Attenuating Ketamine-Induced Nephrotoxicity with *Bryophyllum pinnatum* Extract – Biochemical and Histological Investigation

ABSTRACT

Background: Ketamine, a widely used anesthetic agent, has been shown to induce nephrotoxicity, characterized by increased kidney function markers and structural damage. Despite its therapeutic applications, the adverse effects of ketamine on the kidneys necessitate the exploration of potential protective agents. *Bryophyllum pinnatum* (B. pinnatum), an herbal plant with a long history of medicinal use, has demonstrated various therapeutic properties, including antioxidant, anti-inflammatory, and nephroprotective effects. However, its role in mitigating ketamine-induced kidney damage remains inadequately explored. **Methods**: Sixty male Wistar rats were assigned to six groups. Group 1 served as the control, while Group 2 received ketamine (20 mg/kg) for 7 days to induce renal toxicity. Groups 3-6 were treated with ketamine plus different doses of B. pinnatum extract (50, 100, 200 mg/kg) for 21 days. Biochemical markers, including blood urea nitrogen (BUN), creatinine, urea, sodium (Na), and potassium (K), were measured, and histopathological evaluations were conducted on kidney tissues. **Results**: Ketamine administration significantly increased BUN (11.50±0.17 mg/dL), creatinine (100.00±2.89 µmol/L), urea (43.00±2.08 mg/dL), Na (164.00±4.16 mmol/L), and K (2.83±0.34 mmol/L) compared to controls ($p<0.05$). Treatment with B. pinnatum at doses of 100 and 200 mg/kg significantly reduced these markers, with the highest dose showing values near control levels (BUN: 5.33±0.24 mg/dL, creatinine: 64.67±4.26 µmol/L, urea: 12.23±0.15 mg/dL, Na: 131.00±0.58 mmol/L, K: 1.10±0.07 mmol/L, $p<0.05$). Histologically, B. pinnatum treatment attenuated ketamine-induced renal damage, with marked improvements in tissue architecture. **Conclusion**: *B. pinnatum* exhibited significant nephroprotective effects, as evidenced by the reduction of kidney function markers and improved histological features, suggesting its potential as a therapeutic agent in managing ketamine-induced renal toxicity.

Key words: Ketamine, Nephrotoxicity, *Bryophyllum pinnatum*, Nephroprotective effects, Kidney function markers, Histopathology

INTRODUCTION

The kidney is a vital organ responsible for essential functions such as filtration of blood, excretion of metabolic waste, regulation of electrolytes, maintenance of fluid balance, and production of hormones like erythropoietin and renin, which play critical roles in hematopoiesis and blood

pressure regulation, respectively [1,2]. Its intricate architecture and functionality make it susceptible to damage from various pathological conditions. Injuries or diseases affecting the kidney can significantly impair these functions, potentially leading to life-threatening complications such as chronic kidney disease, acute kidney injury, or even systemic dysfunctions [3,4].

Pathologies of the kidney may arise due to direct insults, secondary to systemic diseases, or as adverse effects of drugs used to manage other conditions. Unexpected kidney and liver injuries are often observed in cases of drug abuse or excessive use of medications. Notably, some drugs administered for therapeutic purposes may cause collateral damage to other organs, particularly the liver, owing to shared metabolic pathways. One such drug is ketamine, which is widely used but increasingly recognized for its toxic effects on the liver and kidneys [5-8].

Ketamine is a general anesthetic that produces a dissociative state at subanesthetic doses, characterized by depersonalization and derealization [9-11]. As a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, it exerts its effects through inhibition of NMDA receptors, resulting in altered perception and pain modulation [12-14]. Ketamine is metabolized in the liver to its active metabolite, norketamine, which is subsequently excreted through the renal system. Although ketamine was developed as a safer alternative to phencyclidine, its recreational use has escalated in recent decades, leading to significant cases of misuse and associated organ toxicities [5,7]. Chronic abuse has been linked to a condition known as ketamine-associated cystitis or ketamine-induced ulcerative cystitis, characterized by inflammation and ulceration of the bladder wall, extending to the ureters and kidneys. Furthermore, large doses of ketamine have been documented to cause hepatotoxicity, raising concerns about its widespread use [15-17].

Maintaining kidney health is paramount, as renal pathologies resulting from injuries such as glomerulonephritis, ischemia-reperfusion injury, or drug-induced nephrotoxicity can have severe systemic consequences [18-20]. Given the dual susceptibility of the kidney and liver to damage in

cases of ketamine abuse, there is an urgent need for therapeutic strategies to mitigate these effects and restore normal organ function.

To ensure the healthy functioning of the kidneys and liver, researchers have increasingly turned to herbal remedies for their potential to reverse drug-induced injuries. Studies have demonstrated that several medicinal plants possess nephroprotective and hepatoprotective properties, effectively mitigating ketamine-induced organ damage. For instance, plants like *Hypoestes rosea* [21], *Curcuma longa* [22-25], *Glycyrrhiza glabra* [26], and *Phyllanthus niruri* [27], have been reported to exhibit antioxidant, anti-inflammatory, and regenerative properties, reducing oxidative stress and improving organ function in experimental models.

One plant gaining attention for its protective effects against drug-induced toxicities is *Bryophyllum pinnatum* (*B. pinnatum*). Commonly known as the "life plant," *B. pinnatum* is a perennial succulent with a rich history of ethnomedicinal use [28,29]. Its phytochemical composition includes bioactive compounds such as flavonoids, alkaloids, triterpenoids, and phenolic acids, which have been shown to confer anti-inflammatory, antioxidant, and cytoprotective properties. Pharmacological studies have demonstrated the plant's ability to mitigate oxidative stress, reduce inflammation, and promote tissue regeneration, making it a promising candidate for the management of ketamine-induced liver and kidney injuries [29].

This study seeks to investigate the biochemical and histological improvements in ketamine-induced liver injury in Wistar rats via the administration of *B. pinnatum* leaf extract. By exploring the protective and reparative effects of this medicinal plant, the study aims to provide new insights into its therapeutic potential for managing drug-induced organ toxicities.

MATERIALS AND METHODS

Experimental Animals

Sixty male Wistar rats, weighing between 180 and 200 grams, were procured from the Animal House of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt. The animals were housed in clean, disinfected wooden cages lined with sawdust bedding, maintained under controlled conditions of a 12-hour light/dark cycle, 50–60% humidity, and a temperature of approximately 30°C. Prior to the commencement of the experiment, the rats were acclimatized for two weeks with unrestricted access to clean water and standard animal feed.

Chemicals and Plant used

Fresh leaves of *B. pinnatum* were collected from the area behind the Ofrima Building within the Abuja Park of the University of Port Harcourt. The plant was identified and authenticated by Dr. Edwin Nwosu of the Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, and was assigned the voucher number UPH/V/1308. Ketamine and risperidone were procured from Alpha Pharmacy and Stores on NTA Road, Port Harcourt, Rivers State, Nigeria.

Preparation of *B. pinnatum* extract

The plant tissue homogenization technique, as detailed by Pandey and Tripathi [30], was employed to extract fresh juice from the leaves of *B. pinnatum*. Fresh leaves were finely ground using a blender, and the juice was subsequently extracted and filtered through a clean white handkerchief, following the methodology described by Das et al. [31]. The extracted juice was carefully collected into clean reagent bottles and stored under refrigeration to ensure preservation.

Dose selection

The doses of ketamine (20 mg/kg) and risperidone (0.5 mg/kg) used in this study were based on recommendations by Monte et al. [32] and Ben-Azu et al. [33], respectively. Sub-lethal doses of *B. pinnatum* crude extract (50, 100, 200 mg/kg body weight) following guidelines from Salahdeen and Yemitan [34] and were administered in 0.1ml, 0.2ml and 0.4ml volume respectively after a preliminary dose-determination experiment to determine the weight (mg/mL) of *B. pinnatum*.

Experimental Design

The protocol was designed and modified based on the established method by Monte *et al*. [32] and Uahomo and Isirima *et al.* [35]. 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 20mg/kg ketamine once daily intraperitoneally for 7 days. Three (3) animals were sacrificed from each group on the 7^{th} day, and blood samples, as well as liver 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 20mg/kg ketamine once daily intraperitoneally for the next 21-day period. Group 3, designated as the positive control, received 0.5mg/kg risperidone orally 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, group 5 received 100 mg/kg, and group 6 received 200 mg/kg orally once daily for the same 21-day period.

Table 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	Received 20mg/kg ketamine I.P once daily for 21 days
Group 3	Risperidone	9	Received 0.5mg/kg risperidone orally once daily for 21 days
Group 4	BP50	9	Received 50mg/kg body weight of *B. pinnatum* extract
Group 5	BP100	9	Received 100mg/kg body weight of *B. pinnatum* extract
Group 6	BP200	9	Receive 200mg/kg body weight of *B. pinnatum* extract

Collection of Blood and Tissue Sample

Twenty-four hours following the treatments on the 8th, 15th, and 22nd days of the experimental period, the animals were anesthetized using diethyl ether and subsequently euthanized. Blood samples were collected through cardiac puncture, while kidney tissues were harvested for biochemical and histological evaluations.

Biochemical Analysis

Kidney function markers were assessed to evaluate the effects of B. pinnatum leaf extract on renal health in Wistar rats subjected to ketamine-induced toxicity. These markers included Sodium (Na), which plays a crucial role in fluid balance and neuromuscular function, and Potassium (K), essential for maintaining cellular function and membrane potential. Calcium (Ca) levels were analyzed, reflecting kidney involvement in calcium homeostasis. Urea and Creatinine levels served as key indicators of glomerular filtration rate (GFR) and overall renal excretory function. Albumin

levels were also measured, as they serve as a critical marker of protein leakage and kidney function integrity. Finally, Blood Urea Nitrogen (BUN), which reflects protein metabolism and kidney clearance ability, was measured to provide further insight into renal function under ketamine-induced stress and its potential amelioration by B. pinnatum

Kidney Function markers assay

Kidney function markers were analyzed using validated methods: serum sodium and potassium levels were determined using the flame photometric method as described by Maruna and Trinder [36]; calcium was assessed using the O-Cresolphthalein Complexone (OCPC) method following Morin [37]; albumin was determined using the Bromocresol Green (BCG) dye-binding method as described by Doumas et al. [38]; creatinine levels were determined by the Jaffe reaction as outlined by Bonsnes and Taussky [39]; urea concentration was measured enzymatically using the Urease-Glutamate Dehydrogenase (GLDH) method described by Weatherburn [40]; and blood urea nitrogen (BUN) was calculated from urea levels using established conversion factors consistent with Hosten [41].

$$BUN\ (mg/dL)\ = Urea\ (mg/dL)\ x\ 0.467$$

Histopathological Examination

The animals were anesthetized with diethyl ether and subsequently dissected aseptically to remove the kidney tissues. The tissues were then transferred into 10% chloroform and carefully trimmed to a thickness of 2mm to 4mm to facilitate the penetration of the fixative. The kidney tissues were processed through several stages, including fixation, dehydration, clearing, impregnation, embedding, sectioning, and staining with hematoxylin and eosin (H&E), followed by mounting. These standard tissue processing methods were adapted from the protocols outlined by Baker [42] and Isirima and Uahomo [43].

Ethical Approval

The study was conducted in accordance with ethical guidelines established by the National Institutes of Health (NIH) for the ethical treatment of animals in research. Approval for the study

protocol was obtained from the Research Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria, under reference number UPH/CEREMAD/REC/MM91/076.

Method of Data Analysis

The data collected were analyzed using the Statistical Package for Social Sciences (IBM SPSS, Version 26.0). Descriptive statistics, such as means and standard error of mean (SEM), were used to summarize the data from the experimental groups. Inferential statistical tests, including one-way analysis of variance (ANOVA) or t-tests, were conducted to assess differences between groups. Post-hoc tests, such as the Dunnett (2-sided) test, were employed to compare the study groups and identify statistically significant differences, with significance set at $p<0.05$.

RESULTS

Effect of *B. pinnatum* on Kidney function makers in Ketamine-induced Toxicity in Wistar Rats

Table 2 shows the effect of ketamine and different doses of *B. pinnatum* on BUN levels in Wistar rats over three weeks. The control group maintained stable BUN levels at 6.77±0.15 mg/dL throughout the study. The ketamine-only group exhibited a significant increase in BUN levels, reaching 11.50±0.17 mg/dL ($p<0.05$). The risperidone-treated group started with BUN levels similar to the control group (6.77±0.15 mg/dL), but by week 3, these levels increased to 8.77±0.15 mg/dL. The low-dose *B. pinnatum* (50 mg/kg) group showed fluctuating BUN levels, ranging from 5.50±0.17 mg/dL to 8.70±0.12 mg/dL, which remained significantly lower than the ketamine group ($p<0.05$). The medium (100 mg/kg) and high (200 mg/kg) doses of *B. pinnatum* consistently showed reductions in BUN levels, with the high dose nearing control values (5.33±0.24 to 5.73±0.49 mg/dL, $p<0.05$).

Table 3 presents the effects of the treatments on creatinine levels. The control group maintained stable creatinine levels at 69.00±2.08 µmol/L. Ketamine significantly elevated creatinine levels to 100.00±2.89 µmol/L ($p<0.05$). Risperidone-treated rats initially exhibited elevated creatinine levels, but by week 3, these levels slightly reduced to 85.00±1.73 µmol/L ($p<0.05$). All doses of *B. pinnatum* led to reduced creatinine levels compared to the ketamine group, with the high dose showing values closer to control (69.00±3.79 to 64.67±4.26 µmol/L, $p<0.05$).

Table 4 highlights the effects of ketamine and *B. pinnatum* on urea levels. The control group had stable urea levels at 26.00±1.15 mg/dL. Ketamine administration increased urea levels to 43.00±2.08 mg/dL ($p<0.05$). Risperidone initially reduced urea levels, but by week 2, levels increased to 34.00±1.15 mg/dL before stabilizing near control values. The high-dose *B. pinnatum* (200 mg/kg) significantly reduced urea levels, with values ranging from 12.23±0.15 to 23.33±0.88 mg/dL, closer to control by week 3 ($p<0.05$).

Table 5 reports the effects on sodium (Na) levels. The control group maintained stable sodium levels at 139.00±0.58 mmol/L. Ketamine elevated sodium levels to 164.00±4.16 mmol/L ($p<0.05$). Risperidone-treated rats had sodium levels near control (140.00±1.15 to 140.67±0.88 mmol/L, $p<0.05$). All doses of *B. pinnatum* significantly reduced sodium levels, with the high-dose group (200 mg/kg) showing a substantial decrease to 131.00±0.58 mmol/L by week 3 ($p<0.05$).

Table 6 illustrates the effects of ketamine and *B. pinnatum* on potassium (K) levels. The control group had consistent potassium levels at 1.20±0.06 mmol/L. Ketamine significantly increased potassium levels to 2.83±0.34 mmol/L ($p<0.05$). Risperidone initially reduced potassium levels, but by week 3, levels spiked to 3.50±0.12 mmol/L ($p<0.05$). *B. pinnatum* treatment led to reductions in potassium, with the high-dose group (200 mg/kg) showing levels close to control (0.50±0.05 to 0.93±0.09 mmol/L, $p<0.05$).

Table 7 presents calcium (Ca) levels. The control group maintained stable calcium levels at 2.50±0.06 mmol/L. Ketamine caused a significant decrease in calcium levels to 0.77±0.18 mmol/L ($p<0.05$). Risperidone-treated rats showed calcium levels close to control values (2.47±0.09 to 2.43±0.09 mmol/L, $p<0.05$). High-dose *B. pinnatum* significantly restored calcium levels, with values ranging from 2.43±0.12 to 3.43±0.15 mmol/L by week 3 ($p<0.05$).

Table 8 details albumin (ALB) levels. The control group maintained stable albumin levels at 4.10±0.06 g/dL. Ketamine treatment significantly reduced albumin levels to 2.33±0.24 g/dL ($p<0.05$). Risperidone-treated animals showed albumin levels near control values (4.23±0.15 to 4.10±0.06 g/dL, $p<0.05$). The high-dose *B. pinnatum* group significantly restored albumin levels, increasing from 3.57±0.23 to 4.47±0.03 g/dL by week 3 ($p<0.05$).

Table 2: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on BUN (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	6.77±0.15#	6.77±0.15#	6.80±0.15#
20mg/kg Ketamine	11.50±0.17*	11.50±0.17*	11.50±0.17*
0.5mg/kg Risperidone	6.77±0.15#	7.50±0.17	8.77±0.15
50mg/kg *B. pinnatum*	5.50±0.17#	8.70±0.12	6.83±0.52
100mg/kg *B. pinnatum*	6.23±0.15#	7.50±0.68	5.53±0.72#
200mg/kg *B. pinnatum*	5.33±0.24#	6.40±0.75#	5.73±0.49#

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

Table 3: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Creatinine (µmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	69.00±2.08#	69.00±2.08#	69.00±2.08#
20mg/kg Ketamine	100.00±2.89*	100.00±2.89*	100.00±2.89*
0.5mg/kg Risperidone	79.00±2.08*#	82.00±2.31*#	85.00±1.73*#
50mg/kg *B. pinnatum*	74.33±2.33*#	72.00±4.62*#	75.00±10.15*#
100mg/kg *B. pinnatum*	75.00±6.81*#	78.67±1.76*#	67.00±4.58#
200mg/kg *B. pinnatum*	69.00±3.79#	70.67±7.06#	64.67±4.26#

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

Table 4: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Urea (µmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	26.00±1.15#	26.00±1.15#	26.00±1.15#
20mg/kg Ketamine	43.00±2.08*	43.00±2.08*	43.00±2.08*
0.5mg/kg Risperidone	10.10±0.21*#	34.00±1.15*#	27.00±3.61#
50mg/kg *B. pinnatum*	11.50±1.85*#	32.00±1.15*#	25.00±1.73#
100mg/kg *B. pinnatum*	14.00±1.89*#	31.33±2.40*#	23.67±2.33#
200mg/kg *B. pinnatum*	12.23±0.15*#	27.33±2.40#	23.33±0.88#

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

Table 5: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Sodium (Na) (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3
Control	139.00±0.58#	139.00±0.58#	139.00±0.58#
20mg/kg Ketamine	164.00±4.16*	164.00±4.16*	164.00±4.16*
0.5mg/kg Risperidone	140.00±1.15#	136.00±0.58#	140.67±0.88#
50mg/kg *B. pinnatum*	138.00±0.115#	140.00±0.58#	139.67±1.45#
100mg/kg *B. pinnatum*	138.67±1.76#	137.00±0.58#	140.00±0.58#
200mg/kg *B. pinnatum*	136.00±1.15#	134.00±0.58#	131.00±0.58#

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

Table 6: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Potassium (K) (mmol/L) in Wistar rats

Groups	Week 1	Week 2	Week 3

Control	1.20±0.06#	1.20±0.06#	1.20±006#
20mg/kg Ketamine	2.83±0.34*	2.83±0.34*	2.83±0.34*
0.5mg/kg Risperidone	1.10±0.06#	0.90±0.06#	3.50±0.12*
50mg/kg *B. pinnatum*	1.30±0.06#	1.10±0.05#	3.13±0.15*
100mg/kg *B. pinnatum*	1.30±0.115#	0.80±0.06#	3.27±0.18*
200mg/kg *B. pinnatum*	1.23±0.09#	0.50±0.05*#	0.93±0.09#

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

Table 7: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Calcium (Ca) (mmol/L) in Wistar rats

Groups	**Week 1**	**Week 2**	**Week 3**
Control	2.50±0.06#	2.50±0.06#	2.50±0.06#
20mg/kg Ketamine	0.77±0.18*	0.77±0.18*	0.77±0.18*
0.5mg/kg Risperidone	2.47±0.09#	2.80±0.06#	2.43±0.09#
50mg/kg *B. pinnatum*	2.40±0.12#	2.70±0.06#	2.67±0.09#
100mg/kg *B. pinnatum*	2.40±0.06#	3.00±0.06#	2.60±0.17#
200mg/kg *B. pinnatum*	2.43±0.12#	3.30±0.06#	3.43±0.15#

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

Table 8: Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on Albumin (ALB) (g/dl) in Wistar rats

Groups	**Week 1**	**Week 2**	**Week 3**

Control	4.10±0.06#	4.10±0.06#	4.10±0.06#
20mg/kg Ketamine	2.33±0.24*	2.33±0.24*	2.33±0.24*
0.5mg/kg Risperidone	4.23±0.15#	4.40±0.06#	4.10±0.06#
50mg/kg *B. pinnatum*	4.00±0.12#	4.30±0.06#	3.90±0.06#
100mg/kg *B. pinnatum*	4.17±0.20#	4.60±0.06#	4.07±0.09#
200mg/kg *B. pinnatum*	3.57±0.23	4.90±0.06#	4.47±0.03#

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

The effect of ketamine and various doses of *B. pinnatum* on kidney histology in Wistar

The histological assessment of kidney tissues across different experimental groups demonstrated varying degrees of structural alterations, depending on the treatment administered and the duration of exposure to ketamine.

In week 1, the normal control group displayed well-preserved renal tubules and connective tissue, while ketamine exposure at 20 mg/kg without treatment caused ruptured glomerular capillaries, cellular degeneration, and disrupted renal tubules; treatment with risperidone at 0.5 mg/kg mitigated some damage but still showed congestion in renal blood vessels and epithelial abnormalities. *B. pinnatum* at 50 mg/kg caused glomerular hypertrophy and tubule disruption, 100 mg/kg led to dilatation and rupture of glomerular capillaries with fluid deposits, and 200 mg/kg resulted in extensive capillary rupture, cellular degeneration, and significant renal structural distortion.

In week 2, untreated ketamine exposure at 20 mg/kg caused severe structural damage with dilated glomerular capillaries, cellular proliferation, and hypertrophy in renal tubules; risperidone treatment at 0.5 mg/kg showed pronounced glomerular oedema and epithelial distortion. *B. pinnatum* treatment exhibited dose-dependent effects, with 50 mg/kg causing diffuse fluid

accumulation, renal tubule dilation, and inflammation, 100 mg/kg exacerbating capillary rupture and oedema with fluid deposits, and 200 mg/kg leading to distorted glomeruli and tubules with connective tissue abnormalities.

In week 3, ketamine exposure at 20 mg/kg without treatment led to glomerular cell proliferation, hypertrophy, and severe renal tubule congestion; risperidone treatment at 0.5 mg/kg showed persistent glomerular and epithelial distortions. *B. pinnatum* treatment demonstrated dose-dependent responses, where 50 mg/kg caused glomerular capillary rupture, cellular hypertrophy, and epithelial disruption, 100 mg/kg led to glomerular capillary dilation, connective tissue distortion, and tubule deposits, and 200 mg/kg caused ruptured glomerular capillaries, proximal tubule deposits, and renal blood vessel congestion.

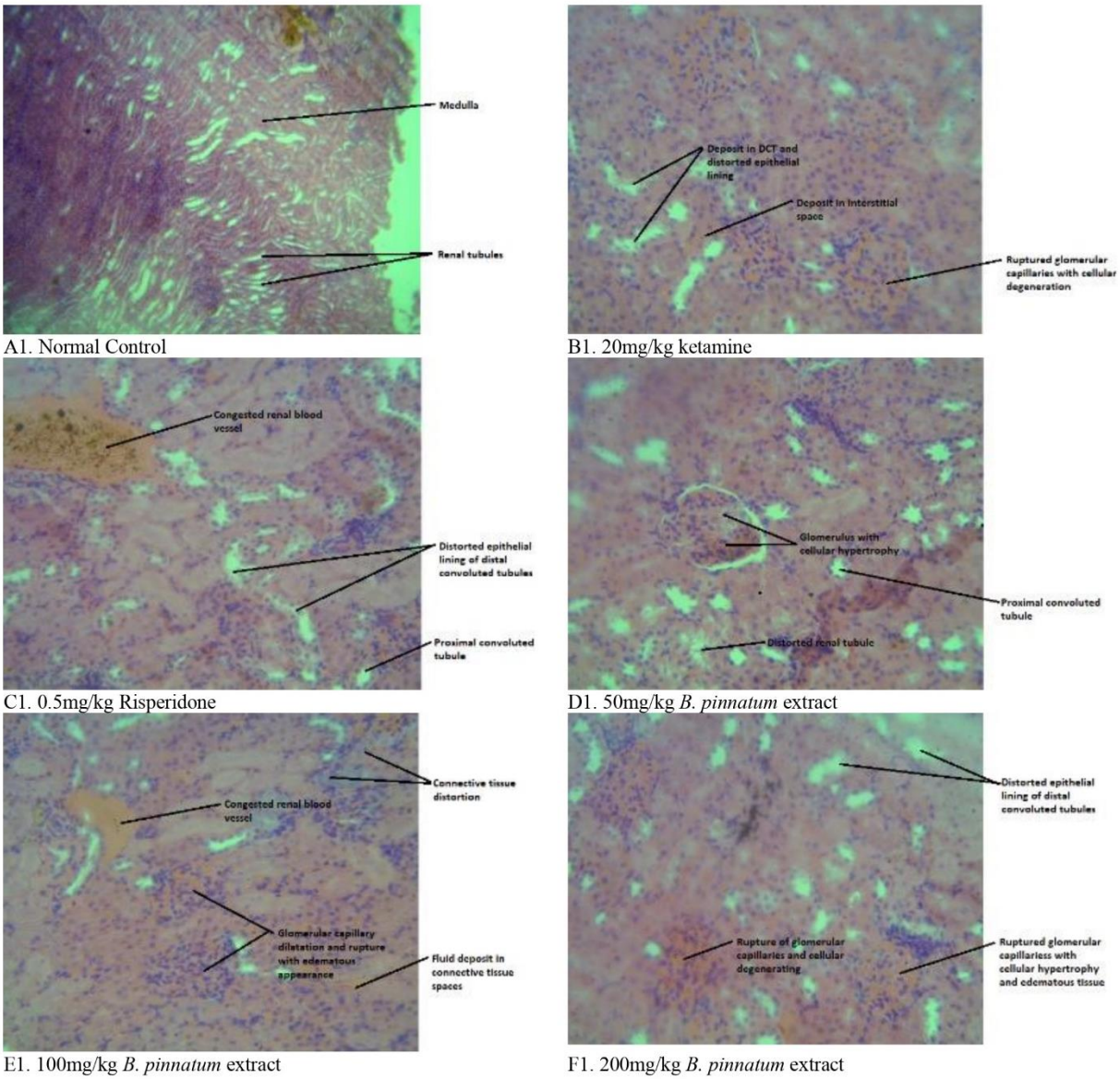

Fig. 1. Photomicrograph of the Kidney tissue across different treatment groups (H & E, x400) in week 1 showing: (A1) normal microstructure with numerous renal tubules and connective tissue in the control group; (B1) abnormalities in the epithelial lining of distal convoluted tubules, congestion in renal blood vessels, and defined proximal convoluted tubules in ketamine-exposed animals treated with risperidone; (C1) hypertrophy of glomerular cells, distortion of renal tubules, and defined proximal convoluted tubules in ketamine-exposed animals treated with a low dose of *B. pinnatum*; (D1) dilatation and rupture of glomerular capillaries, oedematous glomerulus, and congestion in connective tissue space in animals treated with a moderate dose of *B. pinnatum*; (E1) ruptured glomerular capillaries, cellular degeneration, and distortion in distal convoluted tubules in animals treated with a high dose of *B. pinnatum*; (F1) ruptured glomerular capillaries, cellular degeneration, and mild fluid deposition in animals exposed to ketamine without treatment.

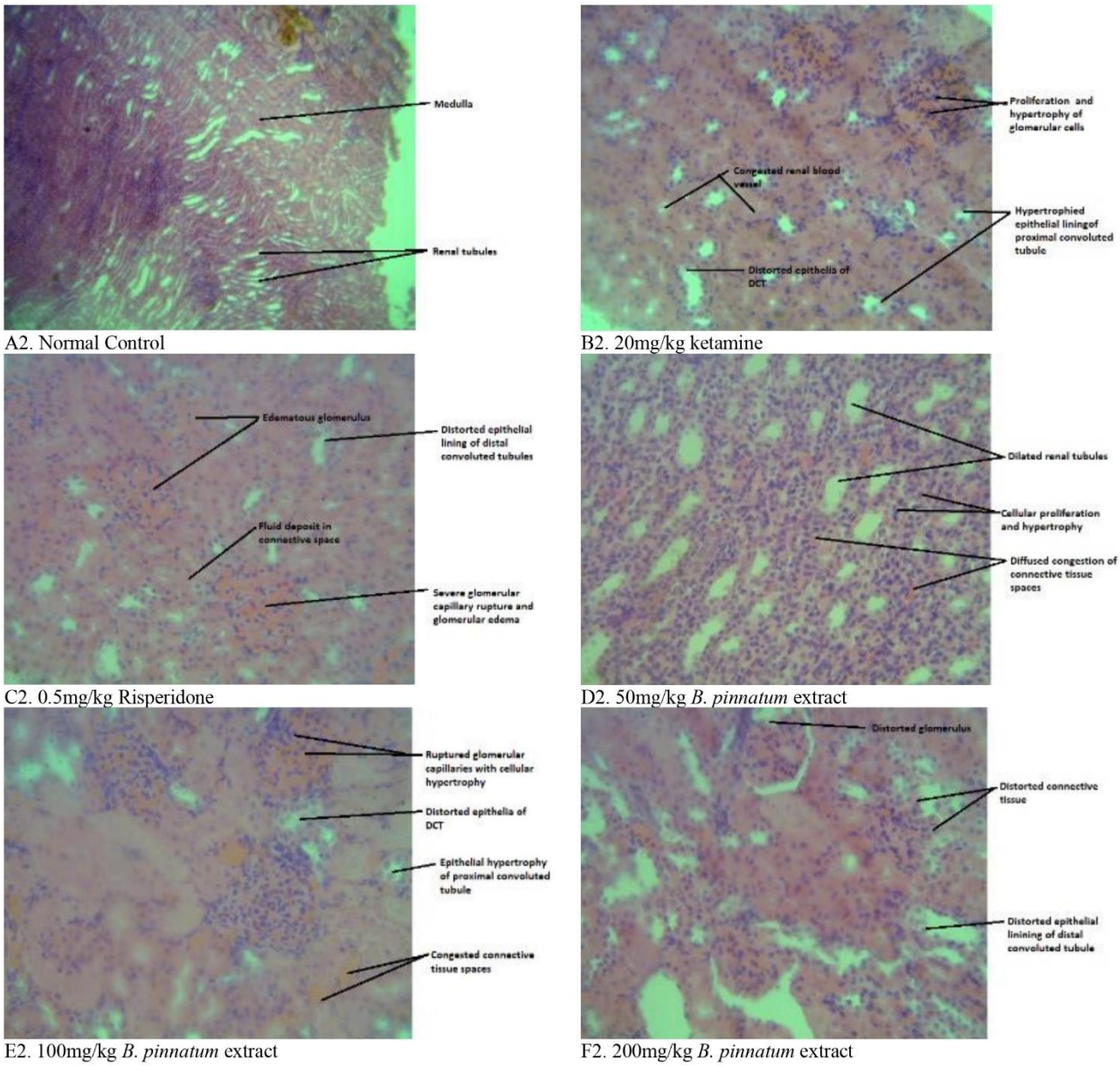

Fig. 2. Photomicrograph of the Kidney tissue across different treatment groups (H & E, x400) in week 2 showing: (A2) normal microstructure in the control group; (B2) ruptured glomerular capillaries, glomerular oedema, and distortion in epithelial lining of distal convoluted tubules in ketamine-exposed animals treated with risperidone; (C2) cellular proliferation, hypertrophy, diffuse fluid accumulation in connective tissues, and dilated renal tubules in animals treated with a low dose of *B. pinnatum*; (D2) ruptured glomerular capillaries, pronounced glomerular oedema, and fluid deposition in connective tissue spaces in animals treated with a moderate dose of *B. pinnatum*; (E2) distortions in glomeruli, epithelial lining of distal convoluted tubules, and connective tissue abnormalities in animals treated with a high dose of *B. pinnatum*; (F2) dilated glomerular capillaries, cellular proliferation, and hypertrophy in the epithelial lining of proximal and distal convoluted tubules in animals exposed to ketamine without treatment.

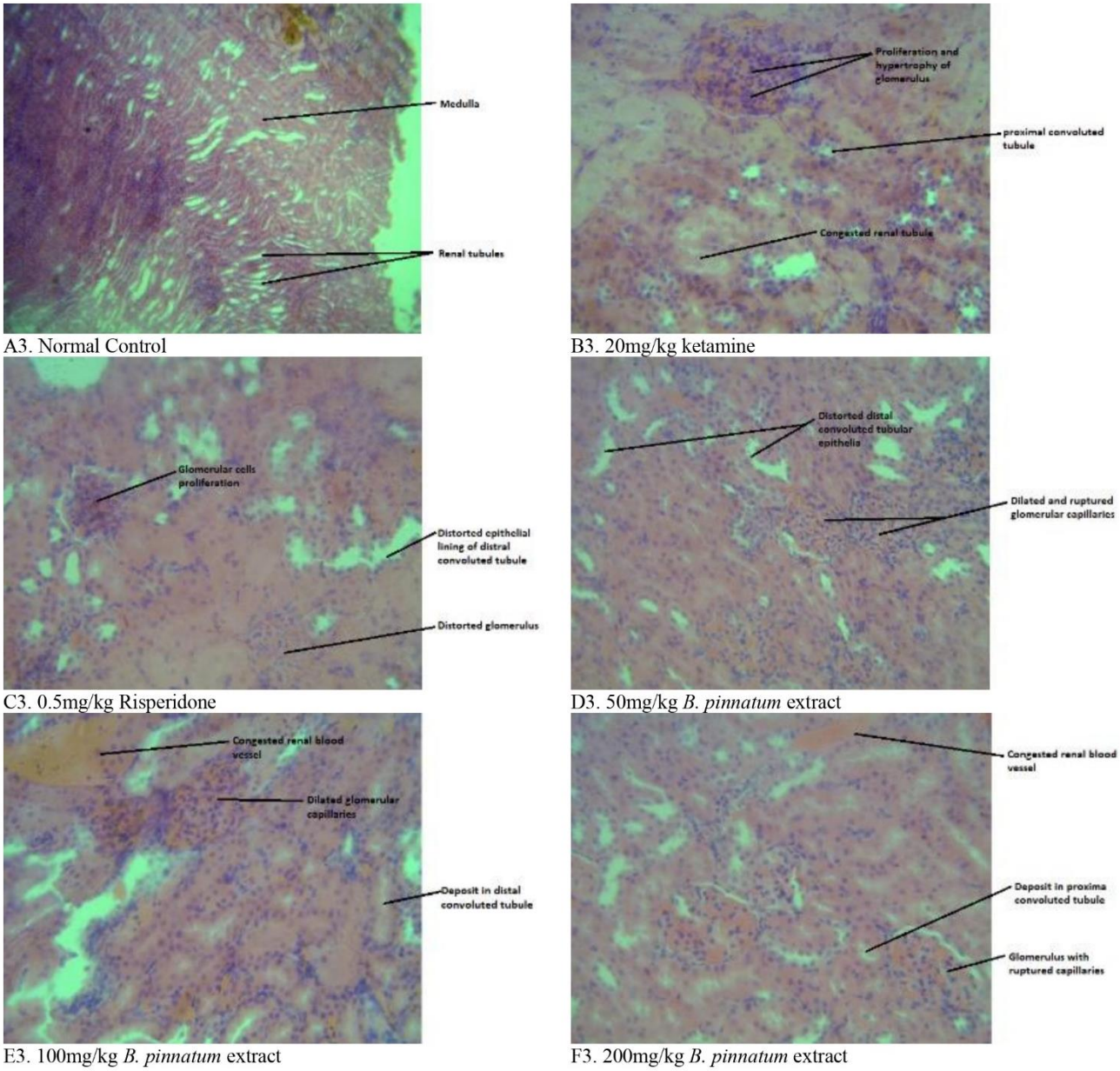

Fig. 3. Photomicrograph of the Kidney tissue across different treatment groups (H & E, x400) in week 3 showing: (A3) normal microstructure in the control group; (B3) distortion in glomerular cells, distortion in epithelial lining of distal convoluted tubules, and microstructural alterations in ketamine-exposed animals treated with risperidone; (C3) ruptured glomerular capillaries, cellular hypertrophy, and distortion in the epithelial lining of distal convoluted tubules in animals treated with a low dose of *B. pinnatum*; (D3) dilated glomerular capillaries, distorted connective tissue, and deposits in distal convoluted tubules in animals treated with a moderate dose of *B. pinnatum*; (E3) ruptured glomerular capillaries, deposits in proximal convoluted tubules, and congested renal blood vessels in animals treated with a high dose of *B. pinnatum*; (F3) proliferation and hypertrophy of glomerular cells, congested renal tubules, and disrupted microstructure in animals exposed to ketamine without treatment.

DISCUSSION

The present study aimed to evaluate the potential protective effects of *B. pinnatum* against ketamine-induced kidney injury in Wistar rats, focusing on both biochemical and histological markers of kidney function and structure. Ketamine, a commonly used anesthetic, is known to cause nephrotoxicity through its mechanisms of oxidative stress, tubular injury, and glomerular damage. Ketamine abuse has been linked to significant kidney damage and dysfunction, including chronic, often irreversible damage such as hydronephrosis, renal failure, shrunken urinary bladder, and hydroureteronephrosis, all affecting kidney function [44,45]. Chronic and illicit use of ketamine can also cause structural damage to the urinary tract, including the bladder, ureters, and kidneys [46]. While the exact mechanism of ketamine-induced kidney damage is not fully understood, it may involve direct effects of the drug or its metabolites, immunological reactions to contaminants, or interactions with other drugs of abuse [44]. Symptoms of ketamine-induced kidney damage include burning micturition, incontinence, and recurrent urinary tract problems [45].

In this study, we assessed the biochemical effects of *B. pinnatum* on key kidney markers, including blood urea nitrogen (BUN), creatinine, urea, sodium, potassium, calcium, and albumin. Additionally, we examined the histopathological changes in kidney tissues to further understand the impact of *B. pinnatum* on renal structure in the context of ketamine-induced damage.

Effect of *B. pinnatum* on biochemical markers of the kidney in ketamine-induced injury

The impact of *B. pinnatum* on various biochemical markers of kidney function was evident in the results of this study, where the administration of ketamine resulted in significant biochemical alterations, indicating renal dysfunction. Ketamine exposure notably increased BUN, creatinine, and urea levels, suggesting impaired renal filtration and excretion, a hallmark of nephrotoxicity.

BUN and creatinine are well-established markers of kidney injury, and elevated levels of these parameters are commonly associated with renal impairment. Furthermore, ketamine caused a significant rise in sodium and potassium levels, reflecting disturbed electrolyte balance, which is often seen in renal injury due to reduced renal excretion capacity. The alteration in calcium and albumin levels also pointed to disrupted renal homeostasis. This observation is corroborated by Jang et al. [47] and Vizgan et al. [48], who reported that ketamine caused nephrotoxicity in rats, characterized by increased BUN, creatinine, and urea levels, and decreased calcium and albumin levels.

The significant increase in BUN, creatinine, urea, sodium, and potassium levels, along with the decrease in calcium and albumin levels induced by ketamine, suggests severe kidney dysfunction in Wistar rats. Elevated BUN and creatinine levels indicate impaired GFR, a critical marker of renal health, suggesting that the kidneys are not effectively filtering waste products from the blood. High urea levels further confirm compromised kidney function and potential accumulation of toxic metabolites [44,47,49]. Increased sodium and potassium levels point to disrupted electrolyte balance and impaired renal tubular function, potentially leading to conditions like hypernatremia and hyperkalemia, which can have severe systemic effects, including cardiovascular complications [44]. The decrease in calcium levels could suggest altered calcium-phosphate metabolism, affecting bone health and neuromuscular function. Reduced albumin levels indicate impaired protein synthesis or increased protein loss due to damaged kidney structures, leading to issues like edema and compromised oncotic pressure [50,51].

Treatment with *B. pinnatum* at both 100 mg/kg and 200 mg/kg doses significantly mitigated these biochemical changes, bringing the levels of BUN, creatinine, and urea closer to the control values. This is in line with previous studies that have shown the nephroprotective effects of *B. pinnatum*,

possibly due to its antioxidant and anti-inflammatory properties [52-54]. The reduction in sodium and potassium levels with *B. pinnatum* treatment suggests an improvement in renal function and electrolyte balance, which could be attributed to the plant's ability to modulate renal tubular transport and reduce cellular damage. The restoration of calcium and albumin levels further supports the protective effects of *B. pinnatum*, as both are critical markers of kidney integrity and function.

The treatment with 200mg/kg *B. pinnatum* appeared to be the most effective in normalizing biochemical markers, with values approaching those of the control group. This suggests a dose-dependent response of *B. pinnatum* in attenuating ketamine-induced renal dysfunction. The significant reduction in these markers of kidney injury points to the potential therapeutic use of *B. pinnatum* in protecting the kidneys from damage caused by nephrotoxic agents like ketamine.

The dose-dependent nephroprotective effects of *B. pinnatum* can be attributed to its diverse array of bioactive compounds such as flavonoids, phenolic compounds, alkaloids, triterpenoids, and bufadienolides. These compounds collectively contribute to its pharmacological actions by acting as potent antioxidants, reducing oxidative stress-induced damage to renal tissues. Furthermore, they possess anti-inflammatory properties that help mitigate inflammation associated with ketamine-induced nephrotoxicity. Previous research has demonstrated the nephroprotective activity of *B. pinnatum* against gentamicin-induced nephrotoxicity in Wistar rat kidneys, highlighting its antioxidant and radical scavenging properties [55,56]. Furthermore, studies suggest that the plant's juice, known for its anti-cholinergic effects, effectively treats hyperactive bladder with fewer side effects compared to conventional drugs.

Effect of *B. pinnatum* on the kidney histology in ketamine-induced injury

The histopathological findings from this study provided crucial insight into the structural effects of ketamine on kidney tissue and the potential ameliorative role of *B. pinnatum*. In the control group, the kidney tissue remained structurally intact, with well-preserved renal tubules and connective tissue. In contrast, ketamine treatment resulted in extensive kidney damage, characterized by ruptured glomerular capillaries, cellular degeneration, and disrupted renal tubules. These histopathological changes suggest profound nephrotoxicity, potentially due to direct cellular toxicity or inflammatory responses leading to impaired renal function and structural integrity. Previous studies have reported similar findings, with Demirkiran et al. [49] observing significant histopathological changes in renal tissue following ketamine exposure, including congestion. In their study, congestion was observed in the control group at a medium level, whereas the ketamine-treated group exhibited statistically lower congestion levels compared to the control group. Kasıkara et al. [57] also reported increased vascular congestion in the kidney of the ketamine group compared to the saline group, reinforcing the nephrotoxic effects of ketamine. Additionally, Yahyaei et al. [58] described similar renal abnormalities, including ruptured glomerular capillaries, cellular proliferation, and hypertrophy in glomerular cells and renal tubules over a three-week exposure period to ketamine.

Risperidone, which was used as a positive control in this study, partially mitigated the ketamine-induced histological damage, but some abnormalities such as glomerular congestion and epithelial distortions were still evident. These findings suggest that risperidone might provide limited protection against ketamine-induced kidney injury, highlighting the need for more effective treatments.

The histological examination of *B. pinnatum*-treated rats revealed dose-dependent effects. The low-dose *B. pinnatum* (50 mg/kg) group exhibited moderate glomerular hypertrophy and tubule disruption, indicating some level of nephroprotective effect, though the damage was still evident. A more pronounced protective effects were observed with 100 mg/kg and 200 mg/kg dose treatment. The 200mg/kg treated group exhibited reduced capillary rupture, cellular degeneration, and renal structural distortion, and the renal tubules showed less disruption. Notably, the 200mg/kg *B. pinnatum* treatment closely resembled the control group in terms of renal tissue integrity, suggesting a significant reduction in ketamine-induced nephrotoxicity.

These histological observations correlate well with the biochemical data, where the high-dose *B. pinnatum* group showed the greatest improvements in kidney function. The protective effects can be attributed to the plant's bioactive compounds such as flavonoids, phenolic compounds, alkaloids, triterpenoids, and bufadienolides, which act as potent antioxidants and anti-inflammatory agents [56,59,60]. These compounds help mitigate oxidative stress and inflammation, reducing cellular damage, hypertrophy, and structural distortions in renal tissues affected by ketamine-induced nephrotoxicity. Furthermore, *B. pinnatum*'s anti-inflammatory and cell-protective effects may promote tissue repair and regeneration, which could explain the structural improvements observed in the kidneys of treated rats.

The ethnomedicinal use of *B. pinnatum* has been supported by previous studies, confirming that its leaf extracts prevent renal calculi formation and ameliorate histological alterations in kidney tissues [59]. Moreover, Anadozie et al. [61] demonstrated the nephroprotective effects of *B. pinnatum*, highlighting its ability to inhibit arginase II activity and mitigate oxidative damage induced by carbon tetrachloride (CCl4) in rats. Furthermore, Hosomi et al. [62] reported the safety of *B. pinnatum* mother tincture (MT) during pregnancy, showing no histological changes in

maternal or fetal structures. Additionally, *B. pinnatum* has been shown to enhance kidney function by reducing glucose and creatinine levels.

CONCLUSION

The present study highlights the potential nephroprotective effects of *Bryophyllum pinnatum* in mitigating ketamine-induced kidney injury. Both biochemical and histological assessments indicate that *B. pinnatum*, particularly at higher doses, can attenuate renal dysfunction and preserve kidney structure. These findings suggest that *B. pinnatum* could be a promising candidate for the development of therapeutic strategies aimed at protecting the kidneys from nephrotoxic damage caused by substances such as ketamine. Further studies are needed to elucidate the precise mechanisms underlying the nephroprotective effects of *B. pinnatum* and to evaluate its potential clinical applications in the treatment of kidney diseases.

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