 4. Neuroprotective Effect of Karanjin on PTZ/MES-Induced Histopathological Alterations in Hippocampal CA1, CA3, and DG Subfields

Normal Control displays intact pyramidal neuron arrangement, clear round nuclei, and normal cellular architecture. Vehicle Control - Seizure Challenged shows severe neuropathology characterized by pyknotic/apoptotic neuronal cores (black arrows), cytoplasmic vacuolation, loss of cell layer compaction, and perineuronal edema. Karanjin 100 mg/kg demonstrates significant structural preservation with intact pyramidal cell layers, prominent nucleoli, and minimal pyknotic changes, highlighting robust histological protection alongside functional seizure suppression (p < 0.001)³³.

1.3. Conclusion

The present study establishes that Karanjin, a furanoflavonoid isolated and purified from *Pongamia pinnata* (L.) Pierre seeds, possesses potent broad-spectrum anticonvulsant, neuroprotective, and antioxidant properties with a superior safety profile. High-purity Karanjin was successfully isolated and characterized. Acute oral toxicity evaluation established a wide margin of safety, with an estimated median lethal dose (LD₅₀) exceeding 2000 mg/kg. Karanjin exerted robust, dose-dependent protection against both Maximal Electroshock (MES) and Pentylentetrazol (PTZ)-induced seizure models. Competitive antagonism assays with Flumazenil confirmed that Karanjin's anti-seizure activity is mediated, at least in part, via positive allosteric modulation of the central GABA_A-benzodiazepine receptor complex, demonstrating potential synergy when combined with reference anti-epileptic drugs. Unlike conventional sedatives and anti-epileptic agents, Karanjin maintained normal locomotor activity and motor coordination without inducing ataxia or central sedation. Calculation of the Protective Index (PI > 9.52) highlights an expanded therapeutic window.

Treatment with Karanjin significantly restored endogenous brain GABA levels, attenuated lipid peroxidation (MDA

accumulation), and normalized central antioxidant enzyme defenses (SOD and Catalase). Histopathological analysis corroborated functional outcomes, showing marked preservation of neuronal density and structural integrity across the hippocampal CA1, CA3, and Dentate Gyrus hippocampal subfields. In summary, Karanjin represents a highly promising bioactive furanoflavonoid lead compound for the design and development of novel, safe, and effective broad-spectrum anti-epileptic therapeutic regimens.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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