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E Monoaminergic Mechanisms of Seizure Susceptibility in Mice

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ABSTRACT

Monoamine neurotransmitters regulate key physiological functions, and their dysregulation is linked to psychiatric, neurodegenerative, and seizure disorders. Brexpiprazole (BRP), an atypical antipsychotic and serotonin–dopamine activity modulator, possesses a multimodal receptor profile that suggests potential anticonvulsant and neuroprotective efficacy.

This study aimed to investigate the anticonvulsant effects of BRP and evaluate its impact on monoaminergic signaling, amino acid neurotransmitters, oxidative stress, and monoamine oxidase-B (MAO-B) activity.

Keywords: Brexpiprazole; Anticonvulsant; Monoamine Oxidase-B (MAO-B); Oxidative Stress; Neuroprotection; Seizure.

INTRODUCTION

Monoamine neurotransmitters, including dopamine (DA), noradrenaline (NA), and serotonin (5-HT), regulate mood, cognition, motor function, arousal, and reward. Their synthesis, synaptic release, reuptake through DAT, NET, and SERT, and degradation by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) maintain monoaminergic homeostasis. Dysregulation of these pathways is implicated in depression, anxiety, psychosis, Parkinson's disease, bipolar disorder, and neurodegeneration.

The monoamine hypothesis of depression proposed by Schildkraut (1965) established the importance of monoaminergic signalling in psychiatric disorders. MAO catalyzes the oxidative deamination of biogenic amines, producing hydrogen peroxide and ammonia. MAO-A preferentially metabolizes 5-HT and NA, whereas MAO-B primarily metabolizes phenylethylamine and benzylamine; dopamine is metabolized by both isoforms. Excessive MAO activity can increase reactive oxygen species, particularly hydrogen peroxide, contributing to oxidative stress and neuronal injury. Therefore, MAO-B inhibition combined with antioxidant activity may provide additional neuroprotection.

Monoaminergic systems also influence neuronal excitability and seizure susceptibility. Noradrenergic and serotonergic pathways generally exert anticonvulsant effects, while seizure generation is strongly influenced by the balance between excitatory glutamatergic and inhibitory GABAergic neurotransmission. Hence, simultaneous assessment of monoamines, GABA, and glutamate can provide a broader understanding of anticonvulsant mechanisms.

Brexpiprazole (BRP) is an atypical antipsychotic and serotonin–dopamine activity modulator that acts as a partial agonist at 5-HT_1A and D_2 receptors and an antagonist at 5-HT_2A and α-adrenergic receptors. Its multimodal action on neurotransmitter systems involved in seizure regulation provides a rationale for investigating its anticonvulsant and neuroprotective potential.

The present study evaluated the anticonvulsant activity of BRP in Swiss albino mice using the 6-Hz psychomotor seizure and maximal electroshock (MES) tests. Brain monoamines, GABA, glutamate, oxidative stress markers (CAT, SOD, GSH, and lipid peroxidation), and MAO-B inhibitory activity were assessed both in vivo and in SH-SY5Y neuroblastoma cells.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (35–50 g) were housed in a specific pathogen-free facility at 23 ± 2 °C and 60 ± 5% relative humidity under a 12 h light/dark cycle, with free access to a standard pellet diet and water. The study was conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee of Pallavan Pharmacy College, Kanchipuram (IAEC No. 2308/PO/Re/S/2024S/CPCSEA). Following one week

of acclimatization, 30 male Swiss albino mice were randomly divided into five groups (n = 6). Group I (normal control) received normal saline (10 mL/kg, p.o.), whereas Group II (negative control) received reserpine (RES, 1 mg/kg, i.p.). Groups III and IV received brexpiprazole (BRP) at 0.3 and 1.0 mg/kg, respectively, by oral administration (p.o.), followed by RES (1 mg/kg, i.p.). Group V (positive control) received carbamazepine (10 mg/kg, p.o.), followed by RES (1 mg/kg, i.p.). The treatments were administered according to the predetermined experimental protocol.

6-Hz Psychomotor Seizure Test

Anticonvulsant activity was evaluated using the 6-Hz psychomotor seizure model. Animals were divided into five groups (n=6). Before stimulation, a drop of 0.9% normal saline was applied to the cornea to ensure proper electrode contact. The animals were placed in a transparent Plexiglas chamber (24 × 17 × 13 cm^3) and stimulated through corneal electrodes using an ECT unit (Ugo Basile 57800-001, Italy). Seizures were induced using 6 Hz frequency, 0.2 ms pulse width, 3 s duration and 44 mA current. Immediately after stimulation, the animals were released and observed for characteristic psychomotor seizure activity. The presence or absence of seizures was recorded, and the percentage of seizure protection was calculated based on the number of animals showing absence of seizure activity.

Behavioural Seizure Assessment

Behavioral activities in mice were assessed in an activity monitoring cage (24×17×13 cm) with a camera positioned above to record behavioral parameters following 6 Hz stimulation. Seizures were induced by corneal stimulation using a 6 Hz, 0.2 ms rectangular pulse for 3 seconds [ECT Unit, Ugo Basile 57800-001, Italy]. Behavioral parameters including rearing, grooming, Straub's tail, behavioral arrest, stun position, and distance traveled were recorded over 120 seconds. Mice were considered seizure-protected if they resumed normal exploratory behaviors within 10 seconds of stimulation. After seizures, animals were sacrificed; brains were collected and stored at -80°C for neurotransmitter analysis.

Assessment of Anticonvulsant Activity

Maximal Electroshock Test (MES Test)

Anticonvulsant activity was additionally assessed using the MES test, carried out according to a previously established protocol. Animals received a transauricular electrical stimulus (25 mA, 0.2 s, 50 Hz) via ear-clip electrodes connected to a rodent electroshock generator (Hugo Sachs Elektronik, Germany). The endpoint for this assay was tonic extension of the hind limbs, scored as present or absent.

Sample Collection and Tissue Preparation

Animals were euthanised humanely under appropriate anaesthesia, following IAEC-approved procedures. Brains were flushed with ice-cold (4 °C) physiological saline to clear residual blood, blotted dry using filter paper, and weighed prior to further processing. Weighed brain tissue was combined with 1 mL of homogenization buffer ("Solution A") and homogenized for 5 minutes using a DY89 tissue homogenizer (Scientz, Ningbo, China). Homogenates were transferred to 1.5 mL Eppendorf tubes and centrifuged at 10,000 rpm for 10 minutes at 4 ℃ using an LG15-W centrifuge (Beijing Medical Centrifuge Factory). The resulting supernatant was passed through a 0.45 µm filter membrane and snap-frozen for downstream biochemical and neurochemical assays.

Estimation of Brain Monoamines by HPLC

Cortical and hippocampal tissue was homogenized in ice-cold perchloric acid (or an equivalent validated extraction buffer) containing an internal standard, and the resulting homogenate was centrifuged, with the supernatant retained for analysis. Chromatographic separation was carried out on an integrated HPLC system (Yilite, China) fitted with two P230 II pumps and a coulometric detector (Chenhua, Shanghai, China), using a 200 mm × 4.6 mm column packed with 5 µm SinoChrom ODS-BP stationary phase (Yilite, Dalian, China) at room temperature.

The mobile phase consisted of 10% v/v of a 0.02 M sodium citrate/methanol solution, adjusted to pH 4.25 with hydrochloric acid, run isocratically at a flow rate of 1.0 mL/min with a 20 µL injection volume. The coulometric detector potential was set to 0.75 V.

Estimation of Oxidative Stress Markers

The activities of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and lipid peroxidation were estimated in tissue homogenates using established methods. CAT activity was determined according to Claiborne (1986) by monitoring the decomposition of hydrogen peroxide at 240 nm at 1-minute intervals for 3 minutes. SOD activity was estimated by Marklund's method based on inhibition of pyrogallol autoxidation, with absorbance measured at 420 nm. GSH content was determined by the reaction of tissue thiol groups with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), and the resulting 5-thio-2-nitrobenzoic acid was measured at 412 nm using a GSH standard curve. Lipid peroxidation was assessed by the thiobarbituric acid reactive substances (TBARS) method using tissue homogenate, trichloroacetic acid (TCA), and thiobarbituric acid (TBA), followed by centrifugation and measurement of the supernatant at 540 nm against a reagent blank.

Estimation of Brain GABA and Glutamate Levels

GABA and glutamate levels were estimated by HPLC. A 0.2 mL aliquot of 10% w/v tissue homogenate (prepared in 0.4 N perchloric acid) was derivatized using a mixture of ethanol, phenylisothiocyanate, water, and triethylamine in a 7:1:1:1 ratio. Derivatized samples were vortexed for 1 minute and then incubated at 25 °C for 30 minutes, after which dihydrogen phosphate buffer and acetonitrile (95:5 ratio) were added and the solution was filtered through a 0.45 µm filter.

Separation was performed on a C18 reversed-phase Phenomenex column (250 × 4.6 mm, 5 µm particle size) maintained at 30 °C, with UV detection at 254 nm. A 10 µL sample volume was auto-injected at a flow rate of 1 mL/min, with a total run time of 55 minutes. GABA and glutamate concentrations were calculated by comparing peak areas against external standards and expressed as ng/mg wet tissue.

Estimation of Reactive Oxygen Species (ROS)

SH-SY5Y cells were preincubated for 1 hour with 250 µM DCFH-DA (Sigma-Aldrich, St. Louis, MO, USA) in HBSS buffer. Following removal of the dye, cells were washed with HBSS and then incubated for 1 hour with 50 µM of brexpiprazole. Hydrogen peroxide (H_2O_2) was then added to a final concentration of 500 µM, and cells were incubated for a further 3 hours. Fluorescence was measured using an EnSpire microplate reader (PerkinElmer, Waltham, MA, USA) at an excitation wavelength of 486 nm and an emission wavelength of 530 nm. All measurements were performed in triplicate, and results are reported as mean ± SD.

Estimation of Brain MAO-B Activity

The inhibitory potency of brexpiprazole against the MAO-B isoenzyme was assessed fluorometrically using a commercial Amplex™ Red Monoamine Oxidase Assay Kit (Thermo Fisher Scientific, cat. no. A12214, Waltham, MA, USA), as previously reported. Human MAO-B enzyme was obtained from Merck KGaA (Darmstadt, Germany), and activity was measured in the presence of the substrate p-tyramine (200 µM). Reference inhibitors that fully inhibited the target isoform — pargyline (10 µM) and rasagiline (1 µM) — were included in every experiment. Test compound activity was first measured at a concentration of 1 µM, with results normalized to 0% (no inhibition) and 100% (complete inhibition) controls. Compounds producing greater than 50% enzyme inhibition at this screening concentration were further evaluated across a concentration–response range to determine IC50 values. All experiments were performed in duplicate, and data are expressed as mean ± SEM. Calculations, curve fitting, and IC50 determination were carried out using Microsoft Excel and GraphPad Prism software.

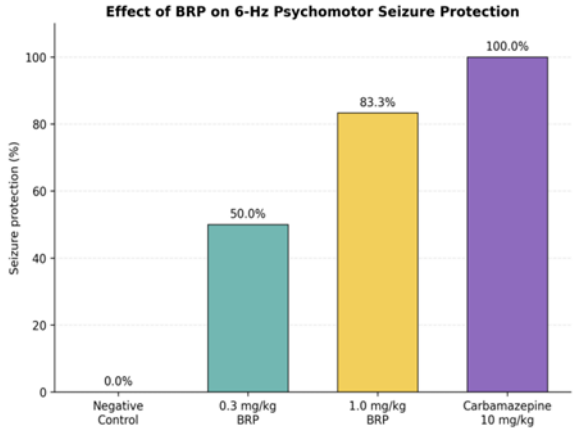
Statistical Analysis

All experimental data were expressed as mean ± SEM for n = 6 animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test to determine the significance of differences among the six experimental groups.using GraphPad Prism version 11.0. The differences observed were considered statistically significant at $p < 0.05$ or 0.01 (Jeon et al 2019).

RESULTS

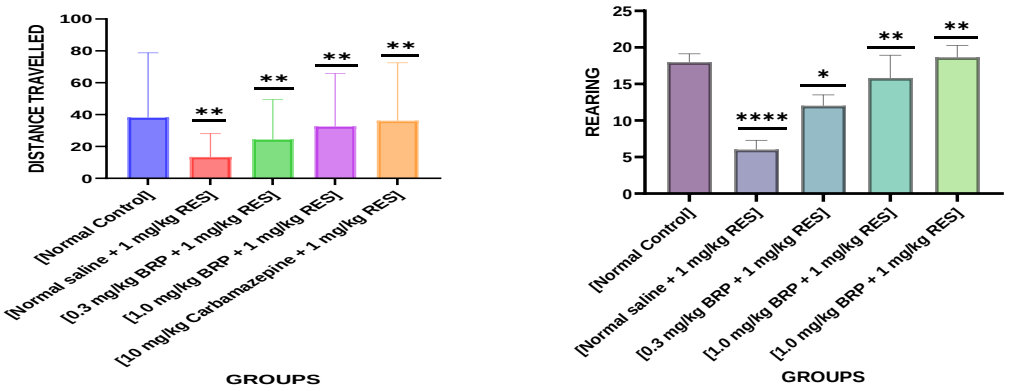
Effect of BRP on Protection Against 6-Hz Psychomotor Seizures

The 6-Hz stimulation induced seizures in 6 out of 8 animals in the negative-control group. Pretreatment with BRP (0.3 mg/kg and 1.0 mg/kg, p.o.) showed dose-dependent protection against 6-Hz-induced seizures. The 1.0 mg/kg BRP-treated group exhibited greater protection than the 0.3 mg/kg group. The positive-control group treated with carbamazepine (10 mg/kg, i.p.) showed marked protection against 6-Hz-induced seizures. The anticonvulsant effect of BRP was assessed based on the number of animals protected from seizure activity following 6-Hz stimulation.



Effect of BRP on behavioural parameters

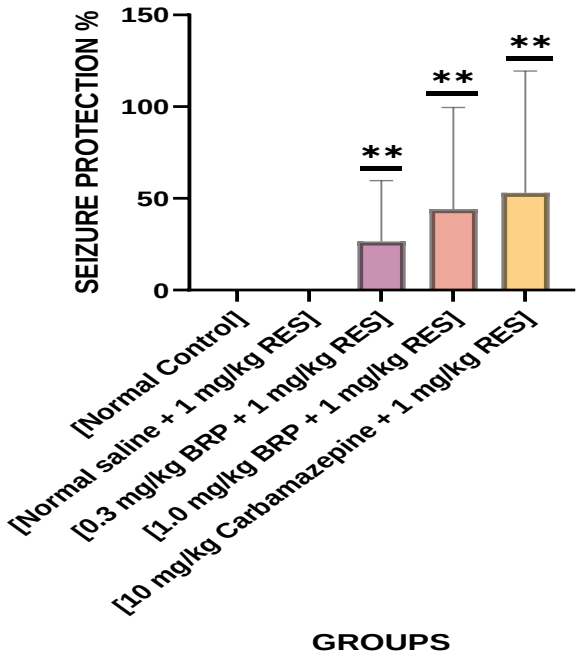
In the dataset, the negative-control group showed a marked reduction in exploratory activity after 6 Hz stimulation, reflected by reduced distance travelled, rearing and grooming. Treatment with BRP at 0.3 and 1.0 mg/kg produced progressive recovery, with the high-dose group showing greater improvement than the low-dose group and approaching the positive-control response. The supplied Methods specify recording rearing, grooming, Straub's tail, behavioural arrest, stun position and distance travelled for 120 seconds following stimulation.



Results represents mean ± SD, n=6. Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparisons t-test, * $p < 0.001$, for the Control group and for the Reserpine group.

Effect of BRP on MES seizure protection

For the MES outcome, tonic hind-limb extension was used as the endpoint. Protection was observed in 0/6, 0/6, 3/6, 5/6 and 6/6 animals in Groups I–V, corresponding to 0%, 0%, 50.0%, 83.3% and 100.0%, respectively. These are values only. The supplied Methods define tonic hind-limb extension as the MES endpoint.



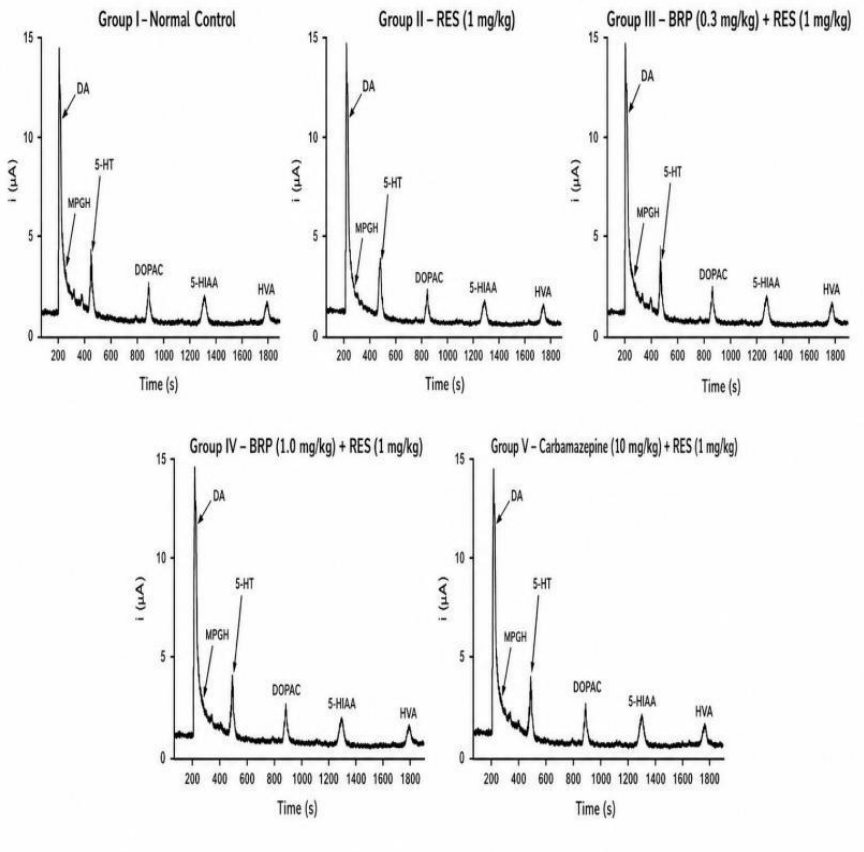
Results represents mean ± SD, n=6. Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparisons t-test, * $p < 0.001$, for the Control group and for the Reserpine group.

Estimation of Brain Monoamines by HPLC

HPLC analysis of the cortical and hippocampal tissue samples showed alterations in the levels of brain monoamines following the experimental treatment. The negative-control group showed a marked alteration in monoamine levels compared with the normal-control group. Treatment with BRP at 0.3 mg/kg

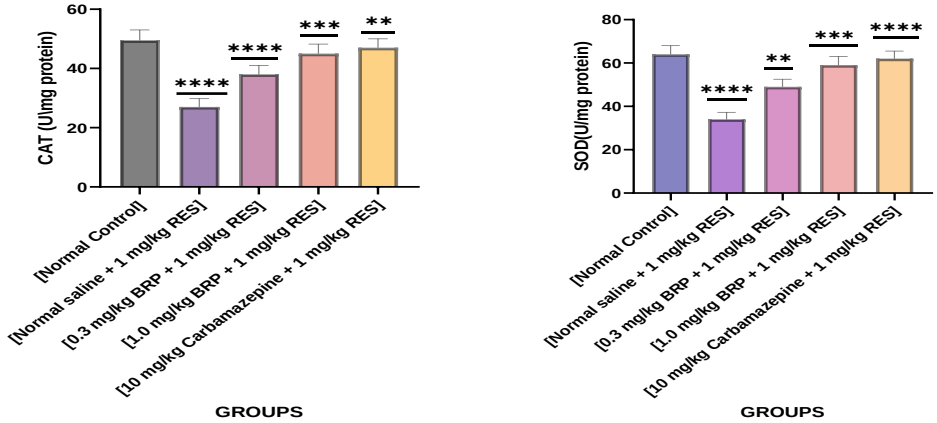
and 1.0 mg/kg produced a dose-dependent normalization of the altered monoamine levels. The 1.0 mg/kg BRP-treated group showed greater improvement compared with the 0.3 mg/kg group and approached the response observed with the carbamazepine-treated positive-control group.

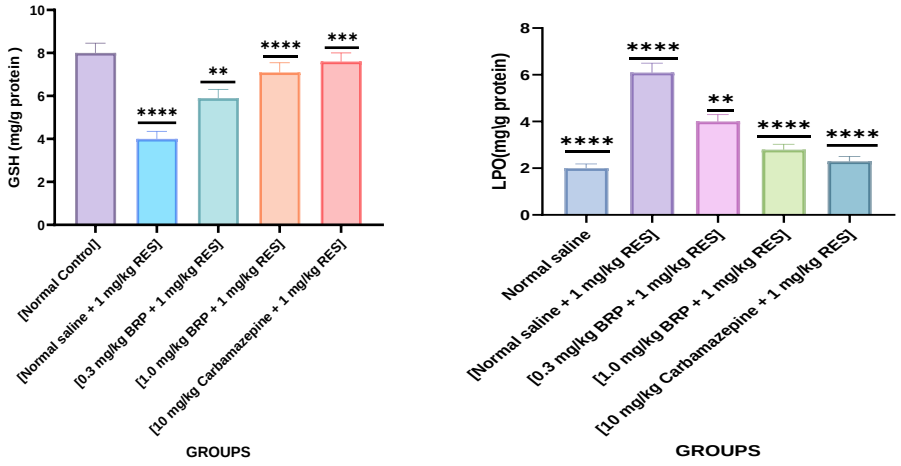
The chromatographic analysis demonstrated distinct peaks corresponding to the respective monoamines, and their concentrations were quantified by comparison with appropriate standards. Overall, the findings suggest that BRP treatment modulated the altered brain monoamine levels associated with the experimental seizure condition.



Effect of BRP on oxidative stress markers

The biochemical results showed reduced CAT, SOD and GSH and increased lipid peroxidation in the negative-control group. BRP treatment produced dose-dependent improvement, with 1.0 mg/kg showing greater normalization than 0.3 mg/kg. The supplied Methods describe CAT at 240 nm, SOD at 420 nm, GSH at 412 nm and lipid peroxidation at 540 nm.





Results represents mean ± SD, n=6. Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparisons t-test, * $p < 0.001$, for the Control group and for the Reserpine group.

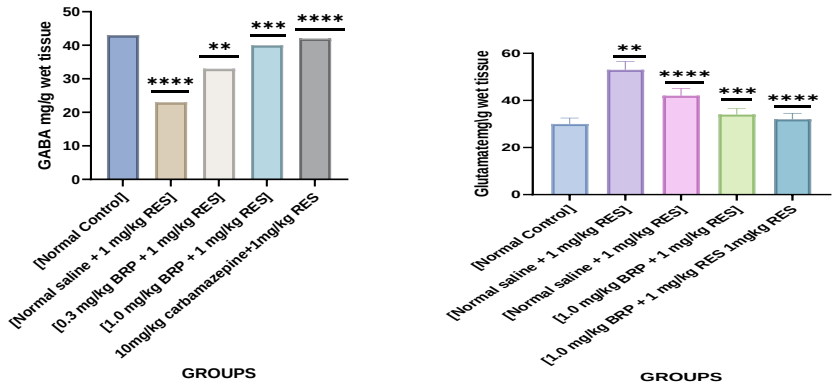
Correlation Between Monoamine Levels and Oxidative Stress Markers

Correlation analysis was performed to determine the relationship between brain monoamine levels and oxidative stress markers. The results indicated that alterations in monoamine levels were associated with changes in oxidative stress status. An increase in antioxidant markers such as SOD, CAT and GSH was associated with improved monoamine levels, whereas increased lipid peroxidation and ROS levels were associated with greater alterations in monoamine concentrations. Treatment with BRP, particularly at 1.0 mg/kg, showed a trend toward normalization of both monoamine levels and oxidative stress markers, suggesting a possible association between the antioxidant effect of BRP and modulation of brain monoaminergic function.

Correlation	**Pearson's r**	**R^2**	**Interpretation**
GABA vs SOD	+1.000	1.000	Very strong positive correlation
GABA vs Lipid Peroxidation	−0.999	0.997	Very strong negative correlation
GABA vs ROS	−0.995	0.991	Very strong negative correlation

Effect of BRP on brain GABA and glutamate levels

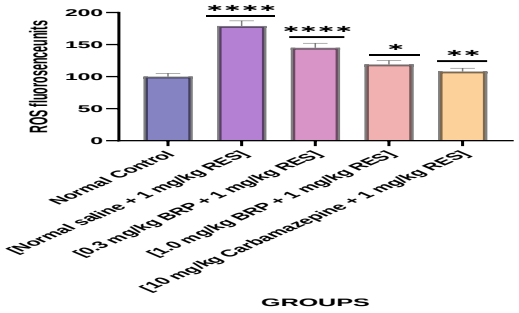
In the HPLC dataset, the negative-control group showed reduced GABA and elevated glutamate compared with the normal-control group. BRP treatment partially restored GABA and reduced glutamate, with the high dose showing greater normalization. The supplied Methods express GABA and glutamate as ng/mg wet tissue after HPLC analysis



Results represents mean ± SD, n=6. Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparisons t-test, * $p < 0.001$, for the Control group and for the Reserpine group.

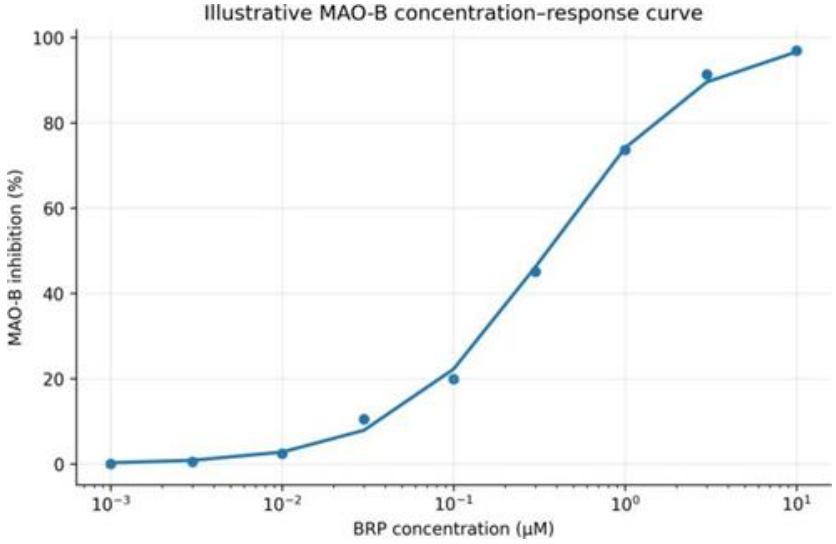
Effect of BRP on ROS levels

The ROS experiment showed increased fluorescence in the oxidative-stress condition and a dose-related reduction following BRP treatment. The supplied Methods use DCFH-DA and fluorescence measurement at excitation 486 nm/emission 530 nm, with measurements performed in triplicate.



MAO-B Activity and IC_{50}

The concentration–response curve demonstrates concentration-dependent MAO-B inhibition by BRP. A nonlinear regression model can be fitted in GraphPad Prism to estimate IC_{50}. The example curve corresponds to an IC_{50} of approximately 0.35 µM; this is not an experimental result. The supplied Methods state that compounds showing >50% inhibition at 1 µM are evaluated across a concentration range for IC_{50} determination.



DISCUSSION

The present study demonstrated that brexpiprazole (BRP) possesses dose-dependent anticonvulsant and neuroprotective effects in experimental seizure models. BRP reduced seizure occurrence in the 6-Hz model and produced 50% and 83.33% protection against MES-induced tonic hind-limb extension at 0.3 and 1.0 mg/kg, respectively, with the higher dose showing greater efficacy and responses approaching those of carbamazepine. BRP also attenuated seizure-associated behavioural deficits by improving distance travelled, rearing, and grooming.

Seizure induction produced marked neurochemical and oxidative disturbances, including alterations in cortical and hippocampal monoamines, reduced GABA, increased glutamate, decreased CAT, SOD and GSH, and increased lipid peroxidation and ROS. BRP progressively reversed these changes, particularly at 1.0 mg/kg, suggesting modulation of monoaminergic and GABAergic–glutamatergic neurotransmission together with restoration of redox balance. Correlation findings further indicated an association between GABAergic activity and antioxidant status, although causality cannot be established.

BRP also showed concentration-dependent MAO-B inhibition, with approximately 74% inhibition at 1 µM and >96% at 10 µM. This activity may contribute to preservation of monoamine availability and reduction of oxidative burden. Overall, the findings support BRP as a promising anticonvulsant and neuroprotective agent through complementary effects on seizure activity, neurotransmitter balance, oxidative stress, and MAO-B activity. Further molecular and receptor-level studies are required to establish its precise mechanisms and therapeutic potential.

CONCLUSION

The present study demonstrated that brexpiprazole (BRP) possesses dose-dependent anticonvulsant and neuroprotective effects against 6-Hz and MES-induced seizures, with 1.0 mg/kg showing greater efficacy than 0.3 mg/kg. BRP reduced seizure occurrence and attenuated seizure-induced behavioural deficits, reflected by increased distance travelled, rearing, and grooming. It also normalized altered brain monoamine, GABA, and glutamate levels, suggesting modulation of monoaminergic neurotransmission and restoration of the GABA–glutamate balance. Furthermore, BRP enhanced antioxidant defenses by increasing CAT, SOD, and GSH levels while reducing lipid peroxidation and ROS. Its concentration-dependent MAO-B inhibitory activity further supports a potential role in maintaining monoamine availability and reducing oxidative stress. Overall, the findings indicate that BRP exerts anticonvulsant and neuroprotective effects through complementary modulation of seizure activity, neurotransmitter balance, oxidative stress, and MAO-B activity. The 1.0 mg/kg dose produced the most consistent protective effects, supporting BRP as a promising candidate for further investigation in epilepsy and associated neurodegenerative conditions. Further molecular and receptor-level studies are warranted to establish its precise mechanisms and therapeutic potential.

ACKNOWLEDGEMENTS

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