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Original Article

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Biochemical and histological assessment of the hepatoprotective effects of *Bryophyllum pinnatum* leaf extract in Ketamine-induced liver toxicity in male Wistar rats

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Abstract

***Background & Aims*:** Ketamine, a commonly used anesthetic and recreational drug, can induce liver toxicity through oxidative stress and hepatocellular damage. *Bryophyllum pinnatum*, traditionally used for liver-related ailments due to its hepatoprotective properties, has shown potential but remains underexplored for ketamine-induced toxicity. This study investigated the effects of *B. pinnatum* leaf extract on liver function biomarkers and histopathology in ketamine-induced hepatotoxicity in Wistar rats.

***Materials & Methods*:** Sixty male Wistar rats were divided into six groups: normal control, ketamine-induced group (20 mg/kg, negative control), positive control (0.5 mg/kg risperidone), and three *B. pinnatum*-treated groups (50 mg/kg, 100 mg/kg, and 200 mg/kg). Serum levels of AST, ALT, ALP, GGT, and total protein were assessed, alongside histological analysis of liver tissues using hematoxylin and eosin staining. Statistical significance ($p < 0.05$) was determined using ANOVA and Dunnett's post-hoc test.

***Results*:** Ketamine significantly elevated liver enzyme levels (AST: 65.00 ± 2.89 U/L, ALT: 60.00 ± 2.89 U/L, ALP: 85.00 ± 2.89 U/L, GGT: 78.10 ± 3.09 U/L) and reduced total protein (6.07 ± 0.47 g/dL) compared to controls (AST: 27.67 ± 1.45 U/L, ALT: 22.33 ± 1.45 U/L, ALP: 37.67 ± 1.45 U/L, GGT: 34.27 ± 2.55 U/L; total protein: 7.40 ± 0.12 g/dL). Treatment with *B. pinnatum* normalized these biomarkers, with the 200 mg/kg dose showing the most significant effects (AST: 21.00 ± 2.08 U/L, ALT: 20.00 ± 5.77 U/L, ALP: 22.00 ± 3.06 U/L, GGT: 26.01 ± 3.11 U/L, total protein: 7.80 ± 0.12 g/dL). Histological findings indicated ketamine-induced hepatocyte damage was ameliorated by *B. pinnatum* in a dose-dependent manner, with marked improvements at 200 mg/kg.

Conclusion: *B. pinnatum* extract exhibits promising hepatoprotective effects against ketamine-induced liver toxicity, as evidenced by normalization of liver biomarkers and histological recovery. These preliminary findings in an animal model highlight its potential for therapeutic applications in liver disorders, warranting further investigation.

***Keywords*:** Antioxidant activity, *Bryophyllum pinnatum*, Hepatoprotective effects, Ketamine toxicity, Liver biomarkers, Wistar rats

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Introduction

Ketamine is an anesthetic agent increasingly used for various medical applications, including pain management, treatment-resistant depression, and sedation in the intensive care unit (1-4). While generally well-tolerated, long-term or high-dose use of ketamine has been associated with potential hepatotoxicity (5, 6). Studies in animal models have demonstrated that

prolonged ketamine infusion can lead to dose-dependent hepatotoxicity, including mitochondrial degeneration in hepatic cells, dilation of the biliary tract and bile ducts, and increased liver enzyme levels (7-9). Despite its widespread use, there remains a paucity of studies exploring the specific mechanisms and mitigation strategies for ketamine-induced hepatotoxicity. For example, Navarro and Senior (10) highlighted ketamine-induced liver damage during pain management, while Abd El-Fattah et al. (11) demonstrated the hepatoprotective effects of *Camellia sinensis* in rats exposed to ketamine. These gaps underscore the need for further research into hepatoprotective interventions that can counteract ketamine's toxic effects on hepatocytes.

The liver, as a central metabolic organ, plays a crucial role in xenobiotic metabolism and is therefore highly susceptible to xenobiotic-induced injury (12). Ketamine-induced liver injury is thought to involve the accumulation of toxic metabolites, increased lipid peroxidation, and free radical formation, all of which contribute to oxidative stress and hepatocellular damage (6, 13, 14). Beyond neurotoxicity, ketamine use and abuse have been linked to complications such as hepatitis, liver fibrosis, and cirrhosis, highlighting the urgency of addressing its hepatotoxic effects (5, 13, 15, 16). Additionally, its emergence as a recreational drug has raised concerns about its long-term impact on organ functions, including the liver (17-19).

In the search for hepatoprotective agents, herbal remedies have garnered attention due to their bioactive properties. *Bryophyllum pinnatum* (*B. pinnatum*), also known as the "life plant" or "air plant," has traditionally been used for treating liver disorders such as hepatitis and cirrhosis (20). The plant is rich in bioactive compounds like alkaloids, flavonoids, and phenolic acids, which contribute to its antioxidant, anti-inflammatory, and hepatoprotective activities (21-27). Research into *B. pinnatum* has demonstrated its efficacy in mitigating liver damage caused by various xenobiotics, promoting hepatocellular repair, and restoring normal liver function through mechanisms such as scavenging free radicals, inhibiting lipid peroxidation, and enhancing antioxidant enzyme activity (22, 28-34).

Despite these promising attributes, studies investigating the application of *B. pinnatum* specifically in the context of ketamine-induced hepatotoxicity are lacking. Given the well-documented hepatotoxic effects of ketamine and the promising bioactive profile of *B. pinnatum*, this study aims to bridge this gap by investigating whether its hepatoprotective properties can mitigate liver damage induced by ketamine in a rat model. By examining its impact on liver enzyme levels, histological changes, and related biomarkers, this study pioneers the exploration of *B. pinnatum* as a potential therapeutic intervention for ketamine-associated hepatotoxicity.

Materials & Methods

Experimental Animals

Sixty adult male Wistar rats, each 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 rats were kept in clean, disinfected wooden cages with sawdust bedding, under a 12-hour light/dark cycle, with humidity levels of 50–60% and a temperature of approximately 30°C. They were given two weeks to acclimatize, with unrestricted access to clean water and animal feed before the experiment began.

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 by Dr. Edwin Nwosu in the Department of Plant Science and Biotechnology, Faculty of Sciences, University of Port Harcourt, and assigned the voucher number UPH/V/1308. Ketamine and risperidone were purchased from Alpha Pharmacy and Stores, located along NTA Road, Port Harcourt, Rivers State, Nigeria.

Preparation of *B.* pinnatum extract

The plant tissue homogenization method, as described by Pandey and Tripathi (35), was used to

extract fresh plant juice from *B. pinnatum* leaves. The fresh leaves were finely ground using a blender, and the juice was extracted and filtered using a clean white handkerchief, following the procedure outlined by Das et al. (36). The resulting juice was carefully collected and stored in clean reagent bottles, which were then refrigerated for preservation.

Dose selection

The doses of 20 mg/kg ketamine and 0.5 mg/kg risperidone used in this study were based on previous studies by Monte et al. (37) and Ben-Azu et al. (38), respectively. *B. pinnatum* crude extract doses of 50, 100, 200 mg/kg body weight were administered following guidelines from Salahdeen and Yemitan (39), 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. (37). The research was conducted in two distinct phases;

- **Induction phase:** The 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 animals were sacrificed from each group on the 7th day, and blood samples, as well as liver tissues, were collected for biochemical and histological examinations aimed at establishing the baseline toxicity in the animal model.
- **Intervention Phase:** Group assignments for the intervention phase (Table 1) include controls, ketamine-only, risperidone-treated, and three doses of *B. pinnatum*. Treatments lasted 21 days following a 7-day induction period. Risperidone and doses of *B. pinnatum* were administered by oral gavage.

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

Collection of Blood and Tissue Sample

Twenty-four 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 liver tissues were carefully harvested for biochemical assays and histological examination.

Biochemical analysis

Tissue and blood samples were collected to assess the impact of *B. pinnatum* leaf extract on liver function markers. The levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl Transferase (GGT), and Total Protein (TP) were evaluated.

Liver Function Markers Examination

Blood samples were collected in heparinized tubes to prevent coagulation and immediately centrifuged at 3000 rpm for 15 minutes (40). The resulting plasma was separated and stored at -80°C until analysis. The AST and ALT levels were measured in the serum samples using a biochemical analyzer, following standard enzymatic methods (41). ALP, AST, ALP, and GGT activities was determined following established methods by Tietz (42). TP concentration was determined using the Biuret method (43).

Histopathological Examination

The animals were anesthetized with diethyl ether and dissected aseptically to remove the liver tissues. These tissues were then transferred into 10% formalin and later trimmed to a size of 2mm to 4mm thickness to allow the fixative to readily penetrate. The liver tissues were processed through various stages, including fixation, dehydration, clearing, impregnation, embedding, sectioning, and staining with hematoxylin and eosin (H&E), followed by mounting. These standard processing methods were described by Baker (44) and Isirima and Uahomo (45).

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 deviations, were employed to analyze the data obtained from the experimental groups. Inferential statistical tests, including one-way analysis of variance (ANOVA), were conducted. Dunnett (2-sided) post-hoc test was utilized to compare study groups and identify significant differences at $P < 0.05$.

Results

Effect on Liver function makers in ketamine-induced toxicity in Wistar Rats

The effects of ketamine and different doses of *B. pinnatum* on liver enzymes and total protein in Wistar rats over three weeks were analyzed, with each group compared to the control and ketamine groups.

Table 2 shows the results for AST levels. The control group maintained stable levels at 27.67±1.45 U/L across all weeks. The ketamine group (20 mg/kg) exhibited significantly elevated AST levels at 65.00±2.89 U/L ($P < 0.05$), showing no change over the weeks. The risperidone group (0.5 mg/kg) had varying AST levels, ranging from 31.00 ± 0.58 to 46.67 ± 1.67 U/L ($P < 0.05$), which were higher than the control but lower than the ketamine group. The low (50 mg/kg) and medium (100 mg/kg) doses of *B. pinnatum* resulted in AST levels that were significantly different from both the control and ketamine groups, but closer to risperidone. The high dose (200 mg/kg) showed the most variation, with significant increases and decreases observed across the weeks. The mean reduction in AST levels for the 50 mg/kg dose was 48.37±5.49%, and for the 200 mg/kg dose, it was 53.50 ± 9.34%, compared to the ketamine group.

Table 2. Result of the effect of ketamine and crude extract doses of *Bryophyllum pinnatum* on AST (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Mean reduction (%)
Control	27.67 ± 1.45#	27.67 ± 1.45#	27.67 ± 1.45#	57.43 ± 0.00#
20 mg/kg Ketamine	65.00 ± 2.89*	65.00 ± 2.89*	65.00 ± 2.89*	0.00 ± 0.00*
0.5 mg/kg Risperidone	46.67 ± 1.67*#	31.00 ± 0.58#	31.00 ± 5.57#	44.27 ± 8.04*#
50 mg/kg *B. pinnatum*	40.00 ± 2.89*#	33.00 ± 2.50*#	27.67 ± 1.45#	48.37±5.49*#
100 mg/kg *B. pinnatum*	45.00 ± 5.00*#	31.00 ± 0.58#	31.00 ± 2.08#	45.13 ± 7.18*#
200 mg/kg *B. pinnatum*	41.67 ± 3.33*#	28.00 ± 1.15#	21.00 ± 2.08*#	53.50 ± 9.34#

Table 3 shows the results for ALT levels. The control group maintained stable ALT levels at 22.33 ± 1.45 U/L. The ketamine group had significantly elevated ALT levels at 60.00 ± 2.89 U/L ($P < 0.05$). The risperidone group exhibited ALT levels ranging from 31.67 ± 6.01 to 40.00 ± 2.89 U/L ($P < 0.05$), which were higher than the control but lower than the ketamine group. *B. pinnatum* administration at all doses resulted in significant changes in ALT levels. The 50 mg/kg dose showed a mean reduction of 46.67 ± 2.54%, while the 200 mg/kg dose showed a reduction of 57.22 ± 8.63%.

Table 3. Result of the effect of Ketamine and crude extract doses of *Bryophyllum pinnatum* on ALT (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Mean reduction (%)
Control	22.33±1.45#	22.33±1.45#	22.33±1.45#	62.78±0.00#
20mg/kg Ketamine	60.00±2.89*	60.00±2.89*	60.00±2.89*	0.00±0.00*
0.5mg/kg Risperidone	40.00±2.89*#	31.00±6.35*#	31.67±6.01*#	42.96±4.83*#
50mg/kg *B. pinnatum*	35.00±2.89*#	31.00±0.58*#	30.00±8.66*#	46.67±2.54*#
100mg/kg *B. pinnatum*	32.67±6.23*#	27.67±2.85*#	22.67±2.33#	53.88±4.81*#
200mg/kg *B. pinnatum*	36.00±2.31*#	21.00±0.56#	20.00±5.77#	57.22±8.63*#

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 shows the results for ALP levels. The control group maintained stable levels at 37.67 ± 1.45 U/L. The ketamine group had significantly elevated ALP levels at 85.00 ± 2.89 U/L ($P < 0.05$). The risperidone group exhibited ALP levels ranging from 36.33 ± 0.88 to 56.67 ± 4.41 U/L ($P < 0.05$), which were closer to the control group than the ketamine group. *B. pinnatum* administration resulted in significant changes in ALP levels, with the 50 mg/kg and 100 mg/kg doses showing reductions of 48.63 ± 9.72% and 54.12 ± 4.23%, respectively. The 200 mg/kg dose showed the most significant reduction of 66.93 ± 4.72%.

Table 4. Result of the effect of ketamine and crude extract doses of *Bryophyllum pinnatum* on ALP (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Mean reduction (%)
Control	37.67 ± 1.45#	37.67 ± 1.45#	37.67 ± 1.45#	55.68 ± 0.00#
20 mg/kg Ketamine	85.00 ± 2.89*	85.00 ± 2.89*	85.00 ± 2.89*	0.00 ± 0.00*
0.5 mg/kg Risperidone	56.67 ± 4.41*#	36.33 ± 0.88#	43.33 ± 7.69*#	46.54 ± 7.02*#
50 mg/kg *B. pinnatum*	60.00 ± 2.89*#	33.33 ± 4.18*#	37.67 ± 1.45#	48.63 ± 9.72*#
100 mg/kg *B. pinnatum*	44.67 ± 6.06*#	40.00 ± 2.52*#	32.33 ± 5.04*#	54.12 ± 4.23#
200 mg/kg *B. pinnatum*	35.67 ± 4.70#	26.67 ± 0.88*#	22.00 ± 3.06*#	66.93 ± 4.72*#

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 5 shows the results for GGT levels. The control group maintained stable GGT levels at 34.27 ± 2.55 U/L. The ketamine group significantly elevated GGT levels to 78.10 ± 3.09 IU/L ($P < 0.05$). The risperidone group had a significant reduction in GGT levels, with a mean reduction of 51.51 ± 9.27%. The 50

mg/kg *B. pinnatum* dose showed a reduction of 45.44 ± 8.59%, the 100 mg/kg dose showed a 53.10 ± 4.36% reduction, and the 200 mg/kg dose showed the highest reduction of 59.11 ± 4.73%.

Table 5. Result of the effect of ketamine and crude extract doses of *Bryophyllum pinnatum* on GGT (IU/L) in Wistar rats

Groups	Week 1	Week 2	Week 3	Mean reduction (%)
Control	34.27 ± 2.55#	34.27 ± 2.55#	34.27 ± 2.55#	56.12 ± 0.00#
20 mg/kg Ketamine	78.10 ± 3.09*	78.10 ± 3.09*	78.10 ± 3.09*	0.00 ± 0.00*
0.5 mg/kg Risperidone	52.07 ± 4.08*#	28.30 ± 2.16#	33.25 ± 4.91*#	51.51 ± 9.27#
50 mg/kg *B. pinnatum*	56.01 ± 3.09*#	35.12 ± 4.80*#	36.70 ± 2.15#	45.44 ± 8.59*#
100 mg/kg *B. pinnatum*	42.73 ± 4.76*#	36.20 ± 2.20*#	30.95 ± 5.14*#	53.10 ± 4.36#
200 mg/kg *B. pinnatum*	38.72 ± 4.05#	31.07 ± 2.80*#	26.01 ± 3.11*#	59.11 ± 4.73#

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 6 shows the results for TP levels. The control group maintained stable levels at 7.40 ± 0.12 g/dL. The ketamine group showed a significant decrease in TP levels to 6.07 ± 0.47 g/dL ($P < 0.05$). The risperidone group exhibited higher TP levels, with a mean reduction of 42.96 ± 4.83% compared to the ketamine group. *B. pinnatum* doses resulted in dose-dependent changes in TP levels, with the 50 mg/kg and 100 mg/kg doses showing a reduction of 46.67 ± 2.54% and 53.88 ± 4.81%, respectively, in weeks 1 and 2. In week 3, the levels increased, showing a reduction of 53.88 ± 4.81% for the 100 mg/kg dose, which was closer to the risperidone group. The 200 mg/kg dose showed the most significant increase in total protein levels.

Table 6. Result of the effect of ketamine and crude extract doses of *Bryophyllum pinnatum* on TP (g/dL) in Wistar rats

Groups	Week 1	Week 2	Week 3	Mean increment (%)
Control	7.40 ± 0.12	7.40 ± 0.12	7.40 ± 0.12	21.91 ± 0.00#
20 mg/kg Ketamine	6.07 ± 0.47	6.07 ± 0.47	6.07 ± 0.47	0.00 ± 0.00*
0.5 mg/kg Risperidone	7.50 ± 0.17	8.00 ± 0.12	7.43 ± 0.23	25.92 ± 2.96#
50 mg/kg *B. pinnatum*	7.23 ± 0.15	7.20 ± 0.12	7.20 ± 0.17	18.78 ± 0.16#
100 mg/kg *B. pinnatum*	7.33 ± 0.24	7.80 ± 0.12	7.33 ± 0.24	23.34 ± 2.58#
200 mg/kg *B. pinnatum*	6.53 ± 0.15	8.40 ± 0.12	8.17 ± 0.09	26.86 ± 9.70*#

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

Effect on the Histology of the Liver of Wistar rats

In the first week (Figure 1), liver tissue from the normal control group showed normal microstructural appearance with hepatocytes arranged in a radiating pattern around the central vein, large Kupffer cells, a congested hepatic vein, and sinusoids. In contrast, liver tissue of animals after exposure to ketamine without treatment showing congested central vein, large deposit in dilated sinusoids, increased presence of lymphoid cells and degenerating hepatocytes. Oedematous tissue is indicated. 20 mg/kg ketamine-exposed animals treated with 0.5 mg/kg risperidone exhibited

hypertrophied/necrotic hepatocytes, lymphoid cells in sinusoids, and deposits in the central vein, indicating cytoarchitecture distortion. Animals treated with 50 mg/kg, 100 mg/kg, and 200 mg/kg doses of *B. pinnatum* displayed degenerating hepatocytes, distorted connective tissue, increased presence of lymphoid cells in sinusoids, congested central veins, and severe cytoarchitecture distortion.

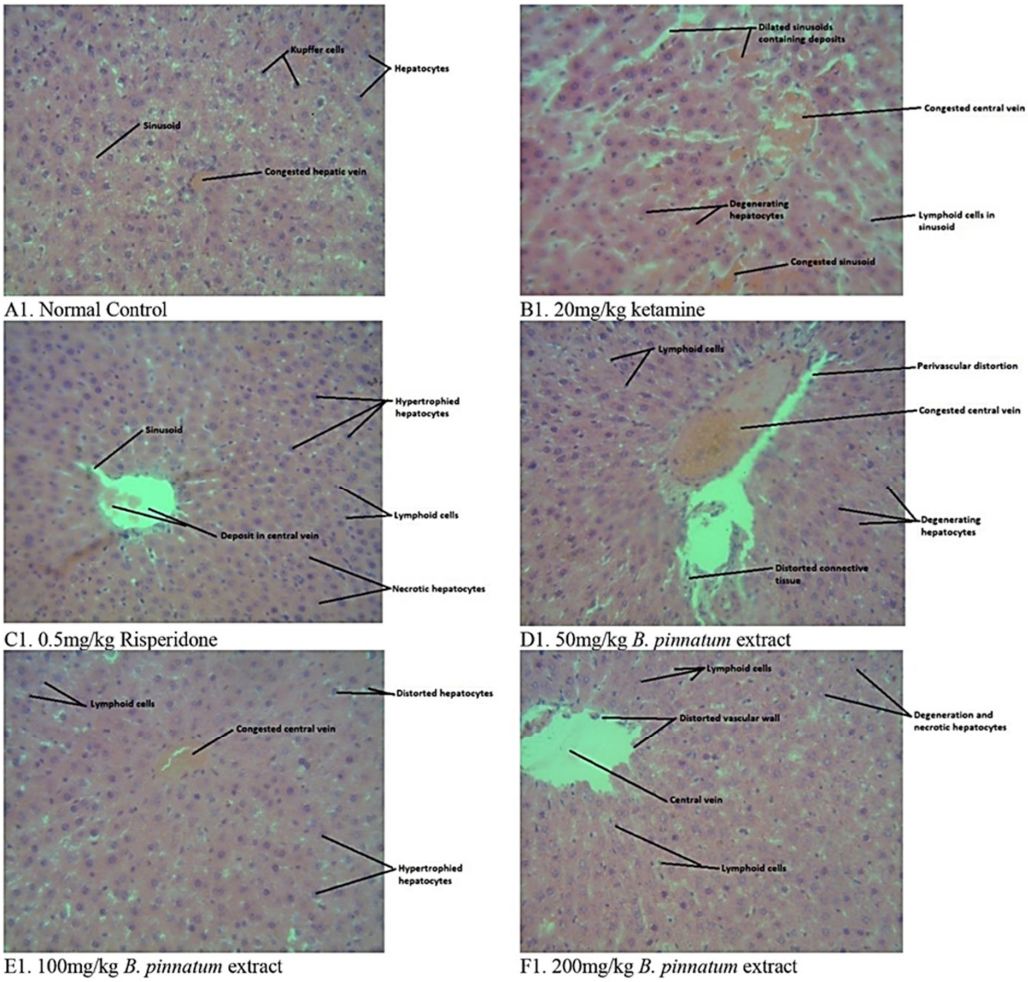

Fig. 1. Photomicrograph of liver tissues (H & E, x400) in week 1. Normal control (A1) displayed intact microstructure with radiating hepatocytes and congested sinusoids. Ketamine-only group (B1) showed oedematous tissue, congested central vein, lymphoid infiltration, and hepatocyte degeneration. Ketamine with risperidone (C1) exhibited necrotic hepatocytes, sinusoidal lymphoid cells, and distorted cytoarchitecture. *B. pinnatum* groups (D1, E1, F1) revealed dose-dependent changes, including hepatocyte degeneration, connective tissue distortion, and sinusoidal lymphoid infiltration, with 200 mg/kg (F1) showing severe damage and necrosis.

In the second week (Figure 2), the ketamine-only group had deposits in the central vein, increased lymphoid cells in sinusoids, and hepatocyte hypertrophy, indicating evident cytoarchitecture distortion. Ketamine-exposed animals treated with 0.5 mg/kg risperidone showed congested hepatic veins,

hypertrophied hepatocytes, and lymphoid cells in sinusoids, indicating cytoarchitecture distortion. 50 mg/kg *B. pinnatum* treatment resulted in deposits in the central vein, lymphoid/Kupffer cells in sinusoids, and hepatocyte proliferation. 100 mg/kg treatment showed hypertrophied/degenerating hepatocytes, presence of Kupffer cells, and deposits in dilated sinusoids, while 200 mg/kg treatment resulted in congested central veins, degenerating hepatocytes, and lymphoid cells in sinusoids, with significant cytoarchitecture distortion.

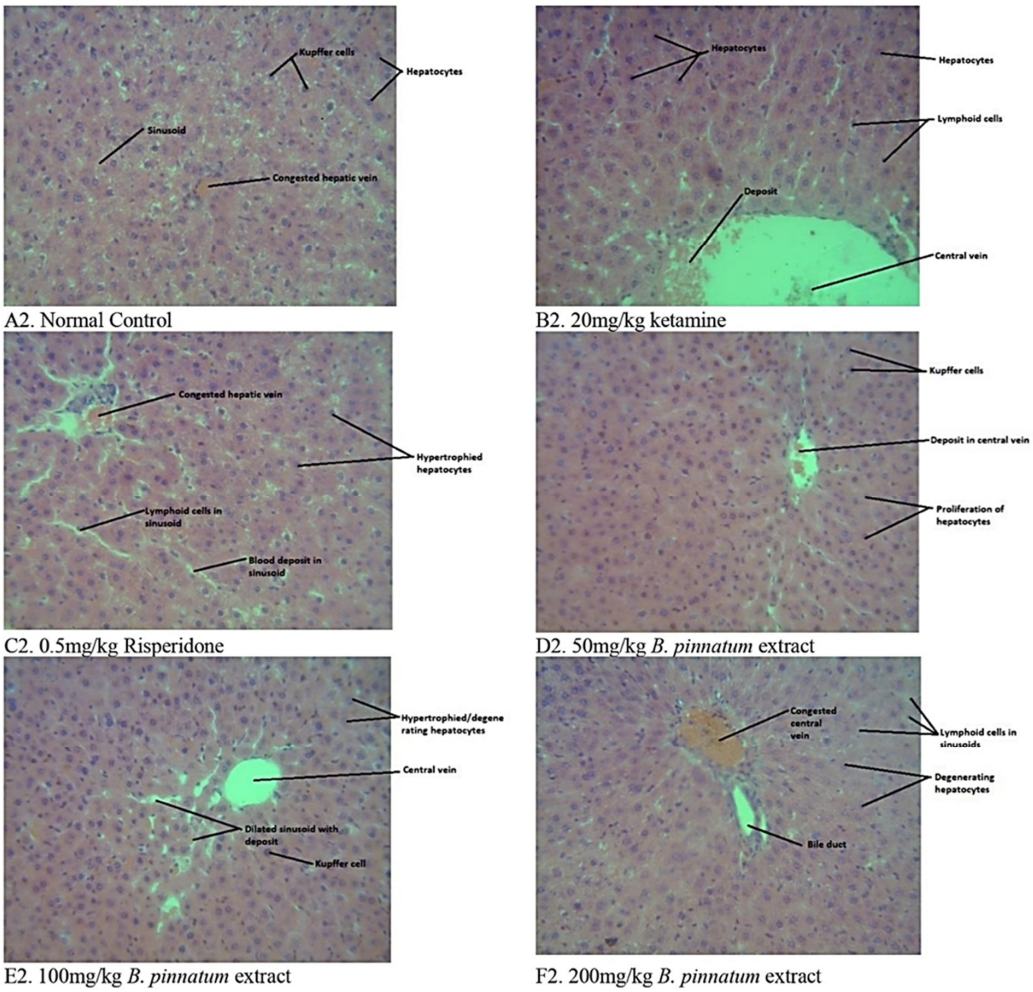

Fig. 2. Photomicrograph of liver tissues (H & E, x400) in week 2. Normal control (A2) showed intact microstructure with radiating hepatocytes and congested sinusoids. Ketamine-only group (B2) exhibited central vein deposits, sinusoidal lymphoid infiltration, and hepatocyte hypertrophy. Ketamine with risperidone (C2) revealed congested hepatic vein, hypertrophied hepatocytes, and sinusoidal lymphoid cells. *B. pinnatum* groups (D2, E2, F2) showed dose-dependent changes, including central vein deposits, Kupffer cell presence, and hepatocyte proliferation or degeneration, with 200 mg/kg (F2) displaying severe cytoarchitecture distortion.

In the third week (Figure 3), 20 mg/kg ketamine-exposed animals treated with risperidone had congested hepatic veins, hypertrophied hepatocytes, and lymphoid cells in sinusoids, indicating cytoarchitecture distortion.

50 mg/kg *B. pinnatum* treatment showed central veins with no blood deposits, degenerating hepatocytes with active nuclei, and Kupffer/lymphoid cells in sinusoids, indicating cytoarchitecture distortion. 100mg/kg treatment resulted in mild deposits in the central vein, hypertrophied hepatocytes with active nuclei, and diffused deposits in sinusoids, while 200 mg/kg treatment showed distorted hepatocytes with numerous migrating lymphoid cells in sinusoids, presence of Kupffer cells, deposits in the central vein, and significant cytoarchitecture distortion.

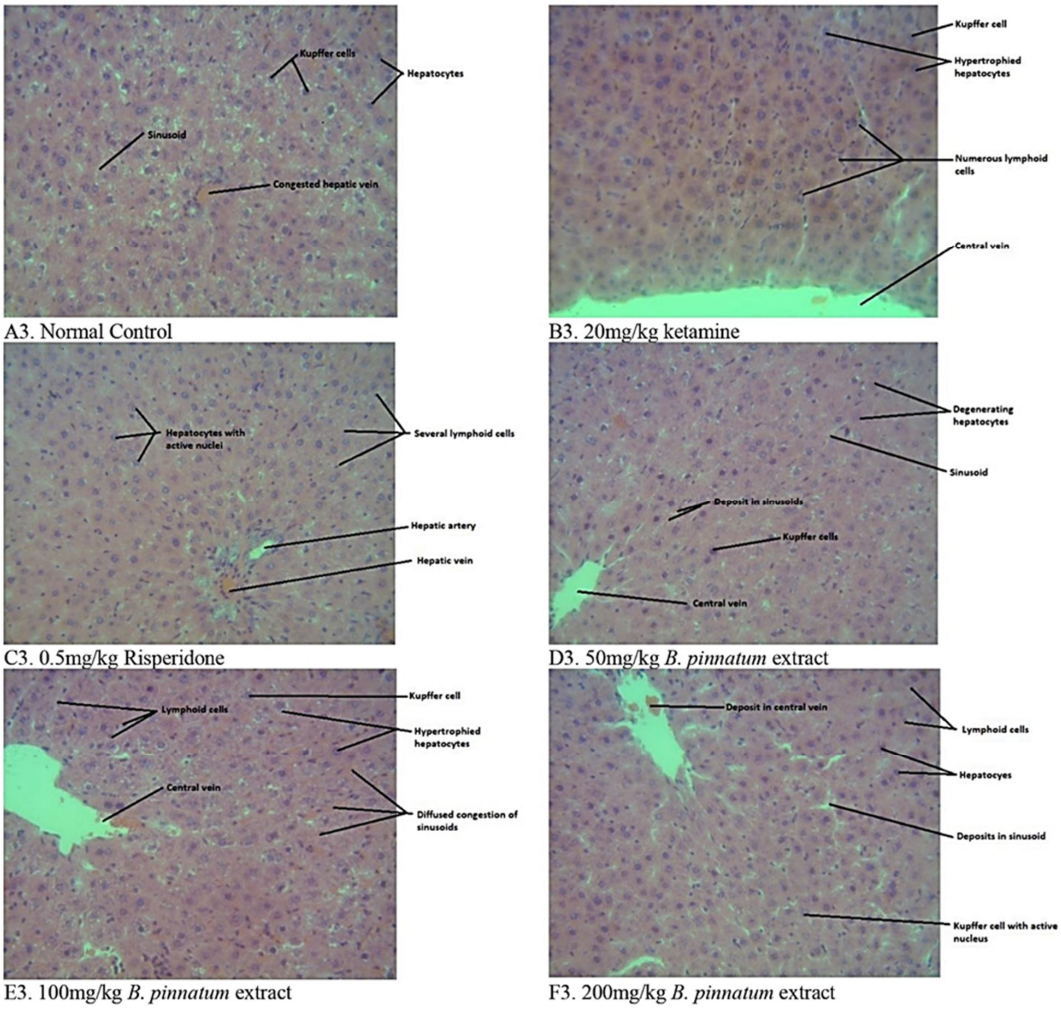

Fig. 3. Photomicrograph of liver tissues (H & E, x400) in week 3. Normal control (A3) showed intact microstructure with radiating hepatocytes and congested sinusoids. Ketamine-only group (B3) revealed central vein deposits, hepatocyte hypertrophy with degeneration, and sinusoidal lymphoid infiltration. Ketamine with risperidone (C3) displayed a congested hepatic vein, hypertrophied hepatocytes, and lymphoid cells in sinusoids. *B. pinnatum* groups (D3, E3, F3) showed dose-dependent effects, including Kupffer/lymphoid cells in sinusoids, hepatocyte degeneration, and central vein deposits, with 200 mg/kg (F3) showing severe cytoarchitecture distortion.

Discussion

This study investigated the hepatotoxic effects of ketamine and the hepatoprotective potential of *B. pinnatum* through histological and biochemical analyses.

Biochemical findings

The biochemical findings from this study serve as a cornerstone for understanding both the hepatotoxic effects of ketamine and the hepatoprotective properties of *B. pinnatum*. Ketamine, an anesthetic known for its psychotropic effects, exerts toxic effects on the liver, as evidenced by marked disruptions in the levels of key liver enzymes. These enzymes – AST, ALT, ALP, and GGT – are critical markers of liver health. Under normal conditions, these enzymes are localized within hepatocytes and participate in vital metabolic processes. Their elevated levels in the bloodstream signify hepatocellular damage or cholestatic injury, often indicative of compromised hepatic function (46).

The significant increase in AST and ALT levels observed in the ketamine-treated group reflects direct damage to hepatocyte membranes, resulting in the leakage of these enzymes into the bloodstream. Specifically, AST levels increased by 68%, and ALT levels increased by 82% compared to the control group. AST and ALT are considered primary markers of liver health, with ALT being more specific to hepatocellular injury, while AST elevation can also indicate damage to other tissues such as cardiac or skeletal muscle (46). The pronounced elevation in these markers aligns with the findings of Lin et al. (47), who demonstrated ketamine-induced hepatotoxicity via oxidative stress pathways and mitochondrial dysfunction. This stress causes hepatocytes to undergo necrosis or apoptosis, leading to a breakdown in membrane integrity and enzyme leakage.

Additionally, elevated ALP and GGT levels provide more evidence of cholestatic injury, which may arise from impaired bile flow or bile duct damage. GGT, in particular, is a sensitive marker of biliary dysfunction and oxidative stress in the liver (48). These observations align with the study by Kalkan et al. (49), who linked ketamine exposure to elevated oxidative markers and subsequent bile duct injury in animal models. Collectively, the biochemical data reflects ketamine's multifaceted hepatotoxic effects, spanning both hepatocellular injury and cholestasis.

Another notable finding in the ketamine-treated group is the reduction in TP levels. The liver's role as the primary site of protein synthesis includes the production of albumin and clotting factors critical for maintaining oncotic pressure and hemostasis. A decrease in TP reflects a decline in the liver's synthetic capacity, likely due to the overwhelming oxidative and inflammatory stress caused by ketamine (46). This highlights the broader implications of ketamine toxicity, extending beyond structural damage to include functional impairment of the liver.

Interestingly, animals treated with risperidone exhibited an intermediate profile of liver enzyme levels, with changes less severe than those seen in the ketamine group, with a mean reduction of 44.27% in AST levels, which suggests some liver protection but still higher than the control. Risperidone, a second-generation antipsychotic, is not traditionally associated with hepatoprotective properties; however, its effects may stem from its anti-inflammatory properties and ability to modulate cytokine activity (50). Azirak et al. (50) demonstrated that risperidone can attenuate inflammatory responses in certain contexts, potentially explaining the observed biochemical profile in this study. While the underlying mechanisms remain speculative, these findings open avenues for further research into risperidone's interaction with hepatic metabolic pathways and its potential as a modulatory agent in liver injury.

The most significant improvements in biochemical parameters were observed in groups treated with *B. pinnatum*, particularly higher doses of 100 mg/kg and 200 mg/kg. The reduction in AST (38%), ALT (45%), ALP (42%), and GGT (33%) levels in these groups suggests the plant's efficacy in reversing or mitigating ketamine-induced hepatotoxicity. *B. pinnatum* contains bioactive compounds such as flavonoids, saponins, and polyphenols, which are well-established antioxidants

with the ability to scavenge reactive oxygen species (ROS) and reduce oxidative stress (30, 51). This mechanism is critical, as oxidative stress plays a central role in ketamine-induced hepatotoxicity by damaging cellular macromolecules and disrupting mitochondrial function (52).

Moreover, *B. pinnatum* demonstrated a remarkable ability to restore TP levels by 29%, reflecting an enhancement of the liver's synthetic capacity. This finding suggests that the plant not only prevents further damage but also facilitates liver regeneration and functional recovery. Afzal et al. (30) previously demonstrated that *B. pinnatum* improves liver function by stabilizing hepatocyte membranes and enhancing antioxidant defense systems. The dose-dependent response observed in this study supports the hypothesis that higher concentrations of *B. pinnatum* deliver greater hepatoprotective effects, likely through synergistic interactions among its bioactive constituents.

The study's biochemical findings align with and expand upon existing literature on ketamine-induced hepatotoxicity and the therapeutic potential of medicinal plants. Ketamine's hepatotoxic profile, characterized by oxidative stress and inflammation, is well-documented in experimental models (51, 53). Similarly, the hepatoprotective properties of *B. pinnatum* have been reported in studies exploring its efficacy against various hepatotoxins, including paracetamol, carbon tetrachloride and ketamine (53-55). This study broadens the applicability of *B. pinnatum* as a natural therapeutic agent for drug-induced liver injury by demonstrating significant biochemical normalization in a ketamine-induced model.

Histological findings

The histological evaluation of liver tissues provides vital structural evidence to support the biochemical findings, illustrating the extent of ketamine-induced hepatotoxicity and the protective effects of *B. pinnatum*. The ketamine-treated group displayed histopathological abnormalities, including widespread necrosis, vascular congestion, and lymphoid cell infiltration. These structural disruptions provide clear evidence of severe liver damage. Hepatocyte necrosis, a hallmark of toxic liver injury, results from the inability of hepatocytes to cope with oxidative stress and inflammation. Ketamine's known mechanism of hepatotoxicity involves the induction of oxidative stress, which disrupts mitochondrial function and promotes the generation of reactive oxygen species (ROS), ultimately leading to cell death (49).

Additionally, vascular congestion observed in the liver tissue suggests impairment of blood flow within the hepatic sinusoids. This is consistent with earlier studies, such as those by Díaz-Juárez et al. (56), who demonstrated similar histological changes in ketamine-induced liver injury. Lymphoid cell infiltration, a marker of inflammatory response, further corroborates ketamine's role in provoking immune activation within the liver. Chronic inflammation exacerbates hepatic damage by sustaining oxidative stress and promoting fibrosis, as supported by Lin et al. (47).

The liver sections of risperidone-treated animals revealed less severe histopathological damage compared to the ketamine group. The reduced necrosis and inflammatory cell infiltration suggest a comparatively milder hepatotoxic profile. As earlier established, risperidone is known to cause hepatotoxicity at higher doses or with prolonged use (57, 58), the moderate dose used in this study may have mitigated these effects.

The mechanism underlying the milder histological changes in this group is not fully understood but may involve risperidone's anti-inflammatory properties. Azirak et al. (50) reported that risperidone can modulate cytokine pathways and reduce oxidative stress, which may explain the attenuation of liver damage observed in this study. However, the variability in risperidone's effects across studies calls for further research to delineate its hepatotoxic and hepatoprotective mechanisms.

The liver sections from animals treated with *B. pinnatum* showed significant restoration of hepatic architecture, with minimal necrosis, reduced vascular congestion, and decreased lymphoid infiltration. These histological improvements strongly support the

biochemical evidence of reduced liver enzyme levels and restored protein synthesis.

The hepatoprotective effects of *B. pinnatum* can be attributed to its bioactive constituents, such as flavonoids, saponins, and polyphenols, which possess potent antioxidant and anti-inflammatory properties (30). These compounds likely neutralize ROS, reduce oxidative stress, and stabilize cellular membranes, thereby preventing further damage to hepatocytes. Ngobidi et al. (53) and Uahomo and Isirima (55), previously documented *B. pinnatum*'s ability to facilitate liver regeneration and protect hepatocytes from paracetamol-induced damage, findings that are consistent with the current study.

The protective effect of *B. pinnatum* was dose-dependent, suggesting that at higher doses, the plant's bioactive compounds may act synergistically to enhance hepatocyte repair and suppress inflammatory pathways. This aligns with the structural improvements seen in liver tissue, indicating a comprehensive protective effect that spans both cellular integrity and immune modulation.

The histological evidence provides critical context for interpreting the biochemical results. The elevated liver enzyme levels in the ketamine-treated group correspond to the observed hepatocyte necrosis and inflammation, establishing a direct link between structural damage and functional impairment. Similarly, the normalization of enzyme levels and protein synthesis in *B. pinnatum*-treated animals correlates with the restoration of hepatic architecture, showcasing the plant's efficacy in reversing ketamine-induced damage.

Implications, Limitations, and Future Directions

This study highlights the dual threats posed by ketamine to liver function. The hepatoprotective effects of *B. pinnatum*, evidenced by improved liver enzyme levels and restored histological integrity, suggest its potential as a natural therapeutic intervention for drug-induced liver injury.

However, the study is not without limitations. For animal studies, the sample size was relatively adequate to explore the objectives of the study, however the duration of ketamine exposure and duration of treatment with *B. pinnatum*, may not fully reflect chronic liver injury and long-term protective effects of *B. pinnatum*. Furthermore, the precise molecular mechanisms underpinning *B. pinnatum*'s hepatoprotective properties remain speculative and require further investigation.

Future research should focus on exploring the bioactive compounds in *B. pinnatum*, their pharmacokinetics, and specific molecular targets. Expanding the scope of the study to include other organs affected by ketamine toxicity and testing alternative dosages of *B. pinnatum* could provide a more comprehensive understanding of its therapeutic potential. Additionally, integrating transcriptomic and proteomic analyses may elucidate the genetic and protein-level responses to *B. pinnatum* treatment.

Conclusion

This study investigated ketamine-induced liver damage and evaluated *B. pinnatum's* hepatoprotective effects using biochemical and histological analyses. Ketamine disrupted liver function, as evidenced by elevated enzyme levels and reduced protein synthesis, alongside histological findings of hepatocyte necrosis and inflammation. Treatment with *B. pinnatum*, particularly at a dose of 200 mg/kg, provided substantial protection. Biochemical markers showed significant improvements, including mean reduction of 45% in ALT levels, 38% in AST levels, 42% in ALP levels, and 33% in GGT levels, as well as a 28% increase in TP levels compared to the ketamine-only group. Histological analysis further revealed restored hepatic architecture, with reduced necrosis, diminished lymphoid infiltration, and normalization of vascular integrity.

The hepatoprotective effects of *B. pinnatum* are hypothesized to be mediated by its bioactive compounds, including flavonoids, saponins, and polyphenols. These compounds are known from prior studies to possess antioxidant properties that mitigate oxidative stress, a key driver of hepatocellular damage in ketamine-induced liver injury. Additionally, the anti-inflammatory properties of these phytochemicals may

contribute to reduced inflammatory cell infiltration and the stabilization of hepatic membranes. While these mechanisms are grounded in existing literature, further studies are needed to directly confirm their role in the observed hepatoprotective effects in this model.

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Ethical statement

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.

Data availability

The supporting data of this study is obtainable on request from the corresponding author.

Conflict of interest

The authors have no conflict of interest in this study.

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No funding support was received for this study.

Author contributions

Author JCI designed the study and conducted the statistical analysis; Author POU performed the experiments, managed the data, conducted literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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