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Integrated *in vivo* and *in silico* assessment of *Rauvolfia vomitoria* extract on NMDA receptors in a PTZ-induced seizure model

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Abstract: *Rauvolfia vomitoria* (RV) is recognised for its anti-seizure properties, largely due to its rich alkaloid content. In this study, we evaluated the effect of an aqueous extract of *Rauvolfia vomitoria* on PTZ-induced seizures in male Wistar rats and used in-silico methods to identify the most promising alkaloid compound for predictive analysis. Male Wistar rats (average weight: 160 g) were divided into four groups (n = 5): saline (control), 25 mg/kg PTZ i.p., 200 mg/kg oral RV pretreatment + 25 mg/kg PTZ i.p., and 40 mg/kg oral carbamazepine pretreatment + 25 mg/kg PTZ i.p. Following treatment, we assessed behaviour via the novel object recognition test (NORT) and evaluated antioxidant enzyme levels, brain electrolyte concentrations, and histomorphology changes. Additionally, we employed molecular docking and pharmacokinetic profiling to assess the drug-like properties of the compounds. NORT results revealed increased exploratory time and a non-significant discrimination index. Antioxidant defences were enhanced, while lipid peroxidation indices showed a non-significant reduction. Major electrolyte concentrations were preserved. Molecular docking identified serpentinine as a high-affinity NMDA receptor ligand, with several other *Rauvolfia vomitoria* alkaloids exhibiting favourable drug-like properties. Oral pretreatment with *Rauvolfia vomitoria* mitigates PTZ-induced seizure, potentially through antioxidant modulation and a slight trend towards recognition memory. *In-silico* analyses highlight *Rauvolfia vomitoria* alkaloids as promising candidates for further experimental validation.

Keywords: Alkaloid; Pretreatment; Seizure; Recognition memory; *Rauvolfia vomitoria*.

1.0 INTRODUCTION

Epilepsy is a chronic neurological disorder of the central nervous system (CNS) characterised by recurrent, unprovoked seizures and represents a major global health burden, particularly in low- and middle-income countries (Anwar et al., 2020). According to the World Health Organization (WHO), approximately 50 million people worldwide, nearly 1% of the global population, are affected by epilepsy, with almost 75% of cases occurring in developing regions (Shan et al., 2024). Beyond the hallmark of motor deficit manifestations, epilepsy is frequently associated with behavioural disturbances, cognitive impairment, and altered neuronal network activity, reflecting widespread dysregulation of brain function (Anwar et al., 2020; Hoxhaj et al., 2023).

Growing evidence implicates oxidative stress as a key contributor to epileptogenesis and seizure-associated neuronal injury. Recurrent seizures promote excessive generation of reactive oxygen species (ROS), with overwhelming endogenous antioxidant defences leading to lipid peroxidation, protein oxidation, and mitochondrial dysfunction (Parsons et al., 2022). However, a few insights into these mechanisms have largely been derived from experimental seizure models.

Pentylenetetrazol (PTZ), a gamma-aminobutyric acid type A ($GABA_A$) receptor antagonist, is widely used to induce seizures and screen potential anti-seizure agents. PTZ-induced seizures disrupt inhibitory neurotransmission and metabolic homeostasis, thereby intensifying oxidative stress and neuronal damage (Shimada & Yamagata, 2018). In addition to impaired GABAergic signalling, excitatory glutamatergic pathways play a critical role in PTZ-induced seizures. Activation of N-methyl-D-aspartate (NMDA) receptors results in excessive calcium (Ca^{2+}) influx, triggering neuronal hyperexcitability and excitotoxic injury (Perucca et al., 2023). This process is further amplified by reduced chloride (Cl^-) conductance at the $GABA_A$ receptor level, shifting the excitation-inhibition balance toward seizure generation. Consequently, NMDA receptor antagonists have long been recognised for their anticonvulsant and neuroprotective properties (Egunlusi & Joubert, 2024).

The use of anti-seizure drugs has been suggested to reduce the likelihood of future seizures when these drugs are administered after the first seizure, particularly in patients with additional risk factors (Leone et al., 2021). Traditional medicine, particularly in Africa, has long used medicinal plants to manage seizures. *Rauvolfia vomitoria* is a plant rich in bioactive alkaloids such as serpentinine, ajmaline and reserpine, which exhibit antioxidant, anticonvulsant, and sedative properties. These phytochemicals, including polyphenols, have demonstrated neuroprotective effects against oxidative stress and inflammation, making them promising candidates for the management of epilepsy (Ekong et al., 2024). However, experimental validation of other phytochemicals in *aqueous extracts of Rauvolfia vomitoria*, along with *in silico* predictions and their therapeutic potential, remains limited.

In the present study, we investigated the protective effects of an aqueous leaf extract of *Rauvolfia vomitoria* against PTZ-induced seizures in male Wistar rats. Behavioural performance, oxidative stress markers, electrolyte balance, and histomorphological changes in the rats were assessed to elucidate potential neuroprotective effects. Furthermore, molecular docking and pharmacokinetic analyses were employed to explore interactions between major *Rauvolfia vomitoria* alkaloids and NMDA receptors. While similar computational approaches have been applied to other neuroactive compounds in medicinal plants, their use for alkaloids derived from *Rauvolfia vomitoria* in the context of seizure modulation remains comparatively underexplored. This integrative approach aims to provide mechanistic insight and identify candidate compounds for further experimental validation.

2.0 MATERIALS AND METHODS

2.1.1 Experimental animal model and design

Twenty healthy male Wistar rats, with an average weight of 160 g, were obtained from the Olabisi Onabanjo University Animal House on the Sagamu Campus, Ogun State, Nigeria. The rats were acclimatised for 14 days and fed pelletised rat chow and water ad libitum in the Physiology Department section of the Animal House at a room temperature of 22 ± 3°C with a 12-hour light/day cycle. All experimental animals were handled based on guided ethical approval obtained at Olabisi Onabanjo University Teaching Hospital, Health Research and Ethics Committee, OUTHREC, with approval details, ref: ID OOUTH/HREC/566/2022AP, for the experimental procedures conducted in this study.

The rats were randomised and divided into four groups of five per cage (i.e., *n*=5). A seven-day animal model of epileptic seizures was generated in this study to evaluate the oral pretreatment effect of *Rauvolfia vomitoria,* as shown in **Table 1**.

Table 1. Experimental animal grouping.

Group (*n*=5)	Treatment	Dose (mg/kg)	Route	Duration (days)
Control	Saline	—	i.p.	3
PTZ	Pentylenetetrazol	25	i.p.	3
RV + PTZ	*Rauvolfia vomitoria* + PTZ	RV: 200; PTZ: 25	RV: oral; PTZ: i.p.	RV: 7; PTZ: 3
Cz + PTZ	Carbamazepine + PTZ	Cz: 40; PTZ: 25	Cz: oral; PTZ: i.p.	Cz: 7; PTZ: 3

Five animals per group (*n*=5) were assigned to control or treatment groups. The control group received saline (i.p.) for 3 days. PTZ (25 mg/kg, i.p.) was used to induce seizures in the PTZ group. In the RV + PTZ and Cz + PTZ groups, animals received *Rauvolfia vomitoria* (200 mg/kg, oral) or carbamazepine (40 mg/kg, oral) for seven days, with PTZ (25 mg/kg, i.p.) administered on days 1-3, 30 minutes after pretreatment. Days 4-7 involved continued *Rauvolfia vomitoria* or carbamazepine administration without PTZ to assess sustained neuroprotective effects. PTZ, pentylenetetrazol; Cz, carbamazepine; RV, *Rauvolfia vomitoria*; i.p., intraperitoneal.

2.1.2 Plant material collection and dosage formulation of aqueous extract of *Rauvolfia vomitoria* per body weight/average weight

The leaves of *Rauvolfia vomitoria* were harvested from an indigenous farm in Sagamu, Ogun State, Nigeria. The plant was confirmed and authenticated at the Forestry Research Institute of Nigeria (FRIN) herbarium in Ibadan, with voucher number 112519. The harvested leaves were air-dried at ambient room temperature for five days. The leaves were manually turned every 12 hours to prevent fungal growth and ensure uniform drying. The dried leaves were pulverised into a fine powder using a laboratory mechanical grinding mill.

Based on the OECD (2002) guidelines and previous evidence from Oghenesuvwe et al. (2014), a low dose of 200 mg/kg body weight was selected for this study. The extract was formulated according to the method of Oghenesuvwe et al. (2014) on extract dissolution and yield, volume, and dosage calculations. Extraction was performed using an aqueous maceration method, where the powdered leaf was dissolved in distilled water for 48 hours at room temperature with intermittent mixing. Following extraction, the mixture was filtered to remove particulate matter, and the extraction yield was determined. The aqueous extract was stored in airtight containers at 4°C until use. A low dose (200 mg/kg) of *Rauvolfia vomitoria* was administered to Wistar rats with an average weight of 160 g. In this study, a rat is recommended to receive 32 mg of *Rauvolfia vomitoria* aqueous extract, corresponding to a dose of 200 mg/kg:

Dosage in mg

$$= \frac{\text{Body weight of animal}}{1000g} \times dose\ (mg)$$

$$= \frac{160\text{g}}{1000g} \times 200\ (mg/kg) = 32\ mg$$

2.1.3 PTZ-induced kindling: Chemicals

25 g of pentylenetetrazol (P6500-Sigma-Aldrich, USA) was obtained from IBRA-HADAD Biochemical Distributors, Nigeria, Limited. Epileptic seizures were induced by intraperitoneal injection of PTZ at 25 mg/kg (Sanya et al., 2016) for three consecutive days following seven days of oral pretreatments, and seizure scores were determined on each PTZ injection.

2.1.4 Oral dosage administration of *Rauvolfia vomitoria* and carbamazepine

Carbamazepine (200 mg) was obtained from a licensed pharmacy in Sagamu, Ogun State, Nigeria. Carbamazepine is a reference antiepileptic drug used in this study due to its well-established anticonvulsant efficacy and its frequent use as a positive control in PTZ-induced seizure models (Bernardi and Barros, 2004). Drug preparation for animal administration was performed according to the method described by Oghenesuvwe et al. (2014). *Rauvolfia vomitoria* and carbamazepine were administered orally. Based on previous experimental studies by Bernardi and Barros (2004), carbamazepine was administered at a dose of 40 mg/kg per body weight. For a rat weighing 160 g, the dose was calculated as follows:

Dosage in mg of carbamazepine

$$= \frac{\text{Body weight of animal}}{1000g} \times \text{dose}\ (mg)$$

$$= \frac{160\text{g}}{1000g} \times 40\ (mg/kg) = 6.4\ \text{mg}$$

In the *Rauvolfia vomitoria* + PTZ group, rats received aqueous *Rauvolfia vomitoria* leaf extract orally at 200 mg/kg per body weight (32 mg per rat; 0.32 mL), while in the carbamazepine + PTZ group, carbamazepine was administered orally at 40 mg/kg per body weight (6.4 mg per rat; 0.32 mL).

2.1.5 Phytochemical screening

Qualitative and quantitative phytochemical screening of *Rauvolfia vomitoria* leaf was conducted to identify

major secondary metabolite classes. All procedures were performed in accordance with classical phytochemical methods as described by Harborne (1973), Sofowora (1993), Trease and Evans (1989), with minor modifications to accommodate sample size.

2.1.6 Procedure for novel object recognition test (NORT)

The novel object recognition test (NORT) was performed to evaluate recognition memory (Lueptow, 2017). The test was conducted in a rectangular open-field arena (60 × 45 × 45 cm) under uniform lighting in a quiet testing environment and consisted of habituation, training, and testing phases. During habituation, each rat in the experiment was individually placed in the empty arena and allowed to explore freely for 5 minutes. 24 hours later, a 10-minute training phase exploring familiar objects in an open-field arena was conducted, which coincided with day one of PTZ-induced seizure induction. Animals were orally pretreated with *Rauvolfia vomitoria* extract and carbamazepine, and 30 minutes later intraperitoneal injection of PTZ was done. Seizure latency and duration were recorded immediately after PTZ injection. The testing phase was conducted 24 hours after training. One of the familiar objects was replaced with a novel object of similar size but different shape and texture. Rats were allowed to explore the arena for 10 minutes after day two pretreatment and PTZ injections, and exploration time for each object was recorded. The arena and objects were cleaned with 70% ethanol between rats to eliminate olfactory cues. Recognition memory was quantified using the discrimination index, calculated as the difference in time spent exploring the novel and familiar objects divided by the total exploration time:

$$\text{Discrimination Index} = \frac{\text{Total Time of Exploration (Novel)} - \text{Total Time of Exploration (Familiar)}}{\text{Total Exploration Time During Testing i. e. (Total Time of Exploration (Novel)} + \text{Total Time of Exploration (Familiar)}}$$

2.1.7 Procedure for the Collection of Tissue Samples and Determination of Antioxidant and Electrolyte

The right hemisphere regions of the brain were excised and homogenised, and the other halves of the brain (left hemisphere) were fixed in 10% neutral buffered formalin solution. The tissue homogenate of the right hemisphere was subsequently centrifuged at 4000 rpm for 10 minutes, after which the supernatant was separated for superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (GSH) determination. SOD activity was determined (Misra & Fridovich, 1972), and CAT activity in brain tissue was determined (Aebi, 1984) via the thiobarbituric acid (TBA) method, which measures MDA reactive products (Ohkawa et al., 1979). The level of reduced GSH was measured via standard methods. The sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), and pH levels of the brain homogenate supernatant were determined using an automated electrolyte analyser (SFRI ISE6000, France). Electrolyte values were determined in mmol/L.

2.1.8 Procedure for histomorphology of brain tissue

The histology of the fixed brain tissue in 10% neutral buffered formalin solution was utilised for hematoxylin & eosin (H&E) staining and cresyl violet staining. The prefrontal and hippocampal brain regions were stained to examine cellular morphology. Histological assessments were descriptive and aimed at capturing qualitative morphological changes rather than quantitative measures.

2.2 Molecular docking: Protein and ligand preparation

Since our study revealed that phenols and alkaloid compounds were present in high quantities in *Rauvolfia vomitoria* (**Table 2**), these compounds were curated for evaluation at the receptor, NMDA. The alkaloid compounds of *Rauvolfia vomitoria* reported by Kern-Fern in the 2023 Tropical Database, together with selected phenolic compounds and the reference drug carbamazepine, were used for ligand-receptor interaction analyses. The ligands were obtained from the renowned database PubChem library, in SDF format. Additionally, the 3-dimensional complex structure of the human GluN1-GluN2A NMDA was procured from the Protein Data Bank PDB with PDB ID: 7EU7 (Zhang et al., 2021). This protein structure is made of chains A-D with varying catalytic complex ligands expressed as co-ligands in this study. Schrodinger Maestro 11.5 software was used for protein and ligand visualisation, and Discovery Studio was used for protein, ligand, and coligand preparation. Energy minimisation of the ligands curated was performed via a universal force field (UFF), followed by the conversion of the ligands to AutoDock ligands to the Protein Data Bank, Partial Charge & Atom Type (PDBQT) format with an integrated Open Babel tool in PyRx.

2.2.1 Receptor active site generation and molecular docking

The active site for the amino acid residue was generated via Discovery Studio from the protein and its complexed ligands. Thus, the coordinates and size of the NMDA active site of this protein were generated via the selection of amino acid residues involved in co-ligand interactions at the protein active site via the generation of the receptor grid box; *x*=27.34; *y*=53.1; and *z*=78.6 A. Virtual screening of curated compounds was performed for receptor-ligand interactions using AutoDock Vina Wizard integrated into PyRx. PyRx is a virtual screening tool that was also used for molecular docking, with exhaustiveness set to 8, and binding affinity was evaluated in kcal/mol.

2.2.2 Physicochemical properties, pharmacokinetic analysis, and drug likeness

The curated compounds used for molecular docking in this study were subjected to AdmetSAR server (Cheng et al., 2012) for physicochemical and drug likeness, i.e., Lipinski's rule of five by screening. All compounds from *Rauvolfia vomitoria* reported in this study were analysed for pharmacokinetic properties using the SwissADME server (Daina et al., 2017).

2.3 Statistical analysis

The experimental results are presented as the means ± standard error of mean (SEM). Comparisons of differences among all groups were performed via one-way ANOVA, and multiple comparisons were performed via Tukey (HSD) post hoc analysis. All the statistical results were analysed via SPSS version 25. A statistically significant value was determined at $p<0.05$. GraphPad Prism version 10.1.0 was used to generate the graphs.

3.0 RESULTS

3.1 Phytochemical Screening Analysis of *Rauvolfia vomitoria*

Analysis of *Rauvolfia vomitoria* phytochemicals, as shown in **Table 2**, revealed the presence of phenols and alkaloid bioactive compounds as reported in their highest quantity compared with the other bioactive compounds.

Table 2. Quantitative and qualitative phytochemical analysis of *Rauvolfia vomitoria*.

Phytochemical	Quantitative content (mg/100 g)	Qualitative Presence
*Alkaloids	63.22	+
Saponins	43.01	+
Tannins	60.32	+
Phlobatannins	ND	–
Steroids	34.38	+
Cardiac glycosides	42.89	+
*Phenols	65.44	+
Flavonoids	45.49	+
Reducing sugars	ND	–
Terpenoids	36.38	+

+: presence of phytochemicals; –: absence of phytochemicals; ND: Not Detected; *: highly abundant phytochemicals

3.2 Effects of aqueous extracts of *Rauvolfia vomitoria* on PTZ-induced seizure and recognition memory

In this study, the anticonvulsant effect of *Rauvolfia vomitoria* was evaluated by comparing PTZ-treated rats with controls and *Rauvolfia vomitoria* pretreated groups. As shown in **Figures 1A and 1B**, PTZ administration significantly reduced seizure latency ($p<0.05$) and increased seizure duration compared with controls. Pretreatment with *Rauvolfia vomitoria* attenuated these PTZ-induced seizure effects, as evidenced by an increase in seizure latency and a reduction in seizure duration in the *Rauvolfia vomitoria* + PTZ groups relative to the PTZ-only group ($p<0.05$), indicating a protective effect of *Rauvolfia vomitoria* against PTZ-induced seizures.

Recognition memory was assessed using the novel object recognition test, in which rats were trained to discriminate between familiar and novel objects. As shown in **Figure 1C**, rats pretreated with a low dose of *Rauvolfia vomitoria* (200 mg/kg) exhibited significantly greater exploration of the novel object than the PTZ-only group ($p<0.05$), suggesting an improvement in exploratory behaviour and a trend towards recognition. However, as shown in Figure 1d, no statistically significant differences in the discrimination index were observed among the groups ($p>0.05$). Despite the lack of statistical significance, the *Rauvolfia vomitoria*-treated groups showed a trend toward improved recognition memory compared with the PTZ-only group.

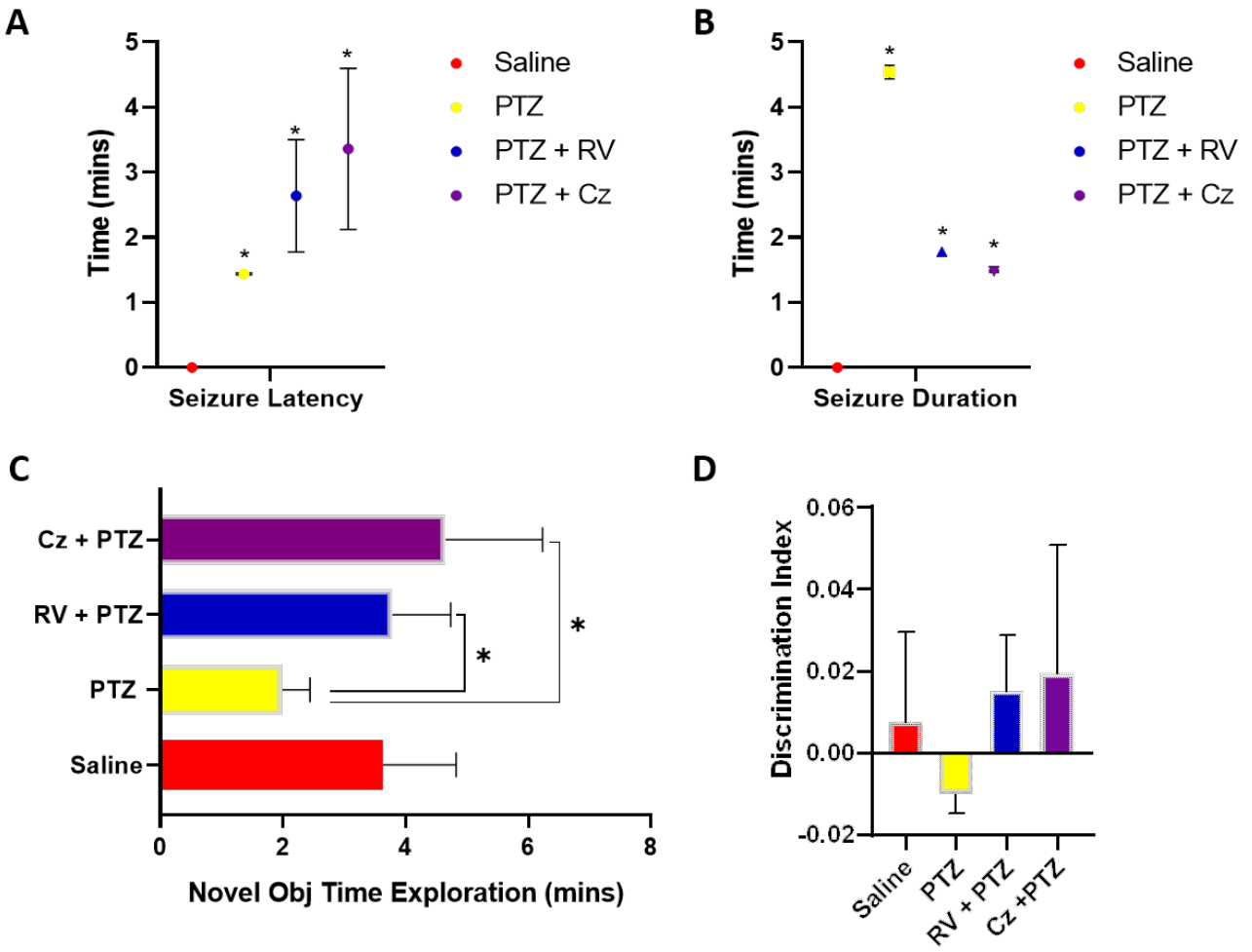


Figure 1. *Rauvolfia vomitoria* pretreatment effect on seizure parameters and recognition memory in PTZ-induced rats (n=5). (A) Seizure latency and (B) seizure duration (minutes) across experimental groups. (C) Novel object recognition test (NORT) total exploration time (minutes) and (D) discrimination index across experimental groups. * Indicates $p < 0.05$ compared with the PTZ-only group.

3.3 Effects of the aqueous extract of *Rauvolfia vomitoria* on PTZ-induced seizure, brain lysate antioxidant and electrolyte properties

Figure 2 shows the effects of *Rauvolfia vomitoria* and PTZ on antioxidant markers in brain tissue, with comparisons made between controls, PTZ-only, and *Rauvolfia vomitoria* + PTZ groups. Compared with the control group, PTZ altered antioxidant levels, whereas *Rauvolfia vomitoria* pretreatment modulated these effects. Superoxide dismutase (SOD) activity in the *Rauvolfia vomitoria* + PTZ group was significantly decreased relative to the control group (p<0.05), indicating a partial restoration toward control levels. In contrast, catalase (CAT) activity did not differ significantly between the *Rauvolfia vomitoria* + PTZ and PTZ-only groups (p>0.05). Glutathione (GSH) levels were significantly elevated in the *Rauvolfia vomitoria* + PTZ group compared with the control group (p<0.05), suggesting enhanced antioxidant capacity following *Rauvolfia vomitoria* pretreatment. Malondialdehyde (MDA) levels did not differ significantly among the groups (p>0.05), indicating that *Rauvolfia vomitoria* had no detectable effect on lipid peroxidation under the conditions tested.

Figure 3 presents the electrolyte profiles across the groups. Comparisons between the PTZ group, the Rauvolfia vomitoria + PTZ group, and the control revealed no significant differences in sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), or chloride (Cl^-) concentrations (p>0.05). Similarly, *Rauvolfia vomitoria* pretreatment did not significantly alter electrolyte levels compared with the PTZ-only group (p>0.05). Brain tissue pH values also did not differ significantly between the *Rauvolfia vomitoria* + PTZ and control groups (p>0.05).

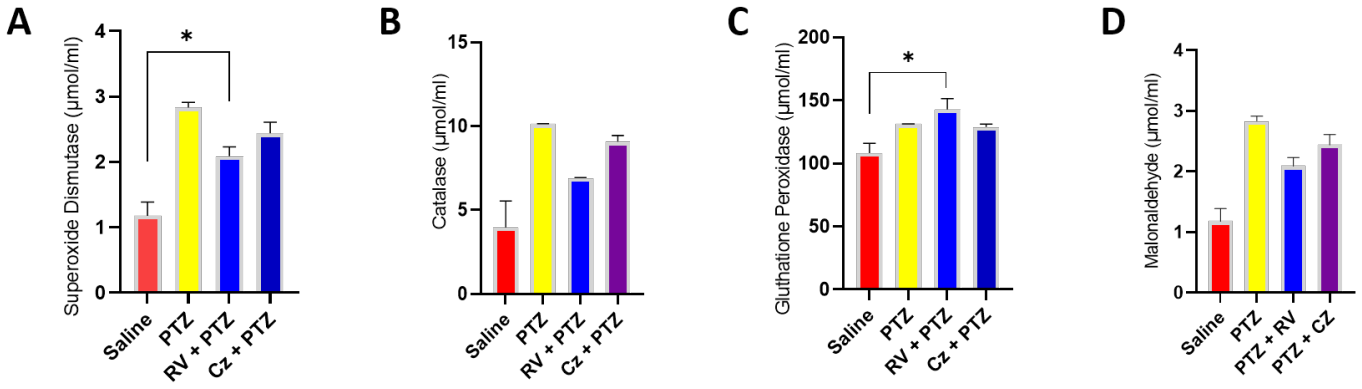


Figure 2. *Rauvolfia vomitoria* pretreatment effects on antioxidant activity in PTZ-induced rats (n=5). (A) Superoxide dismutase (SOD) activity, (B) Catalase (CAT) activity, (C) Glutathione (GSH) activity, and (D) Malondialdehyde (MDA) levels across experimental groups. Data are presented as mean ± SEM. * indicates $p < 0.05$ compared with the PTZ-only group.

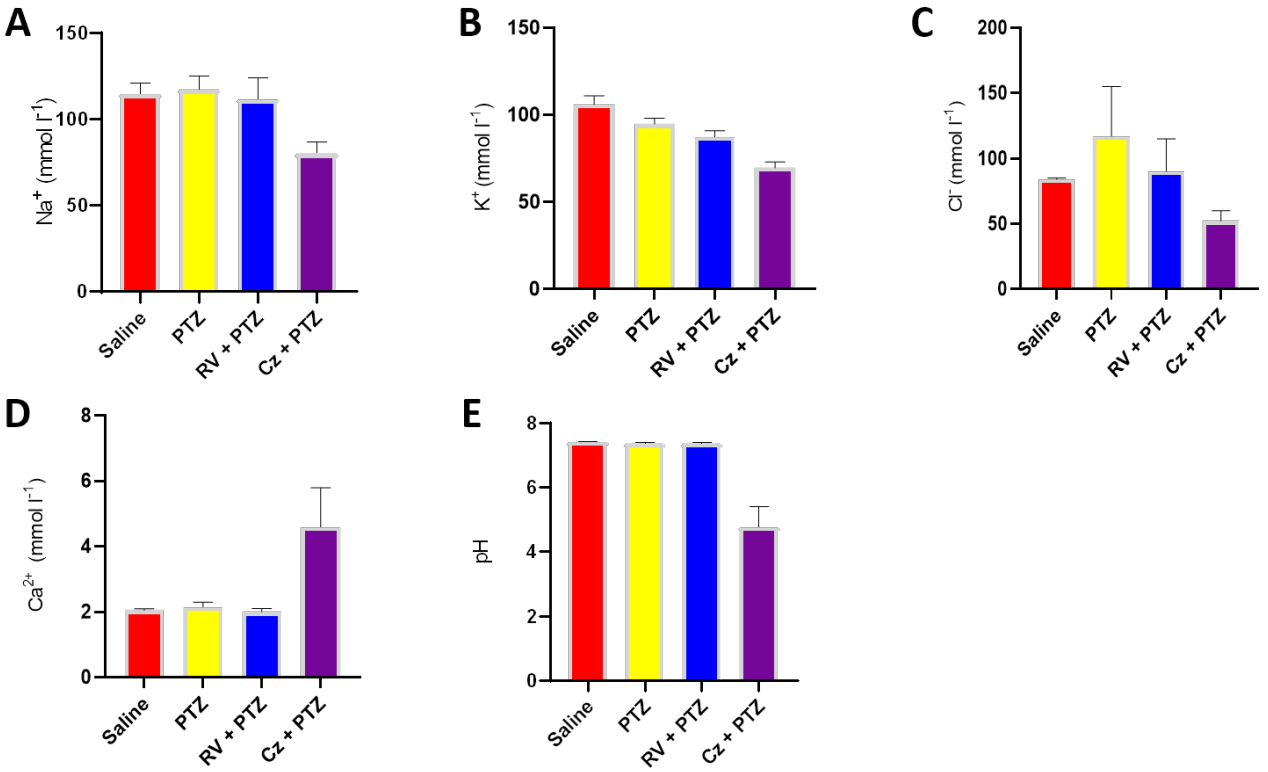


Figure 3. *Rauvolfia vomitoria* pretreatment effects on electrolyte levels in PTZ-induced rats (n=5). (A) Sodium (Na^+), (B) Potassium (K^+), (C) Chloride (Cl^-), (D) Calcium (Ca^{2+}), and (E) pH across experimental groups. Data are presented as mean ± SEM.

3.4 Effects of the aqueous extract of *Rauvolfia vomitoria* on the Histomorphology of the prefrontal cortex and hippocampus in PTZ-induced seizures

Figure 4 and **Figure 5** show hematoxylin and eosin (H&E) and cresyl violet stains, respectively, at ×400 magnification of the coronal section of the prefrontal cortex in pretreated rats given *Rauvolfia vomitoria* + PTZ (**Figure 4C** and **Figure 5C**), which revealed reversed pyramidal cells with slight cellular debris and non-neuronal cells, as observed. These findings were compared with those of normal, well-differentiated pyramidal cells and non-neuronal cells in the control group of rats given saline; however, carbamazepine + PTZ (**Figure 4D** and **Figure 5D**) showed slight regeneration of pyramidal cells, normal vacuoles, and non-neuronal cells. In contrast, the PTZ-induced group presented atrophied cells.

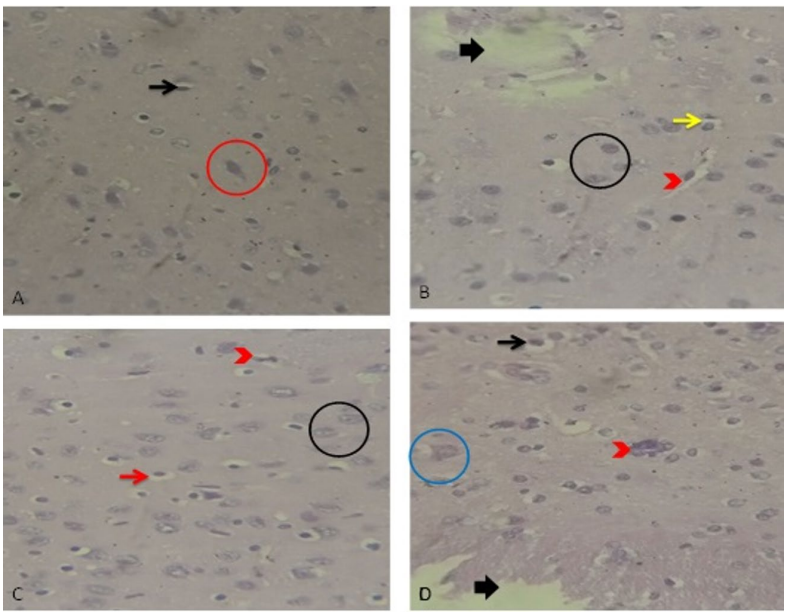


Figure 4. Micrograph of coronal section from prefrontal cortex (H&E stain, ×400 magnification). (A) Control (saline) group shows normal, well-differentiated pyramidal neurons (red circle) with basophilic cytoplasm and nuclei. Small non-neuronal nuclei are observed within the neuropil (thin black arrow). (B) PTZ only group shows distortion and vacuoles (thick black arrow), vacuoles containing cellular debris (red arrowhead), small non-neuronal nuclei (thin yellow arrow, same as red arrowhead) and a neuron (black circle). (C) *Rauvolfia vomitoria* and PTZ-treatment group shows reversed and regenerated pyramidal cells (black circle), non-neuronal cells (thin red arrow) with slight cellular debris (red arrowhead). (D) Carbamazepine and PTZ-treatment group shows slight regeneration of the pyramidal neurons (blue circle), vacuoles (thick black arrow), and normal small non-neuronal nuclei within the neuropil (black thin arrow).

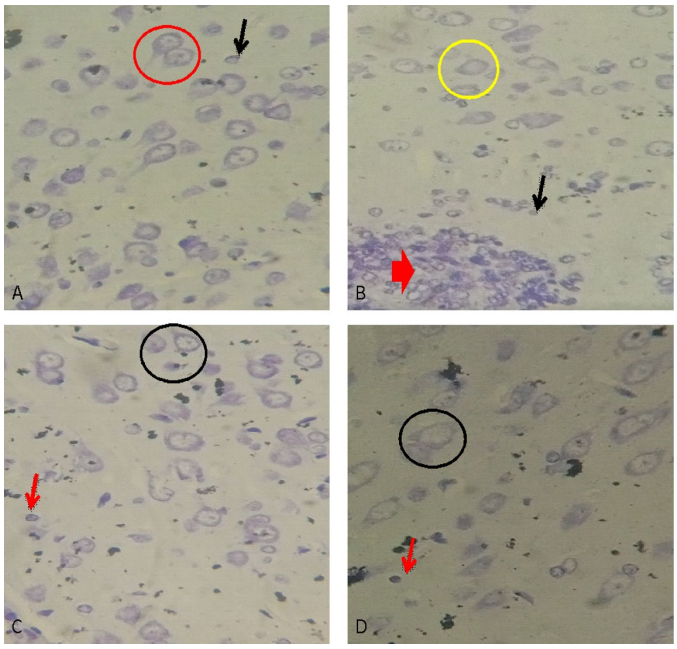


Figure 5. Photomicrograph of coronal section from prefrontal cortex (cresyl violet stain, ×400 magnification). (A) Control (saline) group shows normal, well-differentiated pyramidal neurons (red circle) with basophilic cytoplasm and nuclei. Small non-neuronal nuclei are observed within a neuropil-like area (thin black arrow). (B) PTZ only group shows a distortion and atrophied pyramidal neurons (yellow circle), non-neuronal cells (thin black arrow) and necrotic aggregation of neurons (thick red arrow). (C) *Rauvolfia vomitoria* and PTZ-treatment group shows reversed and regenerated pyramidal neurons (black circle), and non-neuronal cells (thin red arrow). (D) Carbamazepine and PTZ-treatment group shows slight regeneration of the pyramidal neurons (black circle), normal non-neuronal cells (thin red arrow).

Figure 6 and **Figure 7** show pretreated rats given *Rauvolfia vomitoria* + PTZ and carbamazepine + PTZ with hematoxylin and eosin (H&E) and cresyl violet stains, respectively, at ×200 and ×400 magnification of the coronal section of the hippocampus. Compared with normal *cornu ammonis* (CA1, CA2, CA3) and well-differentiated non-neuronal cells found in the control group, *Rauvolfia vomitoria* + PTZ group presented reversed and regenerated *cornu ammonis* (CA1, CA2, CA3) with slight H&E-stained cellular debris. Additionally, carbamazepine + PTZ show slightly regenerated *cornu ammonis* (CA1, CA2, and CA3) cells as observed in histology.

3.5 Inhibitory effects of alkaloid compounds on the NMDA receptor

16 bioactive compounds were screened for molecular docking, of which eight (8) with high binding affinity (ΔG) were analysed for pharmacokinetics analysis. The 16 bioactive compounds included 14 alkaloid bioactive compounds, namely, reserpine, deserpidine, ajmalicine (raubasine), reserpinine (rescinnamine), reserpiline, sarpagine (raupine), ajmaline, alstonine, serpentine, serpentinine, isoreserpiline, geissoschizol, oxindole, pseudoindoxyl; one phenol bioactive compound; and one standard drug, carbamazepine, which were analysed in this study.

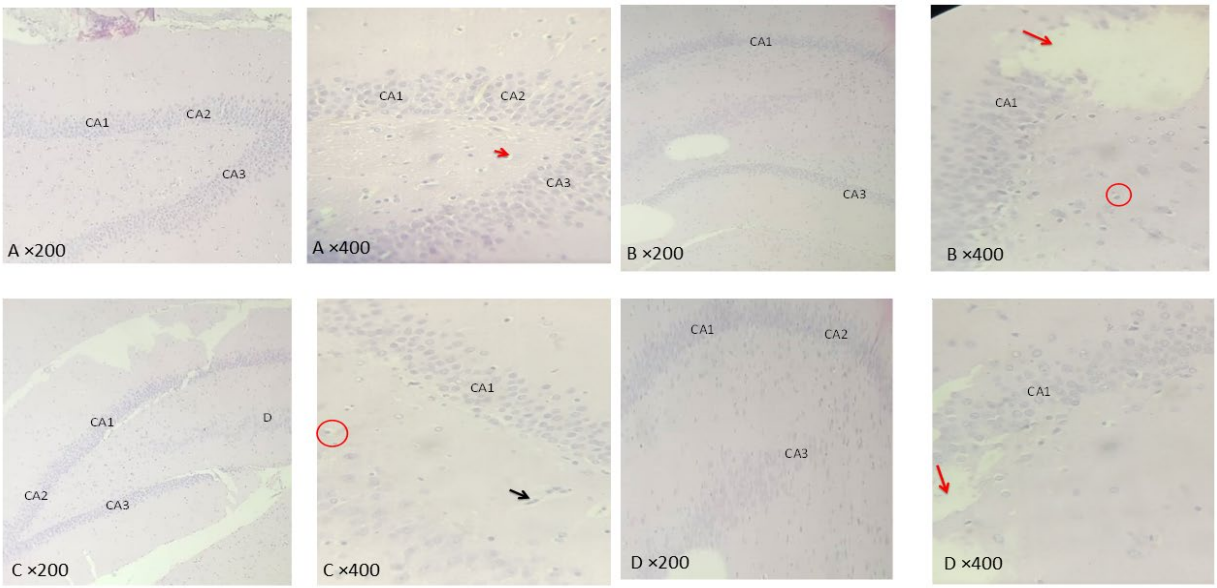
The active site of the NMDA receptor was generated from its complex co-crystallised structure, obtained from the Protein Data Bank, using co-ligands. Among the sixteen bioactive compounds, seven alkaloid compounds had the highest binding affinity (ΔG) in comparison with the standard drug carbamazepine, as shown in **Figure 9**. **Figure 8** shows the natural complex co-crystalised structures, i.e., co-ligands that interact with chains C and D of the protein structure; hence, the utilisation of the entire protein chain residues in our study. These residues include Arg523, Ser688, Ser687, Thr518 and Leu642. The grid boxes generated from the analysis had the following sizes: x = 27.34, y = 53.1, and z = 78.6. Molecular docking, i.e., analysis of the existing molecular interaction between the best ligand conformer (most stable) and the receptor complex, was performed in comparison to the reference drug of interest, carbamazepine. This analysis, shown in **Figure 9**, on drug-receptor interactions, revealed the top seven compounds: serpentinine, alstonine, ajamlicine, ajamline, deserpidine, geissoschizol, and reserpinine, which have binding affinities greater than or equal to that of the standard drug carbamazepine. Serpentinine had binding scores ranging from ΔG values of -10.9 to -9.9 kcal/mol, whereas the reference standard drug had binding scores ranging from ΔG values of -8.4 to -7.4 kcal/mol. More importantly, the conformer of serpentinine with the highest binding affinity was ΔG (-10.9 kcal/mol), and the reserpinine conformer and carbamazepine with the lowest binding affinity were ΔG (-8.4 kcal/mol).

The ADMET properties of the docked compounds presented in **Table 3** are further analysed in **Table 4** for physicochemical properties and the rule of five (drug likeness). The pharmacokinetic profile predictions also included blood-brain barrier potential, gastrointestinal tract absorption rate, oral toxicity, and P-gp.

4.0 DISCUSSION

Identifying effective anti-seizure therapies with reduced adverse effects remains a major challenge, particularly in resource-limited settings where medicinal plants are widely used. The traditional use of plant-derived products continues to motivate the experimental evaluation of their safety and biological activity (Chipiti et al., 2021). In the present study, we investigated the effects of pretreatment with a low-dose aqueous leaf extract of *Rauvolfia vomitoria* (200 mg/kg) on behaviour performance, oxidative stress markers, electrolyte balance, and brain histomorphology in a pentylenetetrazol (PTZ)-induced seizure model in male Wistar rats. In addition, *in silico* molecular docking and pharmacokinetics analysis were employed to explore potential interactions between selected *Rauvolfia vomitoria* alkaloids and NMDA receptors.

Consistent with previous reports, phytochemical evidence supports the notion that *Rauvolfia vomitoria* contains abundant alkaloids and polyphenolic compounds, which vary in concentration across plant parts and have been associated with neuropharmacological activity (Egunlusi & Joubert, 2024; Ekong et al., 2024). These findings support the rationale for examining *Rauvolfia vomitoria* in seizure-related models; however, the present study does not experimentally isolate the contribution of individual compounds.



F**igure 6. Photomicrograph of coronal section from hippocampus (H&E Stain, ×200 and ×400 Magnification). (A)** Control (saline) group shows normal *cornu ammonis* (CA1, CA2 and CA3) and well differentiated neuronal cells (B) PTZ-only group shows distortion and degeneration of the *cornu ammonis* (CA1), large vacuoles (thin red arrow) and reduced cellular density within the hippocampal layers (red circle). (C) *Rauvolfia vomitoria* and PTZ-treatment group shows reversed and regenerated *cornu ammonis* (CA1, CA2, CA3) cells, neuronal cells (red circle) with slight cellular debris (thin black arrow). (D) Carbamazepine and PTZ-treatment group shows slight regeneration of the cornu ammonis (CA1, CA2 and CA3).

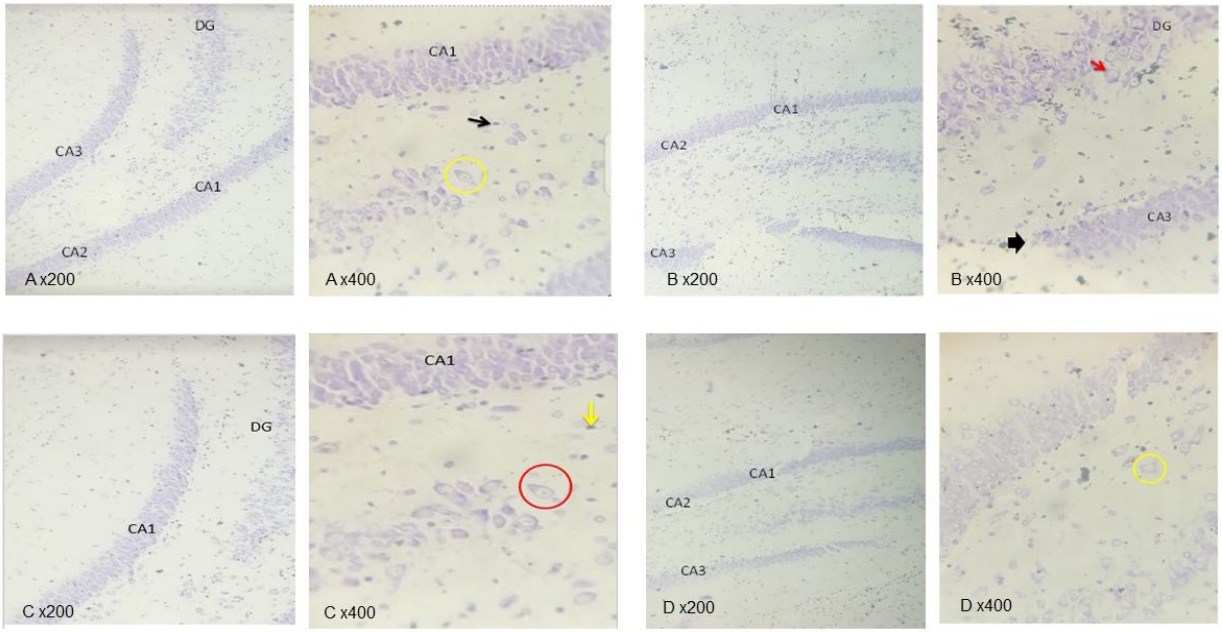


Figure 7. Photomicrograph of coronal section from hippocampus (cresyl violet stain, ×200 and ×400 magnification). (A) Control (saline) group shows a normal *cornu ammonis* of the hippocampal (CA1, CA2 and CA3), Dentate gyrus (DG), neurons (yellow circle) and well-differentiated non-neuronal cells (thin black arrow). (B) PTZ-only group shows distortion and degeneration of the neuronal profile of *cornu ammonis* (CA1, CA2 and CA3) with increased atrophied cells (thin red arrow), large vacuoles (thick black arrow) and reduction of neuronal cells. (C) *Rauvolfia vomitoria* and PTZ-treatment group shows regeneration and improved *cornu ammonis* (CA1), neurons (red circle). (D) Carbamazepine and PTZ-treatment group shows slight regeneration of the *cornu ammonis* (CA1, CA2 and CA3) cells, neurons (yellow circle).

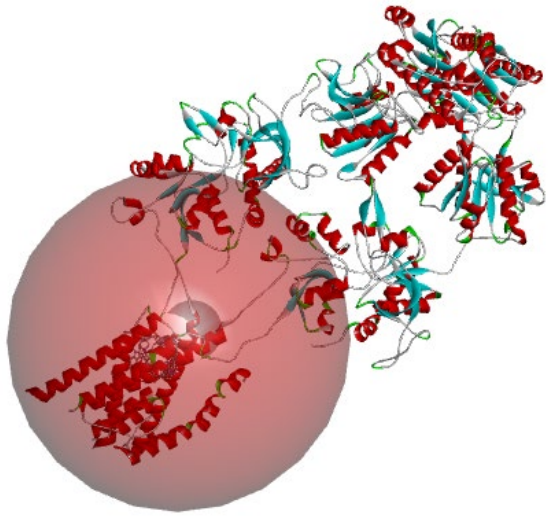


Figure 8. Molecular docked compounds, including carbamazepine and coligand inhibitor saturation, on the human GluN1-GluN2A chain C&D of the NMDA active site.

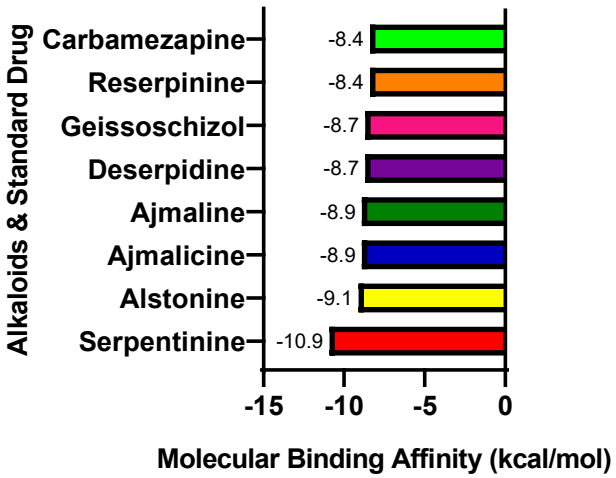


Figure 9: Molecular binding affinities of alkaloids from *Rauvolfia vomitoria* with standard drug.

Behaviour assessment using the novel object recognition test shows no significant differences in the discrimination index across experimental groups. Accordingly, the present findings show only a trend of slightly enhanced behaviour under the tested conditions, although no significant difference. Given prior reports of sedative effects of *Rauvolfia vomitoria* at certain doses (Sridhar et al., 2023; Ekong et al., 2013), it remains possible that dose-dependent or task-specific effects on cognition may exist, warranting further investigation using additional behavioural paradigms and extended testing timelines. Physiologically, it has been mentioned that memory encoding involves activity-dependent changes in synaptic transmission that enhance neural signalling and engage memory-related pathways (Glasgow et al., 2019; Guskjolen & Cembrowski, 2023). However, a more suitable animal model may be required to comprehensively evaluate cognitive neurobehavioral outcomes and the effects of *Rauvolfia vomitoria* on seizures and cognitive impairment (Ekong et al., 2024).

Several studies using the PTZ seizure model have demonstrated its effect on oxidative stress, likely through disruption of CNS cellular metabolism and activation of degradative enzymes. As shown in Figure 2, superoxide dismutase (SOD) is a primary antioxidant defence enzyme that converts superoxide radicals to hydrogen peroxide, which is subsequently decomposed by catalase (CAT) to limit oxidative damage (Nandi et al., 2019). In *Rauvolfia vomitoria* + PTZ-treated rats, reduced hydrogen peroxide accumulation suggests effective CAT-mediated antioxidant activity and protection against seizure-related oxidative injury. Glutathione (GSH), another key cellular antioxidant, was increased in this study, indicating minimal accumulation of reactive oxygen species. Malondialdehyde (MDA), a marker of lipid peroxidation, showed a non-significant decrease, further suggesting reduced oxidative stress in rats pretreated with *Rauvolfia vomitoria.*

Electrolyte analysis revealed no significant differences in sodium, potassium, chloride, calcium, or pH levels between *Rauvolfia vomitoria* pretreated rats and controls. While previous studies have suggested that *Rauvolfia vomitoria* may influence inhibitory neurotransmission (Amole et al., 2009), the present findings do not provide a direct relation to inhibitory effects. Further studies incorporating direct measurements of neurotransmitter activity and receptor function would be required to clarify these potential pathways.

Histomorphology examination of the prefrontal cortex and hippocampus revealed observable qualitative differences in tissue organisation between experimental groups. These findings are descriptive and were not supported by quantitative morphometric analyses. Consequently, interpretations related to neuroprotection, recovery, or functional preservation are beyond the scope of the present study. Our observations do not support earlier reports of dentate gyrus hypertrophy following administration of *Rauvolfia vomitoria* at comparable doses (Ekong et al., 2020). Differences in experimental design doses may account for these discrepancies.

Table 3. Pharmacokinetic analysis of docked compounds.

	Serpentinine	Alstonine	Ajmaline	Ajmalicine	Deserpindine	Geissoschizol	Reserpinine	Carbamazepine
Ames mutagenicity	Yes	Yes	No	No	No	No	No	No
Acute oral toxicity class (OECD)	III	III	II	III	II	III	III	III
Blood-brain barrier permeability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Biodegradability	No	No	No	No	No	No	No	No
Caco-2 permeability	No	Yes	Yes	Yes	No	Yes	No	Yes
Carcinogenicity (binary model)	No	No	No	No	No	No	No	No
Carcinogenicity (trinary model)	Not required	Not required	Not required	Not required	Not required	Not required	Not required	Not required
CYP1A2 inhibition	Yes	No	No	Yes	Yes	No	Yes	No
CYP2C19 inhibition	No	No	No	No	No	No	No	No
CYP2C9 inhibition	No	No	No	Yes	No	No	No	No
CYP2C9 substrate	No	No	No	No	No	No	No	Yes
CYP2D6 inhibition	No	Yes	Yes	Yes	No	No	No	No
CYP2D6 substrate	No	No	Yes	No	Yes	Yes	No	No
CYP3A4 inhibition	No	Yes	No	Yes	No	No	No	No
CYP3A4 substrate	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Hepatotoxicity	No	Yes	Yes	Yes	No	No	No	Yes
hERG inhibition	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Human intestinal absorption	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Human oral bioavailability	No	No	Yes	No	No	No	No	Yes
Predicted LD_{50} (mol/kg)	3.19	2.77	3.38	2.90	3.40	3.04	3.12	1.76
P-glycoprotein inhibitor	Yes	Yes	No	Yes	Yes	No	Yes	No
P-glycoprotein substrate	Yes	No	Yes	Yes	Yes	No	Yes	No

Table 4. Physicochemical properties and drug likeness (rule of five) of the docked compounds.

Compound	Chemical formula	Molecular weight (g/mol)	H-bond acceptors	H-bond donors	iLOGP	Lipinski's rule of five	Veber rule	Bioavailability score	Lead-likeness
Serpentinine*	$C_{42}H_{45}N_4O_5^+$	685.83	6	1.6	3.39	Yes (1 violation: MW > 500)	Yes	0.55	No (2 violations: MW >350, XLOGP3 >3.5)
Alstonine	$C_{21}H_{20}N_2O_3$	348.40	4	0	3.33	Yes (0 violations)	Yes	0.85	Yes
Ajmalicine	$C_{21}H_{24}N_2O_3$	352.43	4	1	3.18	Yes (0 violations)	Yes	0.55	No (1 violation: MW >350)
Ajmaline	$C_{20}H_{26}N_2O_2$	326.43	3	2	0.77	Yes (0 violations)	Yes	0.55	Yes
Deserpidine	$C_{32}H_{38}N_2O_8$	578.65	9	1	3.88	Yes (1 violation: MW >500)	Yes	0.55	No (3 violations: MW >350, rotatable bonds >7, XLOGP3 >3.5)
Geissoschizol	$C_{19}H_{24}N_2O$	296.41	2	2	2.69	Yes (0 violations)	Yes	0.55	Yes
Reserpinine	$C_{35}H_{42}N_2O_9$	634.72	10	1	4.98	No (2 violations: MW >500, H-bond acceptors >10)	No (1 violation: rotatable bonds >10)	0.17	No (3 violations: MW >350, rotatable bonds >7, XLOGP3 >3.5)
Carbamazepine*	$C_{15}H_{12}N_2O$	236.27	1	1	2.10	Yes (0 violations)	Yes	0.55	No (1 violation: MW <250)

* Druglike properties identified with highest and lowest binding scores.

Inhibition of NMDA receptors plays an important role in seizure therapy. Previous studies have shown that ketamine binds NMDA receptors through hydrophobic and hydrogen-bond interactions within the transmembrane domain (Zhang et al., 2021). In this study, serpentinine and related alkaloids exhibited high binding affinities, suggesting interactions with nonpolar residues in the GluN1-GluN2A NMDA receptor TMD, as observed with ketamine. These findings align with reports that alkaloids modulate NMDA receptor pathways (Kukuia et al., 2021).

Several *Rauvolfia vomitoria* alkaloids showed predicted BBB permeability, while multiple compounds were identified as P-gp substrates, which may limit brain exposure through efflux mechanisms. Drug metabolism was evaluated using in-silico predictions of cytochrome P450 (CYP450) interactions. Several *Rauvolfia vomitoria* alkaloids, as well as carbamazepine, were predicted to inhibit one or more major CYP450 isoenzymes, suggesting potential metabolic abilities. Pharmacokinetic and drug-likeness properties were assessed using *in-silico* predictions, which provide preliminary information on absorption, distribution, metabolism, excretion, and toxicity (ADMET; Bitew et al., 2021). Predicted human intestinal absorption and blood-brain barrier (BBB) permeability were used to estimate potential absorption and CNS distribution.

Moreover, the *in silico* toxicity screening indicated predicted hERG inhibition for all *Rauvolfia vomitoria* alkaloids, suggesting potential cardiotoxic liability. Hence, it is inconclusive to infer that *Rauvolfia vomitoria* alkaloid compounds indicate an immediate risk of heart attack; however, understanding the hERG channel-blocking effects of these compounds may reveal how well these alkaloids are used medicinally (Kiss et al., 2013). Predicted AMES mutagenicity was observed for serpentinine and alstonine, while hepatotoxicity signals were predicted for alstonine, ajmaline, ajmalicine, and carbamazepine. Physicochemical and drug-likeness properties were also evaluated using Lipinski's Rule of Five. The "Rule of Five" suggested by Lipinski is mostly used in screening for drug-like candidates, which state that a drug-like compound should have a molecular weight less than 500 Da (MW ≤500 kDa), an ILOGP less than 5.0, and a number of hydrogen bonds and acceptors less than 5 and 10, respectively, although many compounds in *Rauvolfia vomitoria* alkaloids abiding by the stated rules, are not drug-like. Therefore, compliance with these criteria alone does not indicate therapeutic suitability and should be interpreted with caution in this kind of early-stage investigation.

5.0 CONCLUSIONS

Alkaloid compounds in *Rauvolfia vomitoria* are promising bioactive candidates in seizure research. In this study, oral pretreatment with an aqueous extract of *Rauvolfia vomitoria* mitigated PTZ-induced seizures, though memory improvements observed in the novel object recognition test were not statistically significant. Biochemical analyses showed increased antioxidant markers, with no major changes in electrolyte levels. Molecular docking suggested serpentinine and other alkaloids could interact with the NMDA receptor; these predictions are hypothesis-generating and require experimental validation. Interpretation is limited by the small sample size, the use of an aqueous extract, and the absence of electrophysiological and quantitative histological validation. Future studies using isolated compounds and functional assays are needed to clarify the mechanisms and therapeutic relevance of *Rauvolfia vomitoria* in epilepsy.

Ethical Approval: Ethical approval was obtained at Olabisi Onabanjo University Teaching Hospital, Health Research and Ethics Committee, OUTHREC, Ogun State, Nigeria (approval details, ref: ID OOUTH/HREC/566/2022AP).

Data Availability: All data are available in the main text.

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Author Contributions: BO conceptualised the study; IO reviewed the study proposal; BO, IO and RF designed the study, BO performed the literature search; DU, AO and SB performed the data collection; BO performed the data analysis; BO wrote the first manuscript draft; AM, IO and RF edited the manuscript; RF designed and edited the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest associated with this study.

References

Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*, *105*, 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3

Amole, O. O., Yemitan, O. K., & Oshikoya, K. A. (2009). Anticonvulsant activity of *Rauvolfia vomitoria* (Afzel). *African Journal of Pharmacy and Pharmacology*, *3*(6), 319-322.

Anwar, H., Khan, Q. U., Nadeem, N., Pervaiz, I., Ali, M., & Cheema, F. F. (2020). Epileptic seizures. *Discoveries*, *8*(2), e110. https://doi.org/10.15190/d.2020.7

Bernardi, R. B., & Barros, H. M. T. (2004). Carbamazepine enhances discriminative memory in a rat model of epilepsy. *Epilepsia*, *45*(11), 1443–1447. https://doi.org/10.1111/j.0013-9580.2004.52403.x

Bitew, M., Desalegn, T., Demissie, T. B., Belayneh, A., Endale, M., & Eswaramoorthy, R. (2021). Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. *PLoS ONE*, *16*(12), e0260853. https://doi.org/10.1371/journal.pone.0260853

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: A comprehensive source and free tool for assessment of chemical ADMET properties. *Journal of Chemical Information and Modeling*, *52*(11), 3099–3105. https://doi.org/10.1021/ci300367a

Chipiti, T., Viljoen, A. M., Cordero-Maldonado, M. L., Veale, C. G. L., Van Heerden, F. R., Sandasi, M., Chen, W., Crawford, A. D., & Enslin, G. M. (2021). Anti-seizure activity of African medicinal plants: The identification of bioactive alkaloids from the stem bark of *Rauvolfia caffra* using an *in vivo* zebrafish model. *Journal of Ethnopharmacology*, *279*, 114282. https://doi.org/10.1016/j.jep.2021.114282

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 42717. https://doi.org/10.1038/srep42717

Egunlusi, A. O., & Joubert, J. (2024). NMDA receptor antagonists: Emerging insights into molecular mechanisms and clinical applications in neurological disorders. *Pharmaceuticals*, *17*(5), 639. https://doi.org/10.3390/ph17050639

Ekong, M. B., Bassey, O. O., Ebeh, D. I., Usukuma, G. D., Samuel, D. C., Bassey, R. B., Peter, A. I., Mbadugha, C. C., Okokon, J. E., & Akpanabiatu, M. I. (2024). *Rauvolfia vomitoria* phenol extract relieves pentylenetetrazol-induced seizures in Swiss mice and protects some temporal lobe structures. *Acta Epileptologica*, *6*(1), 35. https://doi.org/10.1186/s42494-024-00183-2

Ekong, M., Peter, A., Davies, K., Bassey, E., Aquaisua, A., Akpanabiatu, M., & Ekanem, T. (2013). *Gongronema latifolium ameliorates Rauwolfia vomitoria induced behavior, biochemicals, and histomorphology of the cerebral cortex*. [Conference presentation]. *Journal of Neurochemistry*, *125*(S1), 269. https://doi.org/10.1111/jnc.12186

Glasgow, S. D., McPhedrain, R., Madranges, J. F., Kennedy, T. E., & Ruthazer, E. S. (2019). Approaches and limitations in the investigation of synaptic transmission and plasticity. *Frontiers in Synaptic Neuroscience*, *11*, 20. https://doi.org/10.3389/fnsyn.2019.00020

Guskjolen, A., & Cembrowski, M. S. (2023). Engram neurons: Encoding, consolidation, retrieval, and forgetting of memory. *Molecular Psychiatry*, *28*(8), 3207–3219. https://doi.org/10.1038/s41380-023-02137-5

Harborne, J.B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis* (1st ed.). Chapman & Hall.

Hoxhaj, P., Habiya, S. K., Sayabugari, R., Balaji, R., Xavier, R., Ahmad, A., Khanam, M., Kachhadia, M. P., Patel, T., Abdin, Z. U., Haider, A., & Nazir, Z. (2023). Investigating the impact of epilepsy on cognitive function: A narrative review. *Cureus*, *15*(6), e41223. https://doi.org/10.7759/cureus.41223

Kiss, T., Orvos, P., Bánsághi, S., Forgo, P., Jedlinszki, N., Tálosi, L., Hohmann, J., & Csupor, D. (2013). Identification of diterpene alkaloids from *Aconitum napellus* subsp. *firmum* and GIRK channel activities of some *Aconitum* alkaloids. *Fitoterapia*, *90*, 85–93. https://doi.org/10.1016/j.fitote.2013.07.010

Kukuia, K. K. E., Mensah, J. A., Amoateng, P., Osei-Safo, D., Koomson, A. E., Torbi, J., Adongo, D. W., Ameyaw, E. O., Ben, I. O., Amponsah, S. K., Bugyei, K. A., & Asiedu-Gyekye, I. J. (2021). Glycine/NMDA receptor pathway mediates the rapid-onset antidepressant effect of alkaloids from *Trichilia monadelpha*. *Basic and Clinical Neuroscience*, *12*(3), 395–408. https://doi.org/10.32598/bcn.12.3.2838.1

Leone, M. A., Giussani, G., Nevitt, S. J., Marson, A. G., & Beghi, E. (2021). Immediate antiepileptic drug treatment, versus placebo, deferred, or no treatment for first unprovoked seizure. *The Cochrane Database of Systematic Reviews*, *2021*(5), CD007144. https://doi.org/10.1002/14651858.CD007144.pub3

Lueptow, L. M. (2017). Novel object recognition test for the investigation of learning and memory in mice. *Journal of Visualized Experiments*, *126*, 55718. https://doi.org/10.3791/55718

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*, *247*(10), 3170–3175.

Nandi, A., Yan, L.-J., Jana, C. K., & Das, N. (2019). Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative Medicine and Cellular Longevity*, *2019*, 9613090. https://doi.org/10.1155/2019/9613090

OECD. (2002). *Guidance Document on Acute Oral Toxicity Testing*. OECD. https://doi.org/10.1787/9789264078413-en

Oghenesuvwe, E. E., Ekene, N. E., & Lotanna, A. D. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research*, *4*(18), 100.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Parsons, A. L. M., Bucknor, E. M. V., Castroflorio, E., Soares, T. R., Oliver, P. L., & Rial, D. (2022). The interconnected mechanisms of oxidative stress and neuroinflammation in epilepsy. *Antioxidants*, *11*(1), 157. https://doi.org/10.3390/antiox11010157

Perucca, E., Bialer, M., & White, H. S. (2023). New GABA-targeting therapies for the treatment of seizures and epilepsy: I. Role of GABA as a modulator of seizure activity and recently approved medications acting on the GABA system. *CNS Drugs*, *37*(9), 755–779. https://doi.org/10.1007/s40263-023-01027-2

Sanya, E. O., Soladoye, A. O., Desalu, O. O., Kolo, P. M., & Olatunji, L. A. (2016). Antiseizure effects of ketogenic diet on seizures induced with pentylenetetrazole, 4-aminopyridine and strychnine in Wistar rats. *Nigerian Journal of Physiological Sciences*, *31*(2), 115-119.

Shan, T., Zhu, Y., Fan, H., Liu, Z., Xie, J., Li, M., & Jing, S. (2024). Global, regional, and national time trends in the burden of epilepsy, 1990–2019: An age-period-cohort analysis for the global burden of disease 2019 study. *Frontiers in Neurology*, *15*, 1418926. https://doi.org/10.3389/fneur.2024.1418926

Shimada, T., & Yamagata, K. (2018). Pentylenetetrazole-induced kindling mouse model. *Journal of Visualized Experiments*, *136*, 56573. https://doi.org/10.3791/56573

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa* (2nd ed.). Spectrum Books.

Sridhar, S., Khamaj, A., & Asthana, M. K. (2023). Cognitive neuroscience perspective on memory: Overview and summary. *Frontiers in Human Neuroscience*, *17*, 1217093. https://doi.org/10.3389/fnhum.2023.1217093

Trease, G.E. and Evans, W.C. (1989). *A textbook of pharmacognosy* (13th ed.). Baillière Tindall.

Zhang, Y., Ye, F., Zhang, T., Lv, S., Zhou, L., Du, D., Lin, H., Guo, F., Luo, C., & Zhu, S. (2021). Structural basis of ketamine action on human NMDA receptors. *Nature*, *596*(7871), 301–305. https://doi.org/10.1038/s41586-021-03769-9