RESEARCH ARTICLE

Comparative Evaluation of *In-vitro* Cytotoxic and Neuroactive Effects of Zinc Oxide Nanoparticles and *Tribulus terrestris* Extract

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ABSTRACT

Epilepsy is a chronic neurological illness characterized by recurring seizures that is commonly treated with long-term medicine, such as carbamazepine, which might have negative effects. Because of their potential neuroprotective and anticonvulsant properties, alternative medicines derived from medicinal plants and nanotechnology are gaining popularity. The purpose of this work was to examine the anticonvulsant-like characteristics of zinc oxide nanoparticles (ZnO NPs), carbamazepine, and *Tribulus terrestris* ethanolic extract on human neuroblastoma cells SHSY5Y (CRL-2266). *T. terrestris* extract was prepared using Soxhlet extraction and subjected to phytochemical screening. Zinc acetate and *T. terrestris* extract were used to generate ZnO NPs, which were then evaluated by FTIR, XRD, SEM, EDX, UV-visible spectroscopy, and zeta potential analysis. ZnO NPs, carbamazepine, and plant extract were evaluated for cytotoxicity at doses of 20-100 µg/mL using MTT assays. Morphological alterations were detected using microscopy. All test samples demonstrated dose-dependent decrease of cell viability. At 100 µg/mL, ZnO NPs exhibited the highest inhibition (22.79%), followed by *T. terrestris* extract (20.95%) and carbamazepine (11.87%). Morphological changes were noticeable in nanoparticle-treated cells.The data indicate that ZnO nanoparticles and *T. terrestris* extract have potential anticonvulsant capabilities that warrant further research through in vivo and mechanistic studies.

Keywords: *Tribulus terrestris*, ZnO nanoparticle, anti-convulsant activity, XRD, FTIR.

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INTRODUCTION

Natural products and nanomaterials are becoming most popular as a result of the search for novel and effective therapies for neurological conditions like epilepsy[1]. *Tribulus terrestris*, a widely used medicinal herb, has been demonstrated to have several pharmacological properties, including anti-inflammatory, neuroprotective, and antioxidant effects. This study examined the possible anti-convulsant properties of an ethanolic extract of *Tribulus terrestris* leaves using zinc oxide nanoparticles (ZnO NPs) and plant extract[2]. The production of ZnO nanoparticles has drawn interest because of their special qualities, which include large surface area, stability, and bioactivity, making them attractive options for medicinal uses[3].

To prepare the extract, a thorough phytochemical screening was conducted after Soxhlet extraction using petroleum ether, chloroform, and ethanol. The results confirmed the presence of bioactive compounds, including flavonoids, glycosides, alkaloids, and terpenoids[4]. By mixing *Tribulus terrestris* extract with NaOH in a zinc acetate solution, ZnO nanoparticles were synthesized, and their creation was verified by a unique colour change. To evaluate the stability, particle size, and chemical composition of the produced ZnO nanoparticles, FTIR spectroscopy, zeta potential analysis, and UV-vis spectroscopy were used. The anti-convulsant activities of ZnO nanoparticles as well as that of the Ethanol extract were determined side by side employing SHSY5Y CRL-2266 cells, and their viability was determined by the MTT assay. To provide insight into their potential for controlling seizure disorders and aiding in the development of alternative therapies for neurological illnesses, this study compares the possible anticonvulsant effects of ZnO nanoparticles and *Tribulus terrestris* extract[5].

MATERIALS AND METHODS

Preparation of Extracts

Prof. Sanjay S. Sathe confirmed the fresh *Tribulus terrestris* leaves that were collected from Vita, Maharashtra, India, in September 2024. The leaves were cleaned, ground into a powder, put away for later use, and allowed to dry in the shade for two to three weeks. The plant material was then extracted using the Soxhlet technique using petroleum ether, chloroform, and ethanol. The obtained ethanolic extract was analysed for phytochemical and pharmacological screening. Flavonoids, glycosides, alkaloids, proteins, tannins, gums, phenolic compounds, mucilage, saponins, carbohydrates, and terpenoids were all found in the extract using phytochemical screening[6].

Preparation of Nanoparticles

Zinc acetate was mixed in 50 mL of Milli-Q water and stirred for an hour to create a 1 mM solution. After

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progressively adding 20 mL of NaOH to this solution, 25 mL of plant extract was added. The reaction mixture showed a hue shift following an hour of incubation. The effective production of ZnO nanoparticles (ZnO NP) was demonstrated by the appearance of a yellow colour after three more hours of swirling the solution. Centrifugation was used to separate the resultant precipitate for 30 minutes at 60°C and 3500 rpm. A hot air oven was then used to dry the resultant pellet for two hours at 70°C. The ZnO nanoparticles were stored in sealed containers after drying[7].

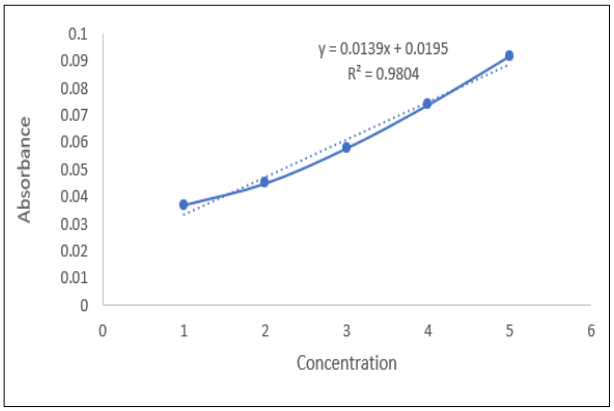


Figure 1: Calibration curve of ZnO nanoparticle

Evaluation of Zinc Oxide Nanoparticles

An analytical instrument known as a Jasco-V-730 UV-vis spectrophotometer A spectrometer. Measurements of absorbance between 200 and 400 nm were performed on a handful of the samples that were chosen for UV-vis analysis[8]. The zeta potential (ZP) makes it easy to determine the stability of colloidal dispersion. The degree of electrostatic repulsion between similarly charged particles in a dispersion is indicated by the ZP[9]. Small molecules and particles are more stable when their ZP is high, which helps to keep them from aggregating together in the solution or dispersion. Repulsion might not be enough when the zeta potential is low, which could lead to flocculation and dispersion instability[10]. Particle size and dispersion must be considered in all nano-pharmaceutical formulations, according to Horiba Scientific. Infrared spectroscopy analysis, or FTIR spectroscopy, is the technique of classifying materials as organic, polymeric, or occasionally inorganic (JASCO FTIR-410). Infrared light is used in the

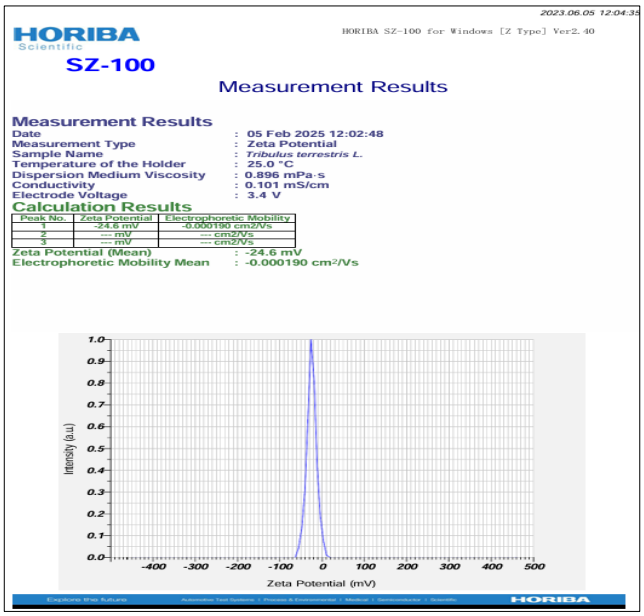


Figure 2: Zeta potential of ZnO nanoparticle

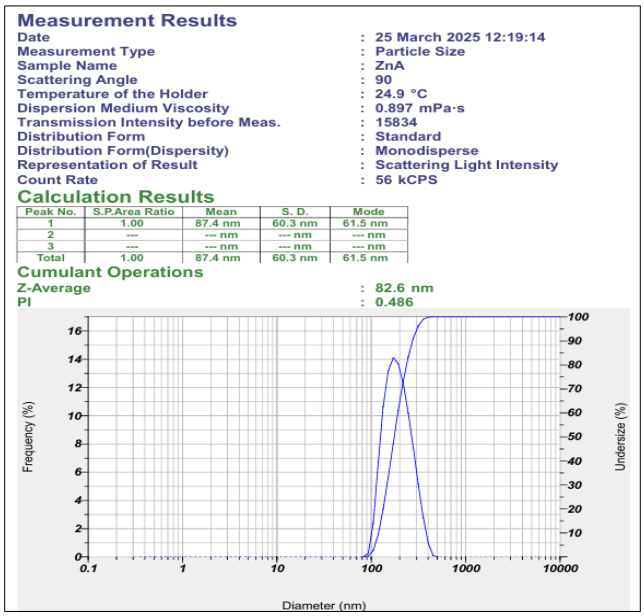


Figure 3: Particle size of ZnO nanoparticle

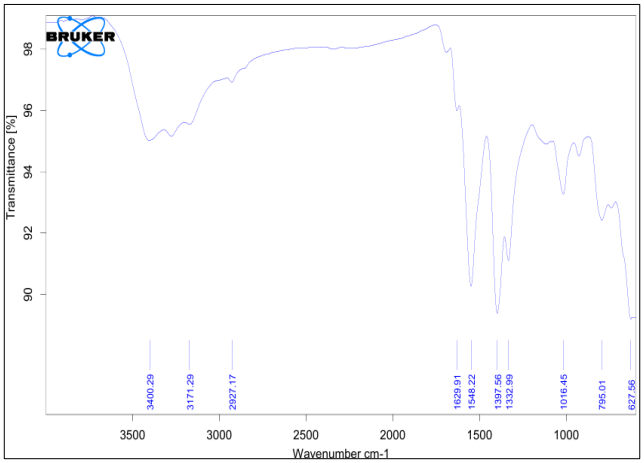


Figure 4: FTIR spectra of ZnO nanoparticle

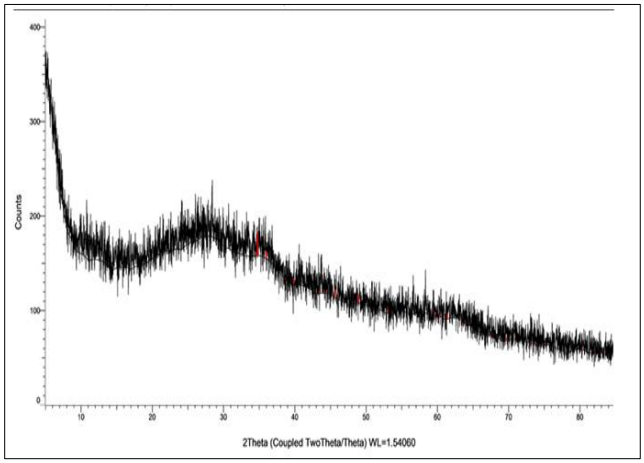


Figure 5: XRD spectra of ZnO nanoparticle

Table 1: Calibration curve with concentration vs absorbance

Concentration (µg)	Absorbance
0.1	0.037
0.2	0.045
0.3	0.058
0.4	0.074
0.5	0.092

study to scan materials and evaluate their chemical composition[11].

Anti-convulsant Activity Procedure

After being cultivated in DMEM with high glucose, FBS, and Antibiotic-Antimycotic solution, and incubated for 24 hours at 37°C with 5% CO_2, cells were seeded in 96-well plates and treated with test samples (20–100 µg/mL) for 24 hours. After four hours of incubation, the absorbance of formazan crystals dissolved in DMSO was measured to assess the anti-convulsant action, and an MTT test was used to assess cell survival after the addition of MTT reagent[12-15].

RESULTS AND DISCUSSION

Tribulus terrestris leaves were extracted using Soxhlet extraction, and the active ingredients were then preserved by shadow drying. The estimated yield value was 5.925g out of 150g. Phytochemical screening results suggested that

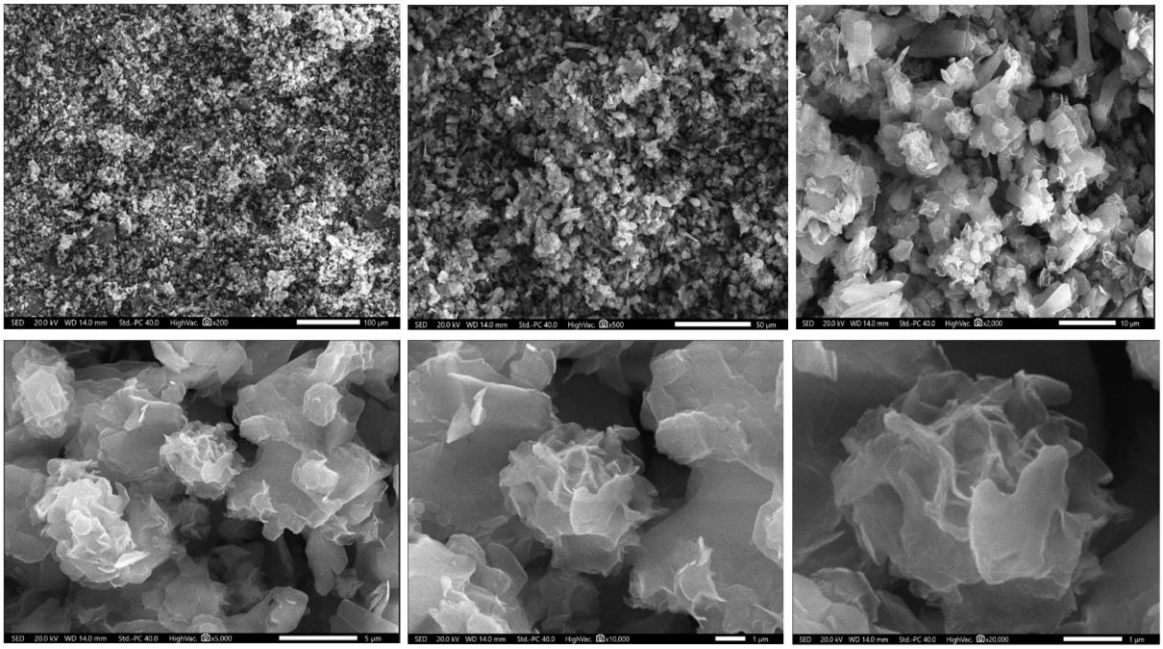


Figure 6: SEM of ZnO nanoparticle

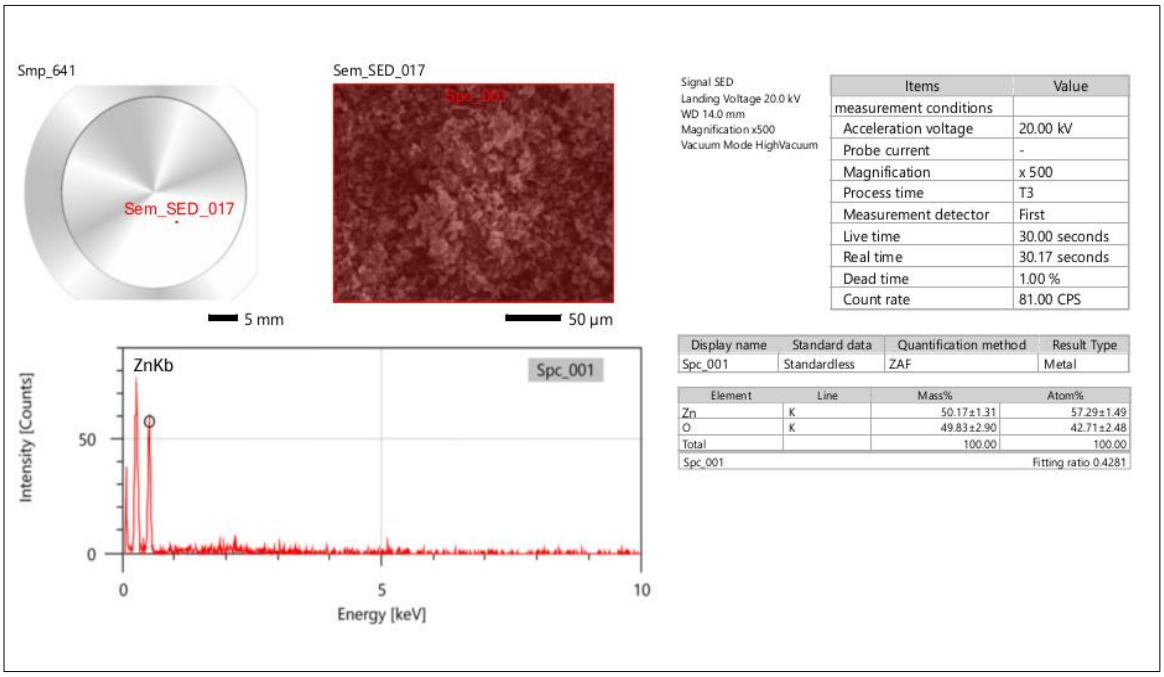


Figure 7: EDX of ZnO nanoparticle

Table 2: Effects of Compound Against SHSY5Y CRL-2266 Cell Lines

S. No.	Sample Code	Conc. (µg/ml)	OD (Mean)	% of Inhibition (%)	% of Viability (%)
1	Control	-	1.532	-	-
2	Standard (Carbamazepine)	20	1.430	6.65	93.35
		40	1.321	13.77	86.23
		60	1.367	10.63	89.37
		80	1.368	10.70	89.30
		100	1.350	11.87	88.13
3	Extract	20	1.411	7.89	92.11
		40	1.364	10.96	89.04
		60	1.302	15.01	84.99
		80	1.261	17.68	82.32
		100	1.211	20.95	79.05
4	Nanoparticles	20	1.395	8.94	93.09
		40	1.334	12.92	90.86
		60	1.287	16.00	87.62
		80	1.228	19.84	86.40
		100	1.183	22.79	83.69

the drug contained flavonoids, alkaloids were in abundance, tannins, glycosides, and saponins were moderately present.

Visual Observation

The color change was seen during the production of the nanoparticles, which signifies their formation. *Tribulus terrestris L.* leaf nanoparticles were successfully extracted. Their characteristics are essential to the drug delivery mechanism.

UV Spectroscopy

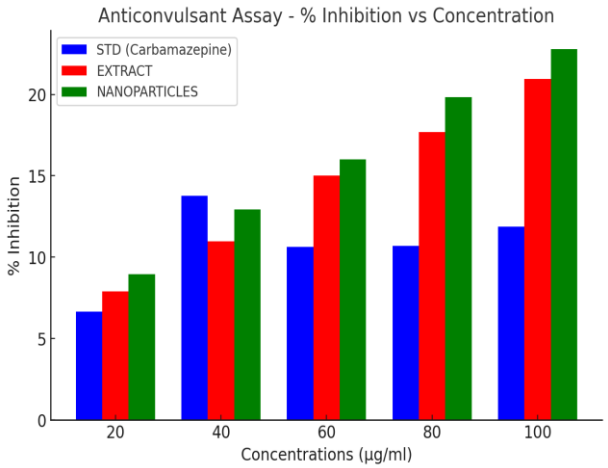


Figure 8: Anticonvulsant activity (% Inhibition vs Concentration)

The synthesis of ZnO NPs is also verified using the UV spectrophotometer method. As a result, the electrons in nanoparticles oscillate at a specific wavelength. The wavelength range where the peak was seen was 200–400 nm. Table 1 and Figure 1 show the specifics of the calibration curve with concentration vs. absorbance.

Zeta Potential

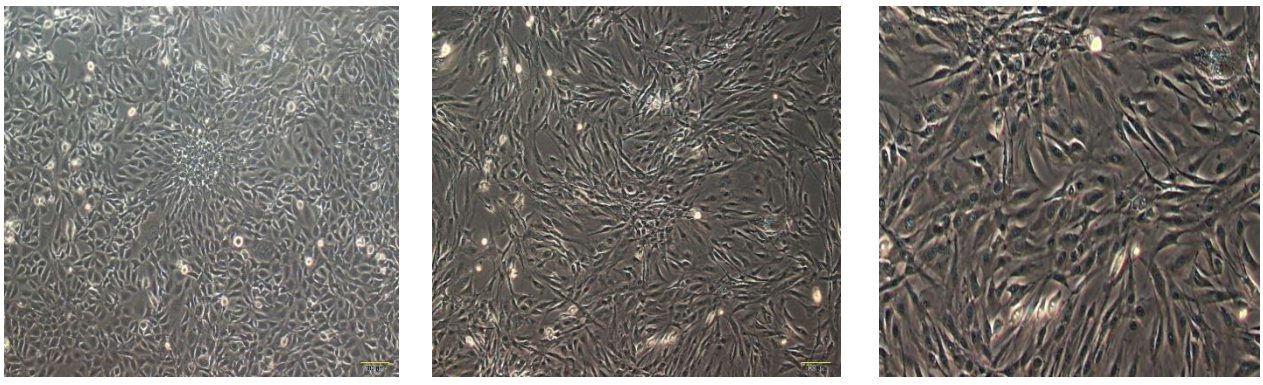
It often takes specialist methods like DLS to determine the size and zeta potential of zinc oxide nanoparticles (ZnO NP) made from extract from *Tribulus terrestris L.* leaves. According to figure 2, the measured zeta potential was -24.6 mV.

Particle Size Determination

Specialized methods, such as DLS. leaves, are often needed to determine the size and zeta potential of zinc oxide nanoparticles (ZnO NP) made from extract from *Tribulus terrestris L*. Figure 3 shows that the measured zeta potential was -24.6 mV.

Fourier Transform Infrared Spectroscopy (FTIR)

The various signals picked up by infrared spectroscopy are produced by the substance-specific vibrations of the molecules. The FTIR spectra of the functional group used in the manufacture of zinc oxide nanoparticles (ZnO NP) revealed a peak between 1000 and 3500 cm-1 (Figure 4). OH, stretching vibrations are indicated by the big peak seen at 3400.29, and the protein's amide-I stretch falls between 1456.72 C and 1456.72 C. The alkane and aldehyde (H-



Control Extract Nanoparticle

Figure 9: *In-vitro* Anticonvulsant Activity Images

C=O) are implied by the peaks at 2927.17. The presence of carbonyl, ester, and amine, respectively. is shown by the peaks at 1629.91, 1332.99, and 1016.45. The faint peaks at 1016.45 and 795.01 represent alkenes, whereas the aromatic compound 736.46 had a straight chain length.

X-Ray Diffraction XRD

Figure 5 indicates the ZnO sample's X-ray diffraction (XRD) pattern, which was captured using Cu Kα radiation (λ = 1.54060 Å). The pattern shows wide diffraction peaks between 10° and 80° in the 2θ range. The ZnO sample is not very crystalline, according to this. The material may have amorphous content, structural disorder, or nanocrystalline domains, as indicated by the peaks' broadness.

Scanning Electron Microscopy (SEM)

Through the use of a focused electron beam, SEM analyzes the size and surface morphology of nanoparticles in great detail. After being cured and covered with a conductive metal, the nanoparticles are scanned to record their surface properties. For mean size, SEM results are similar to dynamic light scattering however, nanoparticles need to be able to survive the electron beam and vacuum without getting damaged (Figure 6).

Energy-Dispersive X-ray Spectroscopy (EDX)

Energy-Dispersive X-ray Spectroscopy (EDX) analysis was performed on the sample's Sem_SED_017 area, as shown in the Figure 7. The two main elements found in the sample are zinc (Zn) and oxygen (O), according to the EDX spectrum. According to the quantitative analysis, the sample's composition is 50.17 ± 1.31 weight percent Zn and 49.83 ± 2.90 weight percent O, or 57.29 ± 1.49 at% Zn and 42.71 ± 2.48 at% O. The spectral data appears to suit the model employed for quantification reasonably well, as indicated by the fitting ratio of 0.4281. The EDX study was performed with a landing voltage of 20.0 kV, a magnification of x500, a live time of 30.00 seconds, and a dead time of 1.00%.

Anti-convulsant Activity

Table 2 and Figure 8 and 9 shows the anti-convulsant activity

CONCLUSION

Using an extract from *Tribulus terrestris*, this work effectively produced ZnO nanoparticles and verified their physicochemical characteristics using a variety of analytical methods. *In vitro* inhibition of SHSY5Y neuroblastoma cell viability was stronger for both the plant extract and ZnO nanoparticles than for carbamazepine under MTT test conditions. This suggests the presence of bioactive components with potential neuropharmacological effects. It is crucial to stress that MTT-based inhibition does not directly evaluate anticonvulsant mechanisms; rather, it represents cytotoxic or viability effects. To confirm the anticonvulsant capability of these materials and to ascertain their safety and effectiveness in comparison to well-established clinical medications such as carbamazepine, more in vivo research, electrophysiological evaluations, and mechanistic studies are necessary.

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