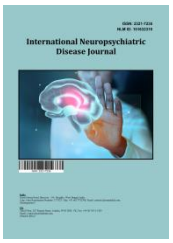

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Neuroprotective Effects of *Bryophyllum pinnatum* against Ketamine-Induced Neurotoxicity in Wistar Rats: Neurochemical, Oxidative Stress, and Histological Investigation

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: This study evaluated the neuroprotective effects of *Bryophyllum pinnatum* (*B. pinnatum*) in ketamine-induced neurotoxicity in Wistar rats, focusing on neurochemical and oxidative stress markers, as well as histological changes in the hippocampus and cerebral cortex.

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Methods: Sixty male Wistar rats were divided into six groups. Group 1 received normal saline (2ml), Group 2 was administered ketamine (20mg/kg), and Group 3 received risperidone (0.5mg/kg). Groups 4, 5, and 6 were treated with *B. pinnatum* at 50mg/kg, 100mg/kg, and 200mg/kg, respectively for a duration of 21 days after induction of toxicity. Neurochemical markers (dopamine, glutamate, GABA, serotonin, noradrenaline, acetylcholine), oxidative stress markers (GSH, MDA, SOD, CAT, GPx), and histological analysis of brain tissues were evaluated after treatment periods of one, two, and three weeks.
Results: Ketamine administration significantly reduced dopamine, GABA, serotonin, and noradrenaline levels while increasing acetylcholine and glutamate levels ($p<0.05$). *B. pinnatum* treatment reversed these effects in a dose-dependent manner, particularly at high doses. Oxidative markers showed reduced GSH and increased MDA levels with ketamine, which *B. pinnatum* mitigated significantly ($p<0.05$). Histologically, ketamine caused neuronal degeneration and microstructural distortion in the hippocampus and cerebral cortex, which were markedly reduced by *B. pinnatum*, especially at 200mg/kg, showing well-preserved neuronal structures.
Conclusion: *B. pinnatum* demonstrates significant neuroprotective effects against ketamine-induced neurotoxicity by modulating neurochemical and oxidative markers and preserving histological integrity in brain tissues, suggesting its potential therapeutic application in neurotoxic conditions.

Keywords: Bryophyllum pinnatum; N-methyl-D-aspartate (NMDA); ketamine; neurotoxicity; neuroprotection; oxidative stress; histology.

1. INTRODUCTION

Psychosis is a severe mental condition characterized by impaired thinking, emotions, and behaviors, often resulting in a disconnect from reality [1]. Ketamine, originally developed as an anesthetic, has gained attention for its rapid-acting antidepressant properties but is also known for inducing a transient psychosis-like state, especially at sub-anesthetic doses [2,3]. This has led to its recreational abuse and raised concerns about potential neurotoxic effects, particularly in chronic users [4,5].

Ketamine's mechanism of action involves antagonism of the N-methyl-D-aspartate (NMDA) receptors in the brain, which disrupts normal neurotransmission pathways [3,6-9]. Studies in rodents have consistently shown that ketamine administration can lead to neurochemical imbalances, oxidative stress, and structural changes in the brain, resembling aspects of neurodegenerative disorders. Zou *et al*. [10] stated that ketamine-induced cell death appears to be apoptotic in nature and closely associated with enhanced NMDA receptor subunit mRNA expression. According to Pascual-Antón *et al*. [11], alterations observed in the frontal cortex and dorsal raphe nucleus (DRN) regions, specifically increased fractional anisotropy (FA), are linked to ketamine's rapid antidepressant effects in rodents. The study posits that these changes in FA within the IL and DRN regions may signify a response to antidepressant therapy. However, a review by Choi *et al*. [12] reported ketamine to have complex and variable effects on fear memory in rodent models.

Given the detrimental effects associated with ketamine use, there is a critical need to explore interventions that can mitigate its neurotoxicity or manage psychosis effectively. Herbal products have emerged as promising candidates due to their rich composition of phytochemicals and antioxidants, which can potentially counteract oxidative stress and modulate neurotransmitter systems disrupted by ketamine.

One such herb is *Bryophyllum pinnatum* (*B. pinnatum*), a succulent plant native to tropical Africa, Asia, and America. *B. pinnatum*, also known as "life plant" or "miracle leaf," is renowned for its pharmacological properties attributed to bioactive compounds like alkaloids, flavonoids, phenolics, and saponins [13]. These compounds have been reported to possess antioxidant, anti-inflammatory, and neuroprotective properties in various studies [14-16].

B. pinnatum's phytochemical profile, encompassing alkaloids known for neuroprotective and analgesic effects [14], flavonoids as potent antioxidants combating free radicals [17], phenolics with anti-inflammatory properties enhancing antioxidant defenses [14], and saponins with diverse pharmacological activities including anti-inflammatory and

cytotoxic effects [18], collectively underscores its therapeutic potential in mitigating oxidative stress-related conditions and neurological disorders.

Due to its reported anti-inflammatory, and neuroprotective effects, and its traditional use in managing inflammatory conditions, and neurological ailments such as Alzheimer's disease and Parkinson's disease, *B. pinnatum* was considered a promising candidate for investigating its neuroprotective capabilities against ketamine-induced neurotoxicity in Wistar rats. This study aimed to assess the effect of *B. pinnatum* potentials in mitigating neurochemical, oxidative stress, and histological integrity in key brain regions in ketamine-induced neurotoxicity in Wistar rats providing insights into its therapeutic application for mitigating ketamine-induced toxicity and associated psychiatric conditions.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Sixty (60) adult male Wistar rats, weighing 180–200g, were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The rats were housed in clean, disinfected wooden cages with sawdust bedding, maintained on a 12-hour light/dark cycle, 50–60% humidity, and a temperature of approximately 30°C. They were allowed to acclimatize for two weeks with free access to clean water and animal feed before the commencement of the experiment.

2.2 Chemicals and Plant used

Fresh leaves of *B. pinnatum* were collected at the back of Ofrima Building within the Abuja part of the University of Port Harcourt environment. The plant was identified and authenticated in the Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, by Dr. Edwin Nwonsu, and assigned voucher number UPH/V/1308. ketamine and risperidone were purchased from Alpha Pharmacy and Stores, located on NTA Road, Port Harcourt, Rivers State, Nigeria.

2.3 Preparation of *B. pinnatum* extract

The plant tissue homogenization method, following the procedure described by Pandey and Tripathi [19], was utilized to extract fresh plant juice from *B. pinnatum* leaves. The fresh leaves were ground into fine particles using a blender, and the juice was extracted and filtered through a white handkerchief, as outlined by Das *et al*. [20]. The resulting juice was collected meticulously and stored in clean reagent bottles, which were then refrigerated for preservation.

2.4 Dose selection of Ketamine, Risperidone, and *B. pinnatum* extract

The doses of ketamine (20 mg/kg) and risperidone (0.5 mg/kg) used in this study were adopted from recommendations by Monte *et al*. [21] and Ben-Azu *et al*. [22], respectively. Sub-lethal doses of *B. pinnatum* crude extract (50, 100, 200 mg/kg body weight) were administered based on guidelines from Salahdeen & Yemitan [23]. Prior to the study, a preliminary dose-experiment was conducted to determine the weight (mg/mL) of *B. pinnatum*.

2.5 Experimental Design

The protocol was designed and modified based on the established method by Monte *et al*. [21]. The research was conducted in two distinct phases;

- **Induction phase:** The sixty (60) animals were randomly assigned to two groups. Group 1 consisted of 12 animals (n=12) and was administered 2ml of distilled water once daily for 7 days. On the other hand, Group 2 comprised 48 animals (n = 48) and received a sub-anesthetic dose of ketamine (20mg/kg) once daily intraperitoneally for 7 days. Three (3) animals were sacrificed from each group on the 7th day, and blood samples, as well as tissues, were collected for biochemical and histological examinations aimed at establishing the toxicity in the animal model.

- **Intervention Phase:** In phase 2, Group 1, originally from phase 1, served as the control and continued to receive 2 mL of distilled water (vehicle-treated) once daily for an extended period of 21 days. Meanwhile, Group 2 from phase 1, consisting of 45 animals, was randomly assigned to five groups of nine animals each (n=9) for further interventions. Specifically, Group 2 continued to be treated with a sub-anesthetic dose of

ketamine (20 mg/kg) once daily intraperitoneally for the next 21-day period to maintain the toxicity model, enabling assessment of the therapeutic and protective effects of risperidone and *B. pinnatum* extracts against ongoing ketamine-induced damage. This ensures observed changes are due to interventions rather than natural recovery, providing a clearer evaluation of their efficacy. Group 3, designated as the positive control, received risperidone (0.5 mg/kg, p.o.) once daily for the same 21-day duration. Groups 4 to 6 were treated with graded doses of *B. pinnatum* extract: Group 4 received 50 mg/kg body weight (low dose), Group 5 received 100 mg/kg body weight (medium dose), and Group 6 received 200 mg/kg body weight (high dose) orally once daily for the same 21-day period.

2.6 Collection of Blood and Tissue Sample

Twenty-four (24) hours after the final treatments on the 8th, 15th, and 22nd days of the experimental period, the animals were anesthetized with diethyl ether and then euthanized. Blood samples were collected via cardiac puncture, and brain tissues were carefully harvested for biochemical assays and histological examination.

2.7 Biochemical Analysis

Tissue samples were collected to assess the impact of *B. pinnatum* leaf extract on neurochemical alterations in neurotransmitter levels including Dopamine, Glutamate, Gamma-Aminobutyric Acid (GABA), Serotonin (5-HT), Noradrenaline, and Acetylcholine (ACh) in ketamine-induced toxicity in Wistar rats. Oxidative stress markers such as Glutathione (GSH), Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were also examined.

2.8 Neurochemical Examination

To measure neurotransmitter of dopamine, glutamate, gamma-aminobutyric acid (GABA), serotonin (5-HT), noradrenaline, and acetylcholine (ACh), the brain tissues of the experimental animals were stored in a freezer at –20°C. The 1004 KARDEŞLER and BAŞKALE / Turk J Med Sci levels of dopamine, glutamate, GABA, and serotonin, noradrenaline, and acetylcholine in the brain tissues (frontal lobe for dopamine, GABA, and serotonin, and hippocampus for glutamate, noradrenaline, acetylcholine) were measured by the means of a test kit. The test kit was based on an enzyme linked immunosorbent assay (ELISA) (Cusabio, China) [24].

2.9 Determination of Oxidative Stress Markers

The sample was blotted dry and homogenized in 1.15% KCl to prepare a 10% w/v suspension. This suspension was centrifuged at 16000 × g in a cooling centrifuge at 0°C. The supernatant obtained was further employed for estimation of malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) [25]. Protein content of the supernatant was estimated by Biuret method. MDA and GSH were expressed as nmol/mg protein and SOD and CAT were expressed as U/mg protein [25].

List 1. Intervention phase experimental design

Group	Identity	No. of Rats	Treatment Protocol
Group 1	Control	9	2ml of normal saline once daily for 21 days
Group 2	Ketamine	9	Receive sub-anesthetic dose of ketamine (20mg/kg) once daily intraperitoneally for 21 days
Group 3	Risperidone	9	Receive Risperidone (0.5mg/kg, per oral) once daily for 21 days
Group 4	BP50	9	Receive 50mg/kg body weight of *B. pinnatum* extract
Group 5	BP100	9	Receive 100mg/kg body weight of *B. pinnatum* extract
Group 6	BP200	9	Receive 200mg/kg body weight of *B. pinnatum* extract

2.10 Histopathological Examination

The animals were anaesthetized with diethyl ether and dissected aseptically to remove the brain tissues which were then transferred into 10% chloroform and later trimmed toa size of 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker [26] and Isirima and Uahomo [27], including, fixation, dehydration, clearing, impregnation, embedding, sectioning, and staining with hematoxylin and eosin (H&E) and finally mounting.

2.11 Method of Data Analysis

The data collected during the study were analyzed using the Statistical Package for Social Sciences (IBM SPSS, Version 28.0). Descriptive statistics, such as means and standard deviations, were employed to analyze the data obtained from the experimental groups. Inferential statistical tests such as analysis of variance (ANOVA) or t-tests was conducted. Post-hoc tests, such as Dunnett (2-sided) test was utilized to compare specific groups and identify significant differences at $p<0.05$.

3. RESULTS

3.1 Effect of Ketamine, Risperidone and *B. pinnatum* on Neurochemical Markers

Ketamine and *B. pinnatum* doses had significant effects on neurotransmitter levels in Wistar rats over three weeks. In the control group, dopamine levels remained stable at 51.00±2.08, whereas the group administered with 20 mg/kg ketamine showed a significant reduction to 20.00±1.15 ($p<0.05$). The positive control group treated with 0.5 mg/kg risperidone had variable dopamine levels, ranging from 42.33±1.45 to 73.00±12.78 ($p<0.05$). *B. pinnatum* administration at low (50 mg/kg), medium (100 mg/kg), and high (200 mg/kg) doses led to dose-dependent changes in dopamine, glutamate, GABA, serotonin, noradrenaline, and acetylcholine levels, with significant variations observed across different doses (Table 1).

In glutamate levels, the control group remained stable at 8.00±0.58, while the 20 mg/kg ketamine group exhibited fluctuations between 10.73±0.67 and 12.00±1.15 ($p<0.05$). The group treated with 0.5 mg/kg risperidone caused variable responses with glutamate levels from 5.57±0.99 to 9.00±0.29 ($p<0.05$). *B. pinnatum* at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg significantly altered glutamate levels, especially in the medium and high dose groups (Table 2). GABA levels remained stable in the control group at 15.00±0.58, whereas 20 mg/kg ketamine caused a significant drop to 6.56±0.73 ($p<0.05$). The 0.5 mg/kg risperidone-treated rats had GABA levels between 11.00±0.58 and 12.40±3.31 ($p<0.05$), with *B. pinnatum* increasing GABA significantly in medium and high doses (Table 3).

For serotonin, the control group stayed stable at 40.00±1.15, while the 20 mg/kg ketamine group showed a significant decrease to 22.00±2.08 ($p<0.05$). The 0.5 mg/kg risperidone treatment resulted in serotonin levels ranging from 24.66±0.88 to 64.33±12.60 ($p<0.05$). *B. pinnatum* administration at 50 mg/kg, 100 mg/kg, and 200 mg/kg led to significant decreases in serotonin levels across all doses (Table 4). Noradrenaline levels in the control group were stable at 31.00±2.08, with the 20 mg/kg ketamine group showing a significant reduction to 17.33±1.20 ($p<0.05$). The 0.5 mg/kg risperidone-treated rats had levels between 21.00±0.58 and 27.50±6.53 ($p<0.05$), and *B. pinnatum* at 50 mg/kg, 100 mg/kg, and 200 mg/kg caused significant changes, especially in low and high dose groups (Table 5).

Acetylcholine levels in the control group remained steady at 5.00±0.58, while 20 mg/kg ketamine administration resulted in a significant increase to 11.37±1.79 ($p<0.05$). The 0.5 mg/kg risperidone-treated rats had variable acetylcholine levels from 4.50±0.12 to 7.10±0.8 ($p<0.05$). *B. pinnatum* at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg significantly decreased acetylcholine levels in the low and medium dose groups, demonstrating the compound's varied impact on neurotransmitter levels across different doses and time points (Table 6).

3.2 Effect of *B. pinnatum* on Oxidative Stress

The administration of ketamine and different doses of *B. pinnatum* had significant effects on GHS levels in Wistar rats over three weeks. In the control group, GHS levels remained stable at 20.00±1.15, while the negative control group treated with 20 mg/kg ketamine showed significantly lower levels at 10.33±0.88 ($p<0.05$). The positive control group administered with 0.5 mg/kg risperidone exhibited varied GHS levels,

ranging from 19.00±0.58 to 25.67±1.2 ($p<0.05$). *B. pinnatum* administration at low (50 mg/kg), medium (100 mg/kg), and high (200 mg/kg) doses resulted in significant changes in GHS levels, with both increases and decreases observed across the weeks (Table 7).

Table 1. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on Dopamine (ng/g) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	51.00±2.08#	51.00±2.08#	51.00±2.08#
Negative Control	20.00±1.15*	20.00±1.15*	41.33±2.03*
Positive Control	42.33±1.45*#	34.67±0.88*#	73.00±12.78*#
Low Dose	32.33±1.45*#	40.00±1.15*#	93.33±9.95*#
Medium Dose	61.00±2.08*#	44.67±0.88*#	76.67±7.69*#
High Dose	71.00±2.08*#	50.00±1.15#	114.67±12.77*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 2. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on Glutamate (µmol/g) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	8.00±0.58	8.00±0.56	8.00±0.58
Negative Control	12.00±1.15	12.00±1.15	10.73±0.67
Positive Control	9.00±0.58	9.00±0.29	5.57±0.99#
Low Dose	12.00±0.58	7.10±0.21	5.23±0.54#
Medium Dose	7.00±0.58#	6.10±0.29#	5.90±0.21#
High Dose	5.00±0.58#	5.10±0.21#	5.23±1.40#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 3. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on GABA (µmol/g) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	15.00±0.58#	15.00±0.58#	15.00±0.58#
Negative Control	9.00±0.58*	9.00±0.58*	6.56±0.73*
Positive Control	12.33±0.88	11.00±0.58	12.40±3.31#
Low Dose	10.67±1.20	12.83±0.44	11.23±0.81#
Medium Dose	15.00±0.58#	13.83±0.44#	12.77±1.01#
High Dose	18.33±0.88#	14.83±0.44#	13.43±0.81#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 4. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on serotonin (ng/g) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	40.00±1.15#	40.00±1.15#	40.00±1.15#
Negative Control	25.33±1.45*	25.33±1.45*	22.00±2.08*
Positive Control	32.33±1.45*#	24.66±0.88*	64.33±12.60*#
Low Dose	20.00±1.15*#	30.00±1.15*#	94.67±1.45*#
Medium Dose	45.00±1.73*#	34.67±0.89*#	110.00±1.15*#
High Dose	55.67±2.33*#	40.00±1.15#	120.00±1.15*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 5. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on Noradrenaline (ng/g) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	31.00±2.08#	31.00±2.08#	31.00±2.08#
Negative Control	17.67±1.45*	17.67±1.45*	17.33±1.20*
Positive Control	24.67±1.45*#	27.50±6.53*#	21.00±0.58*
Low Dose	19.00±1.15*	33.00±0.58#	23.33±0.44*#
Medium Dose	33.00±2.65#	32.33±0.44#	28.00±0.58#
High Dose	40.67±2.33*#	41.33±0.44*#	32.00±0.58#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 6. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on Ach (µmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	5.00±0.58#	5.00±0.58#	5.00±0.58#
Negative Control	12.33±2.60*	12.33±2.60*	11.37±1.79*
Positive Control	6.00±0.58#	7.10±0.8#	4.50±0.12#
Low Dose	7.00±1.53#	6.57±0.84#	4.87±0.15#
Medium Dose	6.00±0.58#	5.13±0.58#	6.00±0.12#
High Dose	4.00±0.58#	5.47±0.38#	7.00±0.15#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

For MDA levels, the control group remained stable at 0.50±0.06, whereas the 20 mg/kg ketamine group exhibited significant increases, ranging from 1.23±0.15 to 1.90±0.06 ($p<0.05$). The positive control group treated with 0.5 mg/kg risperidone showed varied MDA levels between 0.80±0.06 and 1.23±0.15 ($p<0.05$). *B. pinnatum* administration at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg resulted in significant changes in MDA levels, showing both decreases and fluctuations across the weeks (Table 8). SOD levels in the control group remained stable at 18.00±1.15, while the 20 mg/kg ketamine group showed significantly lower levels at 11.00±0.57

(p<0.05). The group treated with 0.5 mg/kg risperidone had varied SOD levels from 19.00±0.58 to 25.00±0.58 (p<0.05), with *B. pinnatum* causing significant increases and decreases at various doses and time points (Table 9).

GPx levels remained stable in the control group at 11.33±0.88, but the 20 mg/kg ketamine group exhibited significantly lower levels at 8.00±0.58 (p<0.05). The 0.5 mg/kg risperidone-treated group showed varied GPx levels from 13.00±0.58 to 18.00±0.58 (p<0.05). Administration of *B. pinnatum* at 50 mg/kg, 100 mg/kg, and 200 mg/kg resulted in significant changes in GPx levels, with both increases and decreases observed across the different weeks (Table 10). For CAT levels, the control group remained stable at 20.00±1.15, while the 20 mg/kg ketamine group showed significantly lower levels at 10.33±0.88 (p<0.05). The positive control group treated with 0.5 mg/kg risperidone exhibited varied CAT levels, ranging from 19.00±0.58 to 25.67±0.58 (p<0.05). *B. pinnatum* administration at low (50 mg/kg), medium (100 mg/kg), and high (200 mg/kg) doses resulted in significant changes in CAT levels, with both increases and decreases noted across the weeks (Table 11).

3.3 Effect of Ketamine and Crude Extract doses of *B. pinnatum* on the Histology of the Hippocampus

The hippocampus tissues of Wistar rats were examined to assess the effects of ketamine, *B. pinnatum*, and risperidone treatments on their histological structures. In the normal control group, the hippocampus tissue maintained a stable microstructure, with clearly defined conus ammonis regions (CA1, CA2, CA3, CA4), subicular area, and granule cells in the dentate gyrus. However, the group exposed to 20 mg/kg ketamine exhibited significant histological alterations. After one week of ketamine

Table 7. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on GSH (µmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	20.00±1.15#	20.00±1.15#	20.00±1.15#
Negative Control	10.33±0.88*	10.33±0.88*	10.33±0.88*
Positive Control	19.00±0.58#	25.67±1.2*#	22.00±0.58#
Low Dose	26.00±0.58#	30.00±1.15*#	30.00±1.15*#
Medium Dose	31.00±0.57*#	35.33±0.57*#	35.33±0.57*#
High Dose	42.00±1.15*#	40.00±1.15*#	40.00±1.15*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 8. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on MDA (µmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	0.50±0.06#	0.50±0.06#	0.50±0.05#
Negative Control	1.90±0.06*	1.23±0.15*	1.23±0.15*
Positive Control	1.23±0.15*	0.80±0.06	1.20±0.06*
Low Dose	0.93±0.09*#	0.30±0.06#	1.00±0.06*
Medium Dose	0.60±0.06*#	0.50±0.06#	0.80±0.06#
High Dose	0.47±0.03*#	0.30±0.06#	0.60±0.06#

*Values are presented in Mean ± SEM; n=3, *=means values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 9. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on SOD (U/mg) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	18.00±1.15#	18.00±1.15#	18.00±1.15#
Negative Control	11.00±0.57*	11.00±0.57*	11.00±0.57*
Positive Control	24.00±0.58*#	19.00±0.58#	25.00±0.58*#
Low Dose	31.00±0.58*#	24.00±0.58*#	30.00±1.15*#
Medium Dose	36.00±0.58*#	30.00±1.15*#	35.33±1.45*#
High Dose	47.00±1.15*#	34.00±0.58*#	40.00±1.15*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

Hint: Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum

Table 10. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on GPx (U/mg) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	11.33±0.88	11.33±0.88	11.33±0.88
Negative Control	8.00±0.58	8.00±0.58	8.00±0.58
Positive Control	14.00±0.58#	13.00±0.58#	18.00±0.58*#
Low Dose	21.00±0.58*#	15.00±0.58#	20.00±0.58*#
Medium Dose	26.00±0.58*#	20.00±1.15*#	25.00±0.58*#
High Dose	37.00±1.15*#	25.00±0.59*#	28.00±0.58*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

***Hint:** Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum*

Table 11. Result of the effect of Ketamine and crude extract doses of *B. pinnatum* on CAT (U/mg) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	20.00±1.15#	20.00±1.15#	20.00±1.15#
Negative Control	10.33±0.88*	10.33±0.88*	10.33±0.88*
Positive Control	19.00±0.58#	25.67±0.58*#	22.00±0.58#
Low Dose	26.00±0.58*#	30.00±1.15*#	30.00±1.15*#
Medium Dose	31.00±0.58*#	35.33±1.45*#	35.33±1.45*#
High Dose	42.00±1.15*#	40.00±1.15*#	40.00±1.15*#

*Values are presented in Mean ± SEM; n=3, *=mean values are statistically significant at p<0.05 when compared to the control values; # =means values are statistically significant at p<0.05 when compared to group 2 (ketamine-induced) values*

***Hint:** Control = 2ml normal saline; Negative control = 20mg/kg Ketamine (i.p); Positive Control = 0.5mg/kg (oral) Risperidone; Low Dose = 50mg/kg crude extract of B. pinnatum; Medium Dose = 100mg/kg crude extract of B. pinnatum; High Dose = 200mg/kg crude extract of B. pinnatum*

exposure, there was noticeable distortion in the CA4 neurons and degeneration in the CA3 neurons, accompanied by the presence of several capillaries in the white matter space. These structural distortions persisted and even worsened over the two- and three-week periods, with increasing infiltration of neuroglial cells.

Treatment with 0.5 mg/kg risperidone following ketamine exposure showed some alleviation of the distortions. After one week, there was proliferation of CA3 neurons and some distortion in CA4 neurons, but the granule cells in the dentate gyrus remained well delineated. Over the two- and three-week periods, risperidone treatment resulted in variable outcomes, with persistent but reduced distortion in CA3 and CA4 neurons and the presence of capillaries and migrating neuroglia in the white matter space. In contrast, administration of *B. pinnatum* at

different doses (50 mg/kg, 100 mg/kg, 200 mg/kg) following ketamine exposure demonstrated dose-dependent histological changes. The low dose (50 mg/kg) of *B. pinnatum* after one week of treatment led to distortion of neurons in CA3 and degeneration in CA2, with well-delineated granule cells in the dentate gyrus, indicating some tissue distortion. At the medium dose (100 mg/kg), the hippocampus tissue appeared more normal with distinct granular cells and conus ammonis areas after one week. However, over two and three weeks, this dose showed increasing signs of neuronal degeneration in CA3 and CA4 regions, with distortion in the granule cells of the dentate gyrus.

The high dose (200 mg/kg) of *B. pinnatum* initially showed mild distortion of tissue microstructure after one week, with distinct granular cells and fluid congestion in the connective tissue. After two and three weeks, the high dose resulted in neuronal degeneration in CA3 and localized distortion in the granule cells of the dentate gyrus, indicating persistent tissue microstructure distortion.

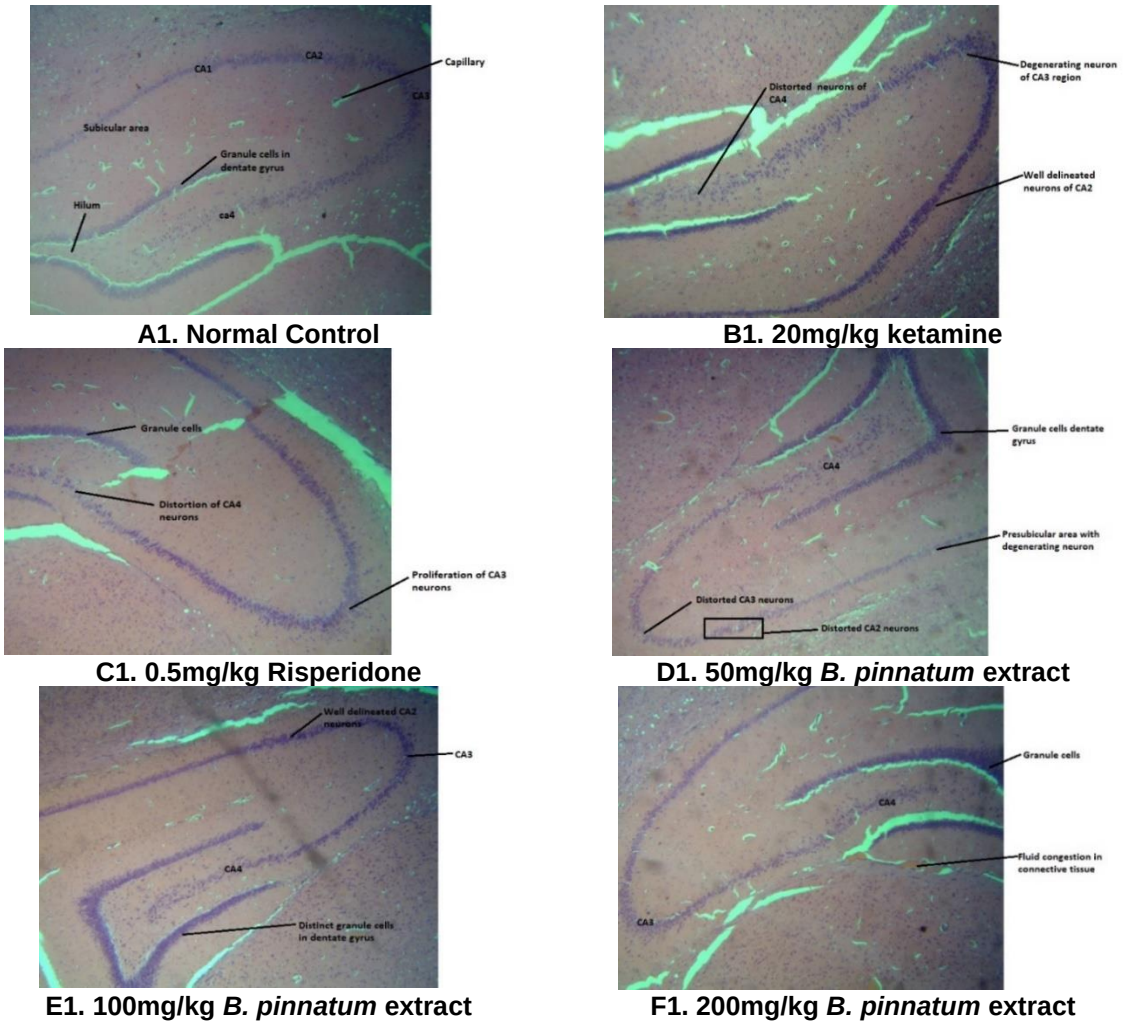
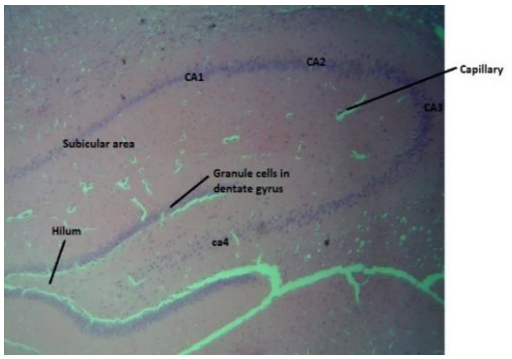
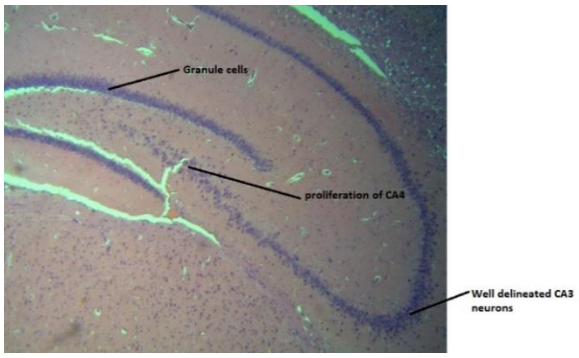


Fig. 1. Photomicrograph of Hippocampus across different treatment groups (H & E, x400) in week 1

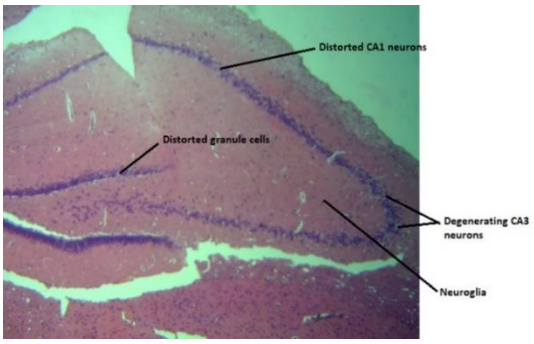
showing: (A) normal microstructure in the control group with intact microstructure including CA1-4 regions and dentate gyrus; (B) neuronal proliferation in CA3 and CA4 with intact dentate gyrus in 20mg/kg ketamine-exposed animals; (C) intact dentate gyrus, CA3 and CA4 neuronal distortion with tissue abnormalities despite 0.5mg/kg risperidone treatment; (D) CA3 neuron distortion, CA2 neuron degeneration, CA4 neuron distortion, and preserved dentate gyrus with tissue cytoarchitecture distortion with 50mg/kg B. pinnatum treatment; (E) normal tissue microstructure with distinct granular cells in CA regions under 100mg/kg B. pinnatum treatment; and (F) normal tissue microstructure with distinct granular cells and mild tissue microstructure distortion with 200mg/kg B. pinnatum treatment



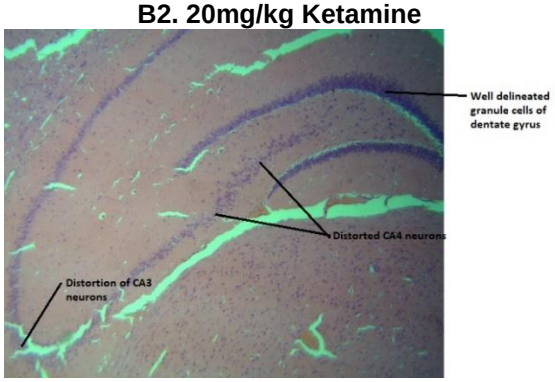
A2. Normal control



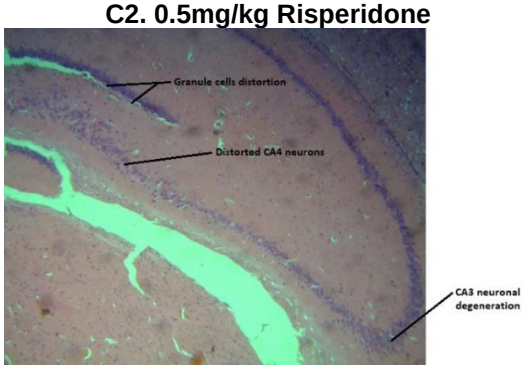
B2. 20mg/kg Ketamine



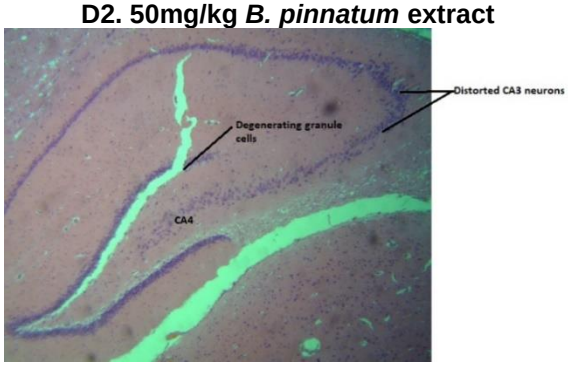
C2. 0.5mg/kg Risperidone



D2. 50mg/kg *B. pinnatum* extract



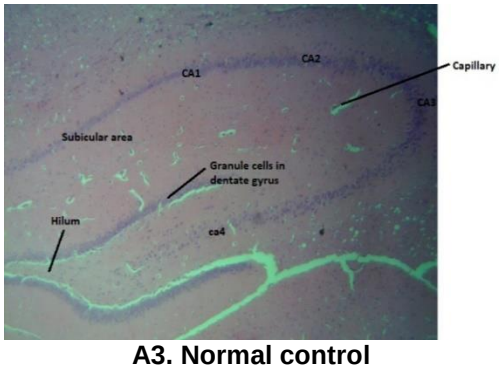
E2. 100mg/kg *B. pinnatum* extract



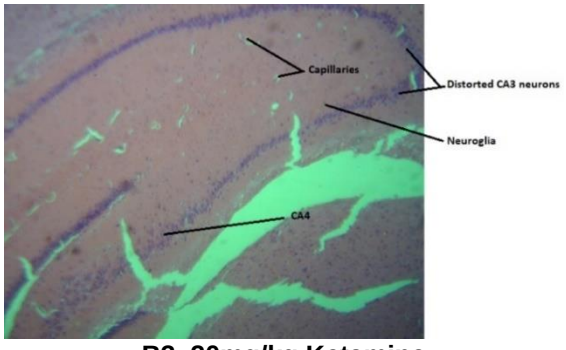
F2. 200mg/kg *B. pinnatum* extract

Fig. 2. Photomicrograph of Hippocampus across different treatment groups (H & E, x400) in week 2

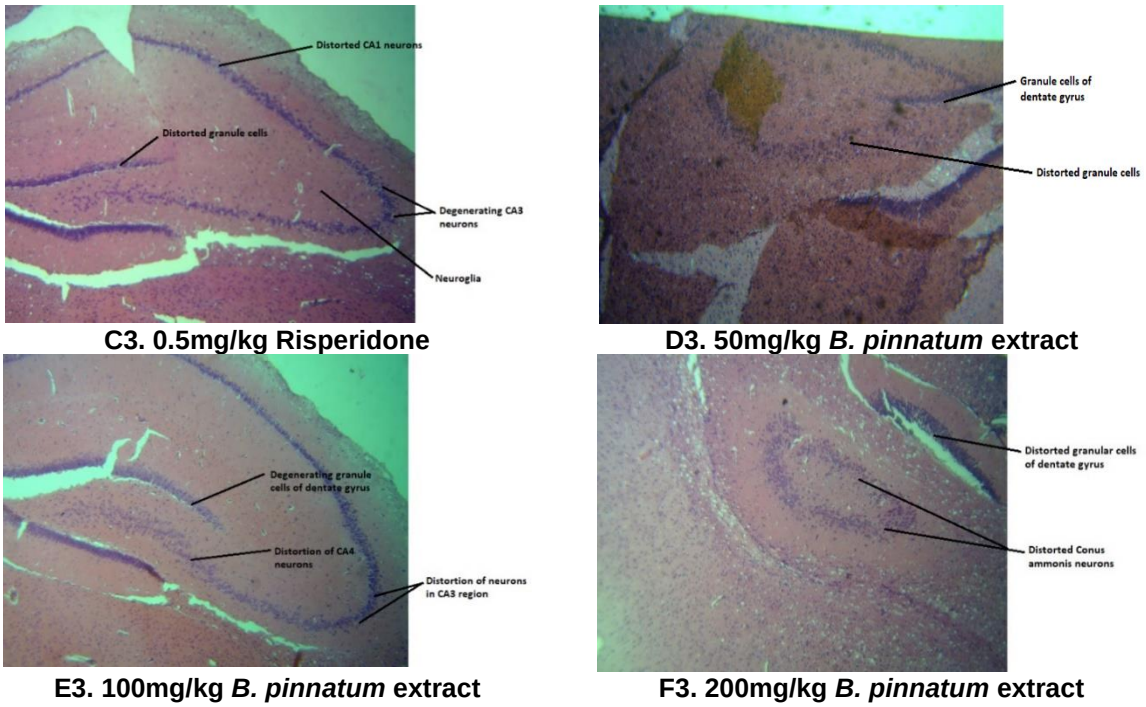
showing: (A2) normal microstructure in the control group; (B2) neuronal proliferation in CA3 and CA4 with intact dentate gyrus in 20mg/kg ketamine-exposed animals; (C2) CA3 and CA4 neuronal distortion with tissue abnormalities despite 0.5mg/kg risperidone treatment; (D2) CA3 neuron distortion with preserved CA4 and dentate gyrus structure with 50mg/kg B. pinnatum treatment; (E2) neuronal degeneration in CA3 and CA4 with distorted dentate gyrus under 100mg/kg B. pinnatum treatment; and (F2) severe CA3 neuronal degeneration and dentate gyrus distortion with 200mg/kg B. pinnatum treatment



A3. Normal control



B3. 20mg/kg Ketamine



C3. 0.5mg/kg Risperidone **D3. 50mg/kg *B. pinnatum* extract**

E3. 100mg/kg *B. pinnatum* extract **F3. 200mg/kg *B. pinnatum* extract**

Fig. 3. Photomicrograph of Hippocampus across different treatment groups (H & E, x400) in week 3

showing: (A3) normal microstructure in the control group; (B3) distortion of tissue microstructure with distorted CA4 and CA3 and infiltration of neuroglial cells by 20mg/kg ketamine; (C3) CA3 and CA4 neuronal distortion. Capillaries are seen in the white matter space with migrating neuroglia with tissue abnormalities despite 0.5mg/kg risperidone treatment; (D3) distorted CA4 neurons in microstructure with 50mg/kg B. pinnatum treatment; (E3) neuronal degeneration in CA3 and CA4 with distorted dentate gyrus under 100mg/kg B. pinnatum treatment; and (F3) neuronal distorted Conusammonis and granules cells of dentated gyrus with 200mg/kg B. pinnatum treatment

3.4 Effect of Ketamine and Crude Extract doses of *B. pinnatum* on the Histology of the Cerebral Cortex

The cerebral cortex tissues of Wistar rats were examined to assess the effects of ketamine, *B. pinnatum*, and risperidone treatments on their histological structures. In the normal control group, the cerebral cortex tissue maintained a stable microstructure with distinct motor neurons (pyramidal neurons) in the pyramidal cell layers, a clear molecular cell layer, and the presence of capillaries in both the molecular and pyramidal cell layers. However, the group exposed to 20 mg/kg ketamine exhibited significant histological alterations. After one week of ketamine exposure, there was noticeable proliferation of neurons in the pyramidal cell layer, congested capillaries, and well-delineated pyramidal neurons with neuroglia cells present in the molecular layer. These structural changes persisted over the two- and three-week periods, with increasing degeneration and necrosis of neurons, especially in the pyramidal cell layer, and the presence of congested capillaries and neuroglia cells.

Treatment with 0.5 mg/kg risperidone following ketamine exposure showed some alleviation of the distortions. After one week, there was proliferation of neurons with a distinct appearance in the pyramidal cell layer and congested capillaries. Over the two- and three-week periods, risperidone treatment resulted in variable outcomes, with ongoing degeneration and necrosis of neurons, the presence of several neuroglia, and cellular overlap. In contrast, administration of *B. pinnatum* at different doses (50 mg/kg, 100 mg/kg, 200 mg/kg) following ketamine exposure demonstrated dose-dependent histological changes. The low dose (50 mg/kg) of *B. pinnatum* after one week of treatment led to proliferation of neurons and pockets of degenerating neurons within the pyramidal cell layer, with several neuroglia present and tissue distortion indicated. At the medium dose (100 mg/kg), the cerebral cortex tissue showed well-delineated large neurons

(pyramidal neurons) with processes and several neuroglia, indicating neuronal cell inflammation. The high dose (200 mg/kg) showed similar features with well-delineated neurons, several neuroglia cells, and few cells in the molecular cell layer, also indicating neuronal inflammation.

After two weeks of treatment with *B. pinnatum*, the low dose resulted in pockets of degenerating neurons and well-delineated pyramidal neurons in the motor cortex, with the presence of neuroglia and congested capillaries. The medium dose showed proliferation of neurons and presence of several neuroglia, with pockets of vacuolation, indicating normal tissue microstructure. The high dose led to hypertrophy and degeneration of neurons in the pyramidal cell layer, with the presence of neuroglia and congested capillaries, indicating distortion of motor cortex neurons.

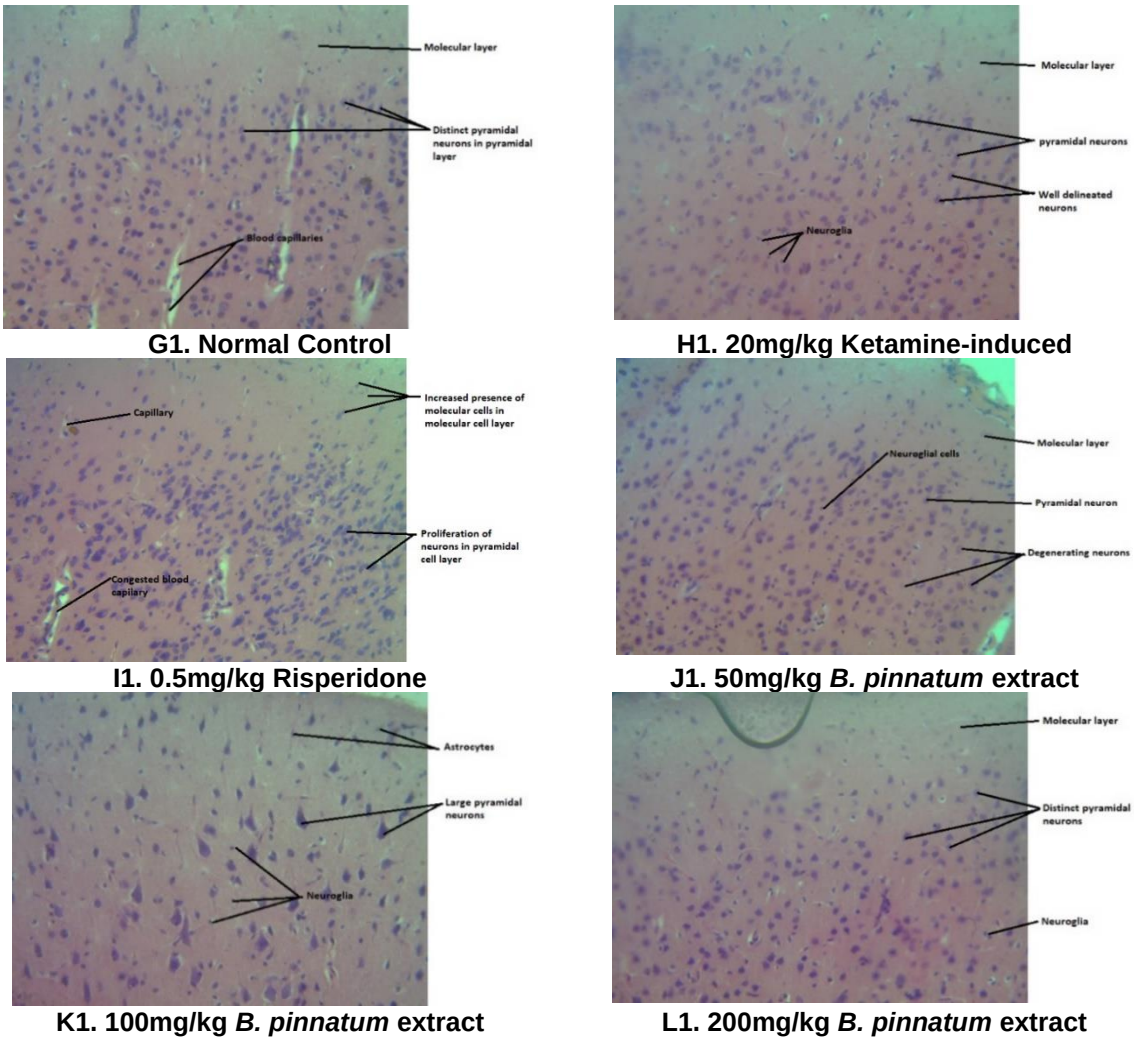
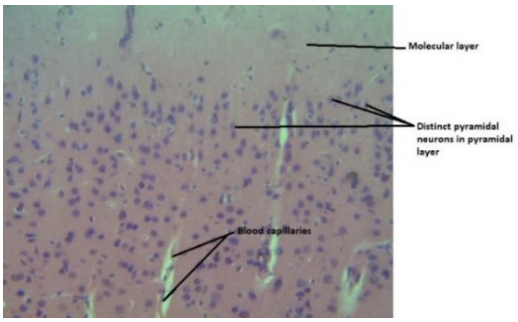
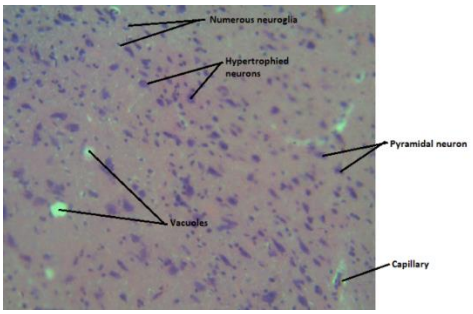


Fig. 4. Photomicrograph of cerebral cortex across different treatment groups (H & E, x400) at week 1

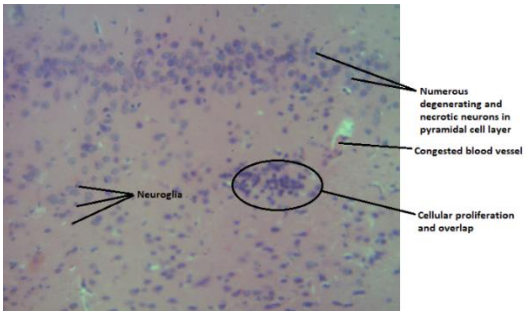
showing: (G1) normal cerebral cortex with distinct pyramidal neurons and a normal molecular cell layer in control group; (H1) lots of pockets of hypertrophied pyramidal neurons, vacuolations and presence of several neuroglia. Congested capillaries are also observed. Distortion of motor cortex neurons indicated in 20mg/kg ketamine treated group; (I1) ketamine-exposed cortex treated with 0.5mg/kg risperidone, displaying proliferated neurons in the pyramidal layer and congested capillaries, with normal tissue structure; (J1) neuron proliferation, pockets of degeneration, and neuroglia cells, indicating tissue microstructure distortion with 50mg/kg B. pinnatum treatment; (K1) well-defined large pyramidal neurons, astrocytes (neuroglia), and signs of neuroglia migration suggesting neuronal inflammation with 100mg/kg B. pinnatum treatment; (L1) intact pyramidal neurons, neuroglia cells, and molecular layer cells, with neuroglia migration indicating potential neuronal inflammation 200mg/kg B. pinnatum treatment



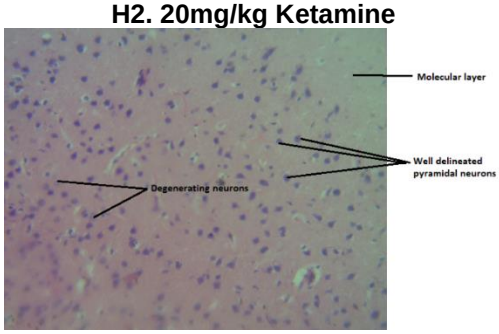
G2. Normal control



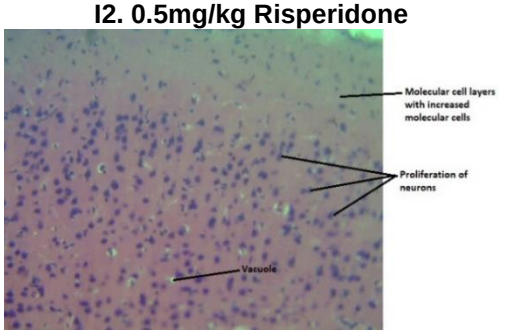
H2. 20mg/kg Ketamine



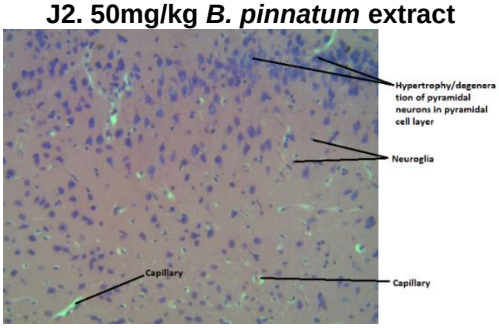
I2. 0.5mg/kg Risperidone



J2. 50mg/kg *B. pinnatum* extract



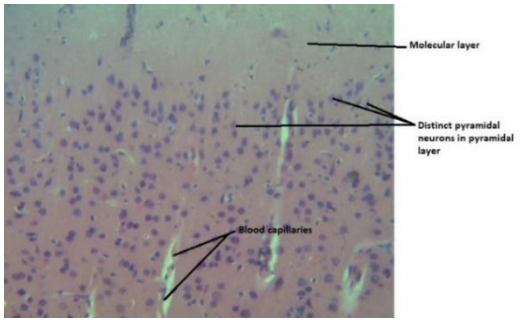
K2. 100mg/kg *B. pinnatum* extract



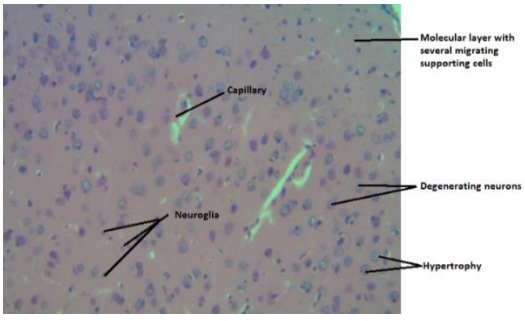
L2. 200mg/kg *B. pinnatum* extract

Fig. 5. Photomicrograph of cerebral cortex across different treatment groups (H & E, x400) at week 2

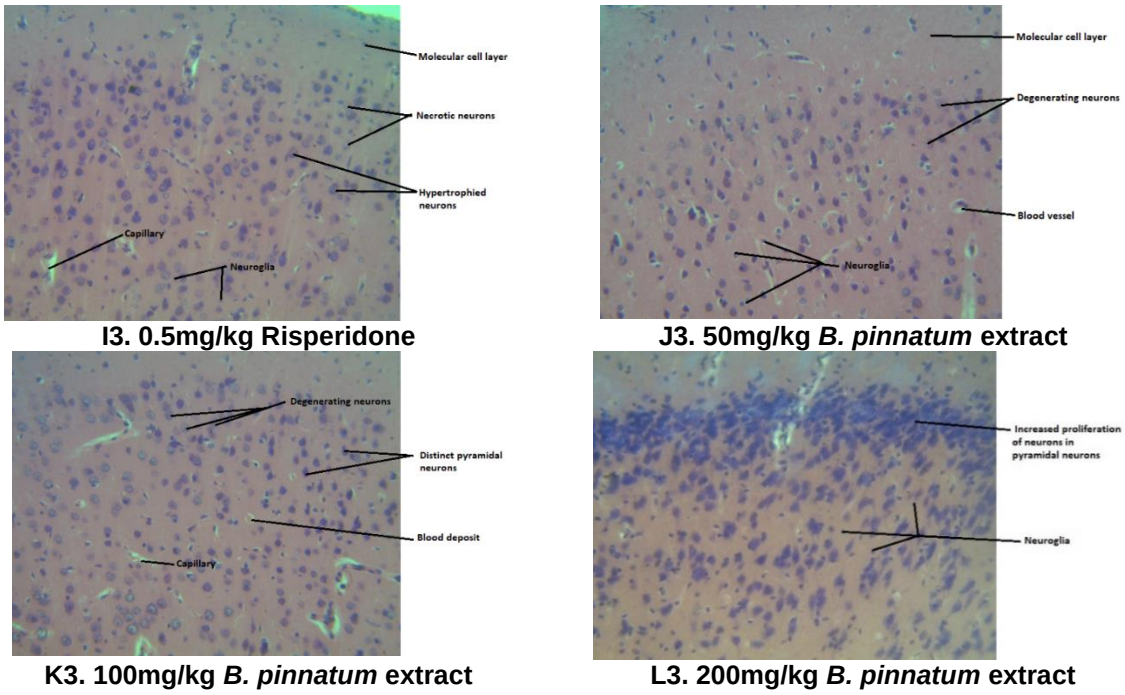
illustrating: *(G2) normal cytoarchitectural appearance with distinct pyramidal neurons and capillaries in both molecular and pyramidal cell layers in the control group; (H2) hypertrophied pyramidal neurons, vacuolations, neuroglia presence, and congested capillaries indicating neuronal distortion in 20mg/kg ketamine-exposed animals without treatment; (I2) neuronal degeneration/necrosis, neuroglia presence, cellular overlap, and congested capillaries in ketamine-exposed animals treated with 0.5mg/kg risperidone; (J2) degenerating neurons, some intact pyramidal neurons, neuroglia presence, and congested capillaries indicating neuronal distortion in ketamine-exposed animals treated with a low dose of 50mg/kg B. pinnatum (K2); neuronal proliferation, neuroglia presence, occasional vacuolation, and normal tissue microstructure in ketamine-exposed animals treated with a medium dose of 100mg/kg B. pinnatum; and (L2) hypertrophy/degeneration of neurons, neuroglia presence, and congested capillaries indicating neuronal distortion in ketamine-exposed animals treated with a high dose of 200mg/kg B. pinnatum*



G3. Normal control



H3. 20mg/kg Ketamine



I3. 0.5mg/kg Risperidone **J3. 50mg/kg *B. pinnatum* extract**

K3. 100mg/kg *B. pinnatum* extract **L3. 200mg/kg *B. pinnatum* extract**

Fig. 6. Photomicrograph of cerebral cortex across different treatment groups (H & E, x400) at week 1

showing: (G3) normal cytoarchitectural appearance with distinct pyramidal neurons and capillaries in both molecular and pyramidal cell layers in the control group; (H3) several hypertrophied and degenerating neurons, numerous neuroglia cells, and microstructural distortion in 20mg/kg ketamine-exposed animals without treatment; (I3) hypertrophy and degeneration of neurons, presence of neuroglia, congested capillaries, and distortion of motor cortex neurons in ketamine-exposed animals treated with 0.5mg/kg risperidone; (J3) degenerating neurons, presence of neuroglia, and disappearance of nuclear content indicating degeneration and necrosis of motor cortex neurons in ketamine-exposed animals treated with a 50mg/kg B. pinnatum; (K3) degenerating neurons alongside some distinct pyramidal neurons, with disappearance of nuclear content and tissue microstructure distortion in ketamine-exposed animals treated with 100mg/kg B. pinnatum; and (L3) neuron proliferation, neuronal overlapping in the pyramidal layer, numerous neuroglia cells, and highly proliferated neurons indicating significant tissue microstructure distortion in ketamine-exposed animals treated with 200mg/kg B. pinnatum

After three weeks of treatment with *B. pinnatum*, the low dose showed extensive degeneration of neurons with several neuroglia present and disappearance of nuclear content, indicating degeneration and necrosis of motor cortex neurons. The medium dose revealed extensive degeneration of neurons and some distinct pyramidal neurons, with tissue distortion indicated. The high dose resulted in proliferation and overlapping of neurons in the pyramidal layer, with numerous neuroglia, indicating high proliferation and overlapping of tissue microstructure.

4. DISCUSSION

Rapid distribution within the central nervous system (CNS) characterizes ketamine, with effects lasting up to 1.5 hours post-insufflation and delayed effects following oral ingestion due to hepatic metabolization [28]. The drug's primary CNS effects are attributed to N-methyl-D-aspartate (NMDA) receptor inhibition and catecholamine reuptake blockade, inducing a psychosis-like state at sub-anesthetic doses [28]. Additionally, reuptake inhibition increases heart rate and blood pressure. Acute ketamine administration results in anesthesia, impaired motor function, cognitive dissociation, depersonalization, and hallucinations at lower doses.

Neurotransmitters are critical indicators in neuroscience, offering profound insights into brain function and disorders by regulating various physiological processes and behaviors [29-32]. Understanding their roles helps unravel the complexities of neural mechanisms and potential therapeutic targets.

In the present study, we evaluated the neuroprotective potentials of *B. pinnatum* in ketamine-induced neurotoxicity. Results from this study revealed that administration of ketamine resulted in a significant reduction in dopamine, serotonin, noradrenaline, and GABA levels, contributing to impaired motor function, mood disturbances, cognitive impairments, and reduced inhibitory control, while increasing glutamate and acetylcholine levels, leading to excitotoxicity and cholinergic overstimulation, respectively. It has been established that ketamine induces neurochemical changes by antagonizing NMDA receptors. This antagonism disrupts normal neurotransmission pathways involving these neurochemical markers, potentially leading to dysregulation of neuronal signaling, neurochemical imbalance, which could lead to various neurological and psychiatric conditions such as mood disorders, anxiety, cognitive impairment, and altered neuronal function. Studies have reported that dopamine reduction can disrupt reward processing, motivation, and movement control [33]. Alterations in glutamate may impact learning and memory, potentially contributing to neurotoxicity [34,35]. A decrease in GABA levels can increase neuronal activity and lead to anxiety and neurological disorders [33]. Reduced serotonin levels are associated with mood disruptions and can contribute to depression and other mood disorders. Lower noradrenaline levels may disrupt attention and increase stress and anxiety. Reduced acetylcholine levels can impair memory formation and cognitive function [33].

To provide intervention for the neurotoxicity induced by ketamine administration, risperidone, a standard drug for managing psychotic disorders, was used. Additionally, doses of *B. pinnatum* were administered. Risperidone is known to primarily act as an antagonist at dopamine D2 and serotonin 5-HT2A receptors, but it also has activity at other receptor sites, contributing to its broad spectrum of effects. Risperidone had complex effects on neurotransmitters initially causing a significant increase followed by a significant decrease in dopamine, glutamate, and GABA levels, significant fluctuations in serotonin and noradrenaline levels, and a decrease in acetylcholine, due to its broad receptor antagonism and interactions across multiple neurotransmitter systems.

B. pinnatum demonstrated dose-dependent modulation of neurotransmitter levels in Wistar rats subjected to ketamine-induced neurotoxicity, with significant effects observed across dopamine, glutamate, GABA, serotonin, noradrenaline, and acetylcholine levels. *B. pinnatum*, particularly at medium (100 mg/kg) and high doses (200 mg/kg), effectively restored dopamine, glutamate, GABA, and noradrenaline levels, suggesting its neuroprotective and neuromodulatory potential, though *B. pinnatum* displayed complex interactions on serotonin and acetylcholine levels, with significant decreases in serotonin across all doses, suggesting potential serotonin suppression or altered metabolism pathways. In contrast, acetylcholine levels decreased significantly in the low (50 mg/kg) and medium doses (100 mg/kg), while the high dose (200 mg/kg) did not exhibit a consistent pattern, indicating a nuanced and dose-specific response that could involve multiple regulatory mechanisms or feedback loops affecting cholinergic signaling.

Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various brain states, including neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease [36,37]. Although the brain accounts for less than 2% of body weight, it consumes about 20% of available oxygen, making it the most susceptible organ to oxidative damage due to its high oxygen demand [38,39]. Increased oxidative stress leads to lipid peroxidation, protein damage, and induction of apoptosis [40,41].

In the present study, MDA were assayed for the estimation of lipid peroxidation levels, while antioxidant enzymatic activity was estimated by assaying for SOD, CAT, GHS and GPx activities in the brain tissues. Ketamine administration resulted in a significant increased MDA levels when compared to the control group. Elevation of MDA levels suggest enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive radicals [42]. Lipid peroxidation is a free radical-related process, which is potentially harmful because if uncontrolled, self-enhancing process causes disruption of membrane, lipids and other cell components. It is also involved in oxidative stress, which plays a role in neural-degeneration [43]. High level of lipid peroxidation (LPO) which is not normal and the simultaneous decline of antioxidants defense mechanism can lead to cellular organelles damage and oxidative stress [40,44]. Treatment with *B. pinnatum* resulted in

decreased MDA levels, indicative of reduced LPO generation, this results in enhanced brain tissues integrity by promoting neuroprotection, preserving membrane integrity, mitigating neuroinflammation, and potentially enhancing cognitive function.

Our findings revealed that Ketamine administration significantly altered oxidative stress markers in Wistar rats, leading to decreased levels of SOD, CAT, GHS, and GPx. This suggests potential oxidative damage and compromised antioxidant defense mechanisms in the tissues. SOD is an antioxidant pivotal in protecting cells against oxidative stress, serves as a sensitive marker for oxidative damage by scavenging superoxide anions and hydrogen peroxide, thereby reducing their toxic effects [45]. CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanism enhanced sensitivity to free radical induced cellular damage [46,47]. It is widely distributed in all animal tissues decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [48]. GPx is equally an important antioxidant enzyme, which reacts with hydrogen peroxide thus preventing intracellular damage caused by the free radicals [49,50].

Treatment with *B. pinnatum* at doses of 50mg/kg, 100mg/kg, and 200mg/kg demonstrated a modulatory effect on these markers over three weeks. Specifically, the low dose (50mg/kg) resulted in a significant increase in SOD, CAT, GHS, and GPx levels, indicating enhanced antioxidant defenses against reactive oxygen species (ROS). The high dose (200mg/kg) produced the most pronounced effects, further boosting SOD, CAT, GHS, and GPx levels, suggesting a potent antioxidative action against ROS-induced oxidative stress. These findings suggest that *B. pinnatum* extract at varying doses can counteract the oxidative stress induced by ketamine.

In the present study, light microscopic examination of routinely Haematoxylin and Eosin, (H&E) of Wistar rats brain regions namely: hippocampus and cerebral cortex-layer V, were undertaken.

The result from the present study showed that ketamine exposure on hippocampal tissue resulted in significant neuronal distortion in CA3 and CA4 regions, accompanied by neuroglial cell infiltration, and capillary presence in the white matter space after three weeks without treatment. The neurodegenerative changes observed in the hippocampus, such as neuronal degeneration and histoarchitectural distortion, following ketamine exposure suggest significant neurotoxicity within the central nervous system. These changes are reflective of alterations in neural tissue integrity, potentially contributing to neuropathological conditions and accelerating brain ageing processes [51,52].

Treatment with *B. pinnatum* at doses of 50mg/kg, 100mg/kg, and 200mg/kg over different durations resulted in varied effects on hippocampal tissue microstructure. Specifically, lower doses (50mg/kg and 100mg/kg) tended to preserve normal tissue architecture with distinct neuronal regions (CA1-4 and dentate gyrus), while the higher dose (200mg/kg) showed signs of neuronal degeneration in CA3 and CA4 regions and distortion of tissue microstructure, indicating a potential dose-dependent impact on hippocampal integrity in ketamine-exposed rats. The observed effects of *B. pinnatum* on hippocampal tissue microstructure, particularly the preservation at lower doses and degeneration at higher doses, likely result from its biochemical constituents such as flavonoids, alkaloids, and phenolic compounds. These compounds are known for their antioxidant and neuroprotective properties. At lower doses (50mg/kg and 100mg/kg), these phytochemicals may scavenge reactive oxygen species (ROS), reduce oxidative stress, and support neuronal health by maintaining normal tissue architecture. However, at the higher dose (200mg/kg), the concentration of these phytochemicals might overwhelm the antioxidant capacity, potentially leading to oxidative stress, neuronal degeneration, and structural distortion in the hippocampus. This dose-dependent effect underscores the importance of dosage considerations in harnessing the neuroprotective benefits of *B. pinnatum* against ketamine-induced neurotoxicity.

In the cerebral cortex, exposure to ketamine induced distinct changes characterized by neuronal proliferation, degeneration, neuroglia presence, and capillary congestion, indicating neurotoxic effects. The implications of these observed effects are suggestive of neurotoxicity by ketamine. Neuronal proliferation and degeneration indicate a disruption in normal neuronal dynamics, potentially compromising cognitive and motor functions [53,54]. The presence of neuroglia and capillary congestion

further underscores inflammatory responses and compromised blood-brain barrier integrity, which may exacerbate neuronal damage and impair brain function over time [54].

Treatment with *B. pinnatum* at doses of 50mg/kg, 100mg/kg, and 200mg/kg over different durations resulted in varied effects on cerebral cortex microstructure. Lower doses (50mg/kg and 100mg/kg) showed preservation of neuronal architecture with occasional degeneration and neuroglia presence, while the higher dose (200mg/kg) led to pronounced neuronal degeneration, neuroglia proliferation, and tissue distortion, suggesting a dose-dependent impact on cortical integrity in ketamine-exposed animals.

The mechanism through which *B. pinnatum* exerts its dual effect on the cerebral cortex microstructure in the context of ketamine-induced neurotoxicity can be hypothesized based on its pharmacological properties and interactions with various cellular pathways. *B. pinnatum* is known to possess antioxidant properties due to its rich content of bioactive compounds like flavonoids, phenolics, and alkaloids [55,56]. These compounds scavenge free radicals and reduce oxidative stress, which is a common mechanism underlying neurotoxicity induced by substances like ketamine. By reducing oxidative stress, *B. pinnatum* at lower doses (50mg/kg and 100mg/kg) may protect neuronal cells from damage and preserve cortical integrity. *B. pinnatum* may modulate neuroprotective pathways such as the Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway, which regulates antioxidant responses. Activation of Nrf2 by *B. pinnatum* can upregulate the expression of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GHS), thereby enhancing cellular defense mechanisms against oxidative stress [57-59]. Ketamine induces neurotoxicity partly through excessive glutamate release and excitotoxicity. *B. pinnatum* extracts have been reported to modulate glutamatergic transmission and reduce excitotoxicity, potentially mitigating the harmful effects of ketamine on neuronal cells.

The dual effect observed (protective at lower doses and detrimental at higher doses) may be attributed to a biphasic response of *B. pinnatum* [56,60]. At lower doses, its antioxidant and anti-inflammatory properties prevail, providing neuroprotection. However, at higher doses (200mg/kg), these effects might be overshadowed by potential cytotoxic or pro-inflammatory effects, leading to neuronal degeneration and tissue distortion.

Therefore, the observed neuroprotective effects of *B. pinnatum* in this study can be attributed to its rich phytochemical composition which possess antioxidant and anti-inflammatory properties. These compounds contribute to the stabilization of neurotransmitter levels by reducing oxidative stress and neuroinflammation, pivotal mechanisms in ketamine-induced neurotoxicity. Previous studies have demonstrated the neuroprotective effects of *B. pinnatum* against various neurotoxins [56,61,62]. These findings suggest that *B. pinnatum* flavonoids can ameliorate oxidative imbalance, down-regulate acetylcholinesterase mRNA transcripts, and improve histological features in the hippocampus and cortex.

5. CONCLUSION

In this study, *B. pinnatum* demonstrated superior neuroprotective effects compared to risperidone against ketamine-induced neurotoxicity, effectively restoring neurotransmitter balance and enhancing antioxidant defenses crucial for mitigating oxidative stress and preserving neuronal integrity. *B. pinnatum* modulated neurotransmitter levels, including dopamine, glutamate, GABA, serotonin, noradrenaline, and acetylcholine, towards normalization, supporting motor control, mood stability, and cognitive function. Additionally, it significantly elevated antioxidant enzymes, reducing lipid peroxidation levels and protecting neuronal membranes. Histological findings indicated *B. pinnatum*'s ability to maintain hippocampal and cortical structures at lower doses, suggesting its potential as a neuroprotective agent deserving further exploration. *B. pinnatum* emerges as a promising candidate for mitigating ketamine-induced neurotoxicity, highlighting its potential as a therapeutic agent for neuroprotection.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was carried out in adherence to ethical guidelines set by the National Institute of Health (NIH) for the ethical treatment of animals in research. The study was approved by the Research Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria with reference number UPH/CEREMAD/REC/ MM91/076.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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