

Reno-Hepatoprotective effects of *Bryophyllum pinnatum* against Hepatic Ischemia- Reperfusion Injury in male Wistar rats

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Abstract

Hepatic ischemia-reperfusion injury (HIRI) is indicated in postoperative complications in hepatic transplantation and surgeries. HIRI can lead to remote renal injury through systemic inflammatory and oxidative cascades. While current therapeutic options are effective, their potential toxic effects can pose additional challenges, thereby hindering hepatic and renal recovery, and highlighting the need for alternative therapies. *Bryophyllum pinnatum* (BP), popularly called “life plant” is widely used in traditional medicine, and is rich in flavonoids, triterpenoids, and bufadienolides, which have shown remarkable antioxidant and anti-inflammatory activities in various animal models. Studies have demonstrated the ability of BP to scavenge free radicals, suppress acute inflammation, and inhibit pro-inflammatory cytokines. Its nephroprotective effects in renal injury models, as well as its hepatoprotective effects in chemically-induced hepatocarcinogenesis have also been documented. Despite these promising findings, its effect against HIRI-induced kidney damage remains unexplored. This study investigates the reno-hepatoprotective potential of BP methanolic leaf extract in male Wistar rats subjected to HIRI.

Forty male Wistar rats were divided into four groups (n=10); group 1- Sham operated, group 2-HIRI, group 3-LDH (low-dose *B. pinnatum* (100 mg/kg), group 4-HDH (high-dose *B. pinnatum* (200 mg/kg). BP was administered orally via gavage for 14 days before HIRI induction. Following treatment, animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg), and HIRI was induced by clamping the hepatic artery, portal vein and bile duct for 60 minutes to induce ischemia after which the clamp was removed for hepatic reperfusion which lasted for 24-hours. Animals were euthanized under deep anesthesia (ketamine 50 mg/kg and xylazine 10 mg/kg, ip) before sacrifice. Then, blood, kidney and liver tissues were harvested for biochemical and histological analysis. Data were analyzed using one way ANOVA with Graph pad prism. Tukey’s post-hoc test was used for multiple comparison. $P < 0.05$ was considered statistically significant.

Biochemical analyses showed a significant restoration in SOD, catalase, and GSH levels and reductions in MDA and inflammatory markers (TNF- α , MPO) in the *B. pinnatum* groups. In addition, altered ALT, ALP, and GGT levels were restored, and urea and creatine levels raised as a secondary effect of HIRI were reduced in the *B. pinnatum*-treated groups. Reduced caspase-3 activity was also observed in *B. pinnatum*-treated groups, indicating lower apoptosis levels. Histological analyses showed improved cytoarchitecture, with preservation of renal corpuscles and reduced inflammation.

Conclusively, *B. pinnatum* exhibits reno-hepatoprotective effects on HIRI-induced kidney damage, potentially modulating oxidative stress, inflammation, and apoptotic pathways.

1 Introduction

Ischemia-reperfusion injury (IRI) is characterized by a complex pathophysiological cascade that aggravates tissue damage during blood flow restoration after a period of ischemia. This phenomenon significantly impacts morbidity and mortality rates in clinical practice, particularly in organ transplantation and major surgical procedures (1). The liver exhibits particular vulnerability to IRI, making

hepatic ischemia-reperfusion injury (HIRI) a major concern in hepatic surgery and liver transplantation (2).

In the pathogenesis of HIRI, oxygen deprivation, during ischemia, triggers ATP depletion, which in turn disrupts ionic homeostasis and causes calcium overload. The reperfusion phase on the other hand, exacerbates injury through reactive oxygen species (ROS) generation, including superoxide anions and hydroxyl radicals, leading to cellular damage through lipid peroxidation and protein oxidation (3). Furthermore, oxidized phospholipids can act as damage-associated molecular patterns (DAMPs), which then go on to initiate inflammatory cascades that exacerbate the injury (4).

The systemic effect of HIRI can also affect renal function, with reported incidences of acute kidney injury following orthotopic liver transplantation ranging from 12–94% (5). This hepatorenal interaction is traceable to multiple pathways, including Kupffer cell activation and subsequent pro-inflammatory cytokine release, such as, tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), which promote systemic inflammation and remote organ injury (6). Additionally, sinusoidal endothelial cell damage promotes microthrombosis and vasoconstriction through von Willebrand factor release and reduced nitric oxide production (6). The damage to the kidneys in this context is mediated by oxidative stress, mitochondrial dysfunction, and heightened susceptibility to apoptosis; a combination that significantly impairs renal function (5, 7).

Current therapeutic strategies to mitigate HIRI-induced renal injury primarily focus on supportive measures and specific pharmacological interventions targeting oxidative stress and inflammation (8). Additionally, ischemic preconditioning and pharmacological approaches to reduce mitochondrial permeability transition pore (MPTP) formation remain limited by variability in individual response and operational complexities in surgical contexts (9). The limitations of these existing treatments, including timing constraints and potential side effects, necessitate the exploration of alternative therapeutic approaches, such as natural compounds with multi-targeted actions capable of modulating both oxidative and inflammatory pathways without the drawbacks of synthetic interventions (10).

Among natural compounds, *Bryophyllum pinnatum*, is well known to possess therapeutic potential suitable for this study. In traditional medicine, *Bryophyllum pinnatum* has long been utilized for various ailments, including liver and kidney disorders (11, 12). Its phytochemical analyses reveal it to be rich in bioactive compounds, including flavonoids, triterpenoids, and bufadienolides (13). These constituents demonstrate significant antioxidant and anti-inflammatory properties, particularly in suppressing pro-inflammatory cytokine production and nuclear factor-kappa B activation (14, 15). In a landmark study by Ojewole (11) to scientifically evaluate the ethnomedicinal applications of *B. pinnatum* leaves, the author concluded that aqueous extract of *B. pinnatum* leaves possessed anti-inflammatory properties, evidenced by the significant inhibition of fresh egg albumin-induced acute inflammation, as well as antinociceptive and anti-diabetic properties. Chibli *et al.* (16) also demonstrated *B. pinnatum*'s anti-inflammatory property in acute and chronic mice ear edema models induced by different irritant agents.

Recent studies have further shown *B. pinnatum*'s capacity to scavenge free radicals and inhibit lipid peroxidation, due to active components such as quercetin, kaempferol, and bryophyllins (17). In a study by Tatsimo *et al.* (18), methanolic extracts of *B. pinnatum* showed strong antioxidative and antimicrobial activities, confirming their traditional use in the treatment of infectious and free radical damages. A recent review by Sharma *et al.* (19) indicated that *B. pinnatum* is effective in the treatment of kidney and liver diseases, and possesses anti-leishmanial, neuropharmacological, antibacterial, immunosuppressive, anti-tumour, and cytotoxic activities.

Its nephroprotective effect is confirmed in the study by Yadav *et al.* (20) which showed in their study on the effect of *B. pinnatum* on ethylene glycol (EG)-induced renal calculi in rats, that *B. pinnatum* effectively reversed EG-induced damage comparable to the standard drug Cystone. Likewise, Anadozie *et al.* (21) concluded that *B. pinnatum* is able to inhibit arginase II activity and consequently prevent carbon tetrachloride (CCl₄)-induced renal oxidative damage in rats. Other studies to examine the renoprotective effects of *B. pinnatum*, such as those by Shukla *et al.* (22), Shinde *et al.* (23), Uahomo and Isirima (24), and Pal *et al.* (25), all show that *B. pinnatum* is able to attenuate renal damage in experimental studies.

Apart from having renoprotective properties, *B. pinnatum* has also been reported to possess hepatoprotective properties. Afzal *et al.* (26) concluded in their study that *B. pinnatum* is able to mitigate chemically-induced hepatocarcinogenesis due to its ability to upregulate antioxidant activities and scavenge free radicals that damage cells. Kombathula and Chernapalli (27) confirmed this in their study and attested that the plant's components—bryophyllin A and β -sitosterol—strongly interact with the Pi3K and C-met pathways respectively to mediate their effect. In another model of butylglycol-induced hepatotoxicity, the researchers observed that groups treated with *B. pinnatum* showed reduced liver injury markers (ALP and AST) compared to the positive control (28). In addition, Ajah *et al.* (29) examined the efficacy of *B. pinnatum* in attenuating damage to the kidneys and liver, as well as the lipid profile of rats fed with high-salt diet. They concluded that the ethanolic extract of *B. pinnatum* was highly potent in preventing cell damage caused by high salt intake. However, its potential role in mitigating HIRI-induced kidney injury remains unexplored.

Since *Bryophyllum pinnatum*'s pleiotropic effects might provide comprehensive protection against both direct and remote organ injury through upregulation of antioxidant enzymes, modulation of oxidative stress, inflammation, and apoptotic pathways, this study aims to evaluate the reno-hepatoprotective effects of *Bryophyllum pinnatum* methanolic extract on kidney injury following hepatic ischemia-reperfusion in male Wistar rats.

2 Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Ketamine and xylazine for anaesthesia were obtained from the Veterinary Department, University of Ibadan. Streptomycin injection,

0.9% normal saline, 10% formalin, and distilled water were procured from the Physiology Laboratory, LAUTECH. Biochemical assay kits for liver function markers (ALT, AST, GGT) and kidney function markers (urea, creatinine) were purchased from Randox Laboratories Ltd., UK. Stains for histological analysis, including Hematoxylin and Eosin, Masson's trichrome, and Periodic acid-Schiff (PAS), were also utilized.

2.2 Experimental Animals and Design

Forty male Wistar rats weighing 120-150g were obtained from animal house of physiology department, LAUTECH, Ogbomoso, Oyo State, Nigeria. The animals were housed under standard laboratory conditions at the animal facility of Faculty of Basic Medical Sciences (FBMS), LAUTECH and were acclimatized for 2 weeks, fed with standard rat chow and watered *ad libitum*. Ethical approval was obtained from Ethics Committee in Animal Care and Use in Research of FBMS with reference number: **ERCFBMSLAUTECH: 058/08/2024**.

The rats were randomly divided into four groups (n = 10 per group):

1. **Sham group:** Sham-operated, no treatment
2. **HIRI group:** Hepatic ischemia-reperfusion injury, no treatment
3. **BP Low Dose + HIRI:** 100 mg/kg *Bryophyllum pinnatum* extract + Hepatic ischemia-reperfusion injury
4. **BP High Dose + HIRI:** 200 mg/kg *Bryophyllum pinnatum* extract + Hepatic ischemia-reperfusion injury

The *Bryophyllum pinnatum* extract was administered orally via gavage for 14 days prior to the induction of HIRI, as described by Anadozie *et al.* (21).

2.3 Plant Material Preparation

Fresh *Bryophyllum pinnatum* leaves were collected from farm house of Faculty of Agricultural Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria. The plant was identified and authenticated by Dr. Mrs. A.F. Ogundola, a botanist at the Department of Pure and Applied Biology, LAUTECH. The plant was assigned the voucher number **LHO 747**, and kept in the Herbarium at the Department of Botany, LAUTECH, Ogbomoso, Nigeria.

The leaves were washed, air-dried, and pulverized into a fine powder using an electric blender. The powdered leaves were macerated in 80% methanol for 72 hours, and the extract was concentrated using a rotary evaporator and further dried in a vacuum oven.

2.3.1 Phytochemical Profiling

Qualitative screening of the *Bryophyllum pinnatum* extract was performed to detect the presence of major phytochemical groups, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, and anthraquinones, using standard chemical tests (Mayer's, Wagner's, alkaline reagent, ferric chloride, foam, Salkowski, Keller-Killiani, Liebermann-Burchard, and Borntrager's tests, respectively).

For quantitative analysis, spectrophotometric methods were employed to determine the total flavonoid content (aluminium chloride colorimetric method), total tannin content (Folin-Ciocalteu method), and total phenolic content (Folin-Ciocalteu method) (Table 1).

Table 1
Qualitative and selected Quantitative analyses of *B. pinnatum*

Phytochemical Group	Qualitative Result	Quantitative Result (where applicable)
Alkaloids	+++	Not quantified
Flavonoids	+++	24.3 ± 1.8 mg QE/g dry weight
Tannins	++	18.7 ± 1.2 mg TAE/g dry weight
Saponins	++	Not quantified
Terpenoids	+++	Not quantified
Phenolic compounds	+++	37.2 ± 2.5 mg GAE/g dry weight
Glycosides	++	Not quantified
Steroids	+	Not quantified
Anthraquinones	+	Not quantified
Key: +++ (strongly present), ++ (moderately present), + (weakly present), - (absent) QE: Quercetin Equivalent, TAE: Tannic Acid Equivalent, GAE: Gallic Acid Equivalent		

2.4 HIRI Induction Procedure

Hepatic ischemia-reperfusion injury (HIRI) was induced following the method described by Şener *et al.* (30) with slight modifications. Briefly, after overnight fasting, rats were anaesthetised with intraperitoneal injections of ketamine (50 mg/kg) and xylazine (10 mg/kg) (31). The abdominal area was shaved and disinfected, and a midline laparotomy was performed. The hepatic artery, portal vein supplying the median and left lateral lobes and the bile duct were clamped using microvascular clamp to induce 70% hepatic ischemia. After 60 minutes of ischemia, the clamp was removed to allow reperfusion which lasted for 24 hours. Sham-operated animals underwent the same procedure without vascular occlusion.

2.5 Sample Collection

Animals were fasted overnight and twenty-four hours after reperfusion, the animals were anaesthetized using intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) (31). Confirmation of anaesthesia was determined by absence of corneal reflex and pedal withdrawal. Blood samples were collected via the retro-orbital puncture in plain bottles. Following this, the liver and kidneys were excised, washed in ice-cold saline, and weighed. Also, the collected blood was allowed to clot and centrifuged at 5000rpm for 10 minutes to obtain serum. Portions of the liver and kidney tissues were fixed in 10% neutral buffered formalin for histological examination.

2.6 Biochemical Analyses

Serum and tissue homogenates were used for various biochemical assays. Liver function markers (ALT, AST, GGT) and kidney function markers (urea, creatinine) were determined using commercial kits on an automated analyzer (Biosystem A25 Random Access Analyzer).

Oxidative stress markers, including superoxide dismutase (SOD) activity, malondialdehyde (MDA) levels, glutathione (GSH) content, and catalase activity, were measured in liver and kidney tissue homogenates using established spectrophotometric methods (32, 33, 34, 35). Total protein content in the tissue homogenates was determined by the Bradford method (36).

Inflammatory markers, including tumour necrosis factor-alpha (TNF- α) in serum and myeloperoxidase (MPO) activity in liver and kidney tissues, were assessed using ELISA and spectrophotometric techniques, respectively (RandD Systems; 37). Serum nitrite levels, as an indicator of nitric oxide production, were measured using the Griess reaction (38). Caspase-3 activity, a marker of apoptosis, was determined in liver and kidney tissue homogenates using a colorimetric assay kit (Sigma-Aldrich, USA).

2.7 Histological Examination

Fixed liver and kidney tissues were processed using standard histological techniques and stained with Hematoxylin and Eosin (HandE) for general morphological assessment. Additional stains, including Masson's trichrome for liver fibrosis and Periodic acid-Schiff (PAS) for renal basement membrane and tubular brush border changes, were also performed. The stained sections were examined under a light microscope (Olympus BX51, Tokyo, Japan) and photographed.

2.8 Statistical Analysis

Data were analysed using GraphPad Prism software (version 9.0, GraphPad Software Inc., San Diego, CA, USA). Results were expressed as mean $\pm$ standard error of the mean (SEM). Differences between groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons.

3 Results

3.1 Liver Function Markers

Alanine aminotransferase (ALT)

There was significant increase in ALT concentration in the HIRI group compared with the sham group.

Aspartate aminotransferase (AST)

AST concentration was significantly increased in the HIRI group when compared with the sham operated group. Significant decrease in AST concentration was also observed in both the low-dose BP and high-dose BP groups.

Gamma-glutamyl transferase (GGT)

There was a statistically significant increase in the GGT concentration of the HIRI group when compared with the sham operated group. GGT was however significantly reduced in the high-dose BP group when compared with the sham and low-dose BP groups.

3.2 Kidney Function Markers

Creatinine

There was a statistically significant increase in creatinine levels of the HIRI group when compared with the sham operated group. The low-dose and high dose groups both significantly reduced creatinine levels when compared with the HIRI group.

Urea

There was a statistically significant increase in the urea concentration of the HIRI group when compared with the sham operated group. Urea concentration was however significantly reduced by both the low-dose BP and high-dose BP groups. Furthermore, the high-dose group significantly decreased urea concentration when compared with the low-dose group.

3.3 Hepatic Oxidative Stress Markers

Hepatic Glutathione (GSH)

Hepatic GSH was significantly reduced in the HIRI group when compared with the sham group. Also, both the low-dose BP and high-dose BP showed a significant increase in GSH concentration when compared to the HIRI group.

Hepatic Total Protein

Hepatic total protein was significantly reduced in the HIRI group when compared with the sham group. Also, both the low-dose BP and high-dose BP groups showed a significant increase in total protein concentration when compared to the HIRI group. Significant decrease was further observed in the high-dose BP group when compared with the low-dose BP group.

Hepatic Malondialdehyde (MDA)

MDA concentration significantly increased in the livers of the rats of the HIRI group when compared with the sham group. Both the low-dose BP and high-dose BP groups, however, were able to significantly

reduce MDA concentration when compared to the HIRI group. Furthermore, there was a significant decrease in the high-dose BP group when compared with the low-dose BP group.

Hepatic Superoxide dismutase (SOD)

Hepatic SOD concentration was significantly decreased in HIRI group when compared with the sham group. In addition to this, both low-dose BP and high-dose BP groups showed a significant increase in SOD concentration when compared with the HIRI group.

Hepatic Catalase

There was a statistically significant decrease in hepatic catalase concentration in the HIRI group when compared with the sham group. There was also a statistically significant increase in catalase concentration in the low-dose BP and high-dose BP groups when compared with the HIRI group.

3.4 Renal Oxidative Stress Markers

Renal Glutathione (GSH)

Renal GSH was significantly reduced in the HIRI group when compared with the sham group. Also, both the low-dose BP and high-dose BP showed a significant increase in GSH concentration when compared to the HIRI group.

Renal Total Protein

Total protein was significantly reduced in the HIRI group when compared with the sham group. Also, both the low-dose BP and high-dose BP showed a significant increase in total protein concentration when compared to the HIRI group.

Renal Malondialdehyde (MDA)

Renal MDA concentration was significantly increased in the HIRI group when compared to the HIRI group. Furthermore, both low-dose BP and high-dose BP groups had a significantly reduced MDA concentration in comparison with the HIRI group.

Renal Superoxide dismutase (SOD)

The renal concentration of SOD was significantly reduced in the HIRI group when compared with the sham group. Only the high-dose BP group, however, showed a significant increase in SOD concentration when compared with the HIRI group.

Renal Catalase

Catalase concentration was significantly reduced in the kidneys of the animals in the HIRI group when compared with the sham group. Also, both low-dose BP and high-dose BP groups showed significant

increase in catalase concentration when compared with the HIRI group.

3.5 Inflammatory Markers

Tumour necrosis factor (TNF- α)

There was a statistically significant increase in TNF- α concentration in the HIRI group when compared with the sham group. Significant decrease was also observed in the low-dose BP and high-dose BP groups when compared with the HIRI group. Furthermore, the high-dose BP group had a statistically significant reduction in TNF- α concentration when compared with the low-dose BP group.

Myeloperoxidase (MPO)

There was a statistically significant increase in MPO concentration in the HIRI group compared with the sham group. Also, the high-dose BP group had its MPO concentration significantly reduced when compared with the HIRI group.

Serum nitrite

There was no statistically significant difference in nitrite concentration among the groups.

3.6 Apoptotic Marker

Caspase-3

There was a statistically significant increase in caspase-3 concentration in the HIRI group when compared with the sham group. The low-dose BP and high-dose BP groups significantly reduced caspase-3 concentration with respect to the HIRI group. In addition, there was a significant decrease in caspase-3 concentration in the high-dose BP group when compared with the low-dose BP group.

3.7 Histology of the Liver

Sham group (SH)

The hepatic tissue appears normal. The hepatocytes (H) appear polygonal with well-outlined vesicular nucleus (N). The hepatocytes are separated by the sinusoids (S) with thin endothelial lining, free from collections and inflammatory cells. The central vein (CV) and bile duct (BD) also appear normal

HIRI group (HH)

The hepatic tissue appears distorted. The hepatocytes (H) appear thickened with well-outlined vesicular nucleus (N). The hepatocytes are separated by the sinusoids (S) with hemorrhagic lesions (circle). There are focal areas of inflammatory cell infiltration (box) and focal vascular congestion (star) in the central vein (CV). The bile duct (BD) appear normal.

Low-dose BP group (LDH)

The hepatic tissue appears normal. The hepatocytes (H) appear polygonal with well-outlined vesicular nucleus (N). The hepatocytes are separated by the sinusoids (S) with thin endothelial lining, free from collections and inflammatory cells. The central vein (CV) and bile duct (BD) also appear normal.

High-dose BP (HDH)

The hepatic tissue appears distorted. The hepatocytes (H) appear polygonal with well-outlined vesicular nucleus (N). The hepatocytes are separated by the sinusoids (S) and it is free of lesion. There is focal vascular congestion (star) in the central vein (CV).

3.8 Histology of the Kidney

Sham group (SH)

The histology of the renal tissue appears normal. The renal corpuscle (arrow) consists of the glomerulus (G) containing podocytes and separated by a well-defined Bowman's space (BS). The renal tubules (RT) are lined by columnar-cuboidal epithelium and separated by interstitium that is free from congestions and collections.

HIRI group (HH)

The histology of the renal tissue appears distorted. The renal corpuscle (arrow) consists of the glomerulus (G) containing podocytes and separated by an ill-defined Bowman's space (BS). The renal tubules (RT) are lined by columnar-cuboidal epithelium and separated by interstitium with focal areas of hemorrhagic lesions (black circle). There are also focal areas of vascular congestions (star) and inflammatory cell infiltration (box).

Low-dose BP (LDH)

The histology of the renal tissue appears normal. The renal corpuscle (arrow) consists of the glomerulus (G) containing podocytes and separated by a well-defined Bowman's space (BS). The renal tubules (RT) are lined by columnar-cuboidal epithelium and separated by interstitium that is free from congestions and collections.

High-dose BP (HDH)

The histology of the renal tissue appears distorted. The renal corpuscle (arrow) consists of the glomerulus (G) containing podocytes and separated by an ill-defined Bowman's space (BS). The renal tubules (RT) are lined by columnar-cuboidal epithelium and separated by interstitium with focal areas of hemorrhagic lesions (black circle).

4 Discussion

The present study demonstrates that HIRI induces significant remote organ dysfunction, particularly affecting renal integrity. The relationship between hepatic damage and renal dysfunction (hepatorenal syndrome), highlights the need for protective interventions that can mitigate the systemic impact of HIRI (39). *B. pinnatum* is rich in flavonoids, alkaloids, and terpenes, which are known for their potent antioxidant and anti-inflammatory properties. Of particular interest is the presence of quercetin and kaempferol derivatives, which have shown renoprotective effects in other models of acute kidney injury (12).

The observed elevation in serum ALT, AST, and GGT levels in the HIRI group confirms substantial hepatocellular damage, consistent with previous findings (40). As demonstrated by Ginès and Schrier (41), severe liver injury can induce systemic vasodilation, leading to reduced effective arterial blood volume and subsequent activation of the renin-angiotensin-aldosterone system (RAAS). This activation results in renal vasoconstriction and compromised renal perfusion. Also, the release of damage-associated molecular patterns (DAMPs) from injured hepatocytes can trigger a systemic inflammatory response. Released pro-inflammatory cytokines and chemokines can directly impact renal function through leukocyte infiltration and tubular cell injury (42).

Consequently, oxidative stress initiated in the liver, evidenced by elevated protein degradation, GGT and MDA levels, as well as reduced GSH, SOD, and catalase levels, can propagate systemically, affecting distant organs including the kidneys. Oxidative stress is one of the primary mechanisms implicated in IRI (3, 43). In the current study, *B. pinnatum* significantly ameliorated oxidative stress in both liver and kidney tissues, as evidenced by reductions in MDA levels and increases in endogenous antioxidant enzymes including superoxide dismutase SOD and catalase. These results are in alignment with earlier studies, which highlight the antioxidant properties of *B. pinnatum* as a contributor to its therapeutic effects (12, 17).

The antioxidative effects observed may be attributed to the plant's phytochemical constituents, including flavonoids such as quercetin and kaempferol, known for their ROS-scavenging capabilities (44, 45). These compounds enhance the activity of nuclear factor erythroid 2-related factor 2 (Nrf2), a major regulator of the cellular antioxidant response. Activation of the Nrf2 pathway also upregulates the expression of genes encoding antioxidant enzymes, which mitigate oxidative stress by neutralising ROS and preserving mitochondrial integrity (46). The increased activity of SOD and catalase observed in this study supports the hypothesis that *B. pinnatum* activates Nrf2, aligning with previous findings that document the role of flavonoids in Nrf2-mediated antioxidative pathways (47, 48).

Moreover, HIRI-induced kidney injury is strongly associated with inflammation, involving the release of pro-inflammatory cytokines (6, 7). Inflammatory cascade analysis revealed that *B. pinnatum* administration led to significant reductions in TNF- α and MPO levels, suggesting an attenuation of the inflammatory cascade. This anti-inflammatory effect may result from the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which has been identified as a central mediator of inflammation in ischemia-reperfusion injury (1, 49).

The phytoconstituents in *B. pinnatum*, including triterpenoids and flavonoids, likely play a role in modulating NF- κ B activation. These compounds have been shown to suppress NF- κ B activity by preventing the phosphorylation and degradation of its inhibitor, I κ B α , thus impeding the transcription of pro-inflammatory cytokines (44). This NF- κ B inhibition reduces the recruitment of neutrophils and macrophages to the site of injury, thereby decreasing MPO levels, as observed in the present study. Furthermore, the downregulation of TNF- α may have limited secondary damage induced by ROS, creating a positive feedback loop that mitigates both inflammation and oxidative stress. Such multi-faceted anti-inflammatory effects underscore the therapeutic potential of *B. pinnatum* in ameliorating HIRI-induced renal injury (11, 12).

Furthermore, our biochemical analyses revealed significant elevations in serum creatinine and urea levels in the HIRI group, indicating compromised glomerular filtration and renal dysfunction. These findings align with previous studies demonstrating the development of acute kidney injury following hepatic ischemia-reperfusion (39, 50). However, the nephroprotective effects of *B. pinnatum* were evidenced by improved levels of creatinine and urea. These markers were significantly reduced in *B. pinnatum*-treated groups compared to untreated HIRI controls, indicating preserved glomerular filtration rate (GFR) and reduced renal damage. Notably, this nephroprotective property was demonstrated in a dose-dependent manner, with the higher dose showing superior efficacy in preserving renal function. This preservation of renal function is consistent with prior findings suggesting that *B. pinnatum* has protective effects on renal tissue under conditions of oxidative stress and inflammation (5, 45).

The reduction in creatinine and urea levels observed in this study may stem from multiple mechanisms, including the antioxidant and anti-inflammatory effects of *B. pinnatum*, as well as its role in inhibiting apoptosis. Apoptosis in ischemia-reperfusion injury is mediated by both intrinsic (mitochondrial) and extrinsic (death receptor) pathways, with caspase-3 serving as an important executor enzyme (4).

In line with this, our study revealed a significant elevation in caspase-3 levels in the HIRI group, indicating enhanced apoptotic activity. The attenuation of caspase-3 activity observed here suggests that *B. pinnatum* may inhibit apoptosis, potentially through modulation of mitochondrial pathways and inhibition of the mitochondrial permeability transition pore (mPTP) opening, as well as the modulation of Bcl-2 family proteins (51, 52). Reduced apoptosis not only preserves renal tubular structure but also minimises functional impairment, which aligns with the observed maintenance of kidney function markers. Histopathological examination provided visual confirmation of these biochemical findings. The HIRI group showed characteristic renal tissue damage, including ill-defined Bowman's spaces and inflammatory cell infiltration. *B. pinnatum* treatment preserved normal renal architecture, supporting its nephroprotective effects at the tissue level.

5 Conclusion

The nephroprotective effects of *B. pinnatum* appear to be mediated through multiple mechanisms: (1) antioxidant properties, evidenced by restoration of GSH, SOD, and catalase levels; (2) anti-inflammatory

effects, demonstrated by reduced TNF- α and MPO levels; and (3) anti-apoptotic activity, shown by normalised caspase-3 levels. These findings suggest that *B. pinnatum* could serve as a promising therapeutic agent for preventing HIRI-induced kidney injury, though further clinical studies are warranted to establish its efficacy in human subjects.

Abbreviations

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
GGT	Gamma-glutamyl transferase
BP	<i>Bryophyllum pinnatum</i>
HIRI	Hepatic Ischemia-Reperfusion Injury
IRI	Ischemia-Reperfusion Injury
MDA	Malondialdehyde
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
GSH	Reduced Glutathione

Declarations

Ethics Approval and Consent to Participate: Ethical approval was obtained from Ethics Committee in Animal Care and Use in Research of FBMS with reference number: **ERCFBMSLAUTECH: 058/08/2024**.

Consent for Publication: Not applicable

Competing Interests: The authors declare that there is no conflict of interest in this manuscript

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Authors' contributions: OVO, OAA, and OSH designed the study; OVO, OAA, RAA, and OSH performed the experiments; RAA, ABA, AWS, and SOO provided new reagents and analytical tools; OVO, ABA, OSH, and OSH analysed the research data; OVO, RAA, OSH, OSH, AWS, and SOO contributed to the writing of the manuscript; all authors read and approved the final manuscript.

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Plates

Plates 1 and 2 are available in the Supplementary Files section.

Figures

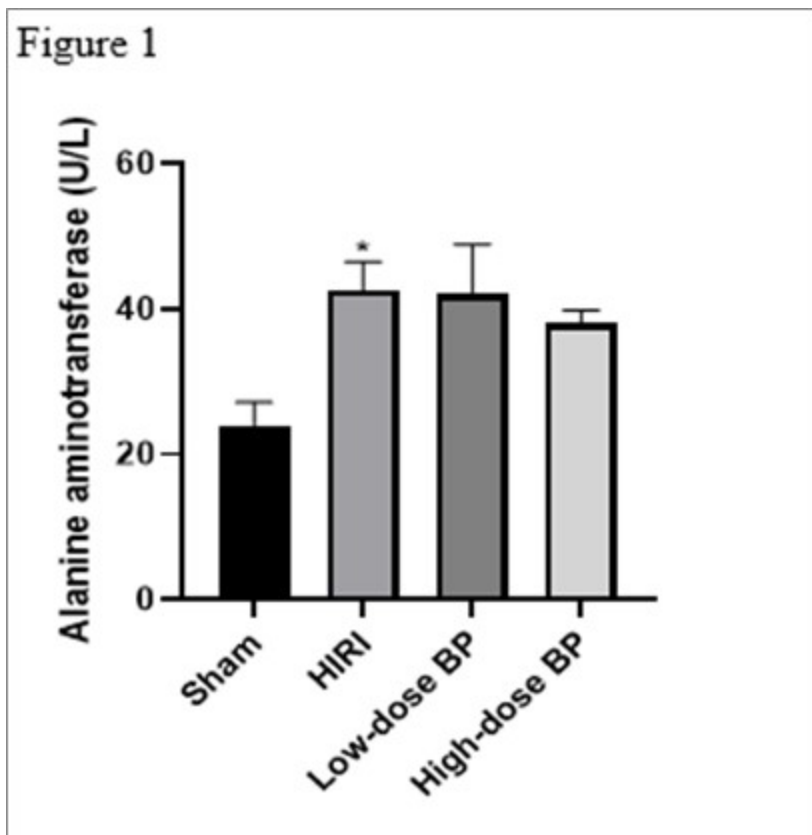


Figure 1

Effect of *Bryophyllum pinnatum* on Alanine aminotransferase (ALT) concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P < 0.001$) vs sham group.

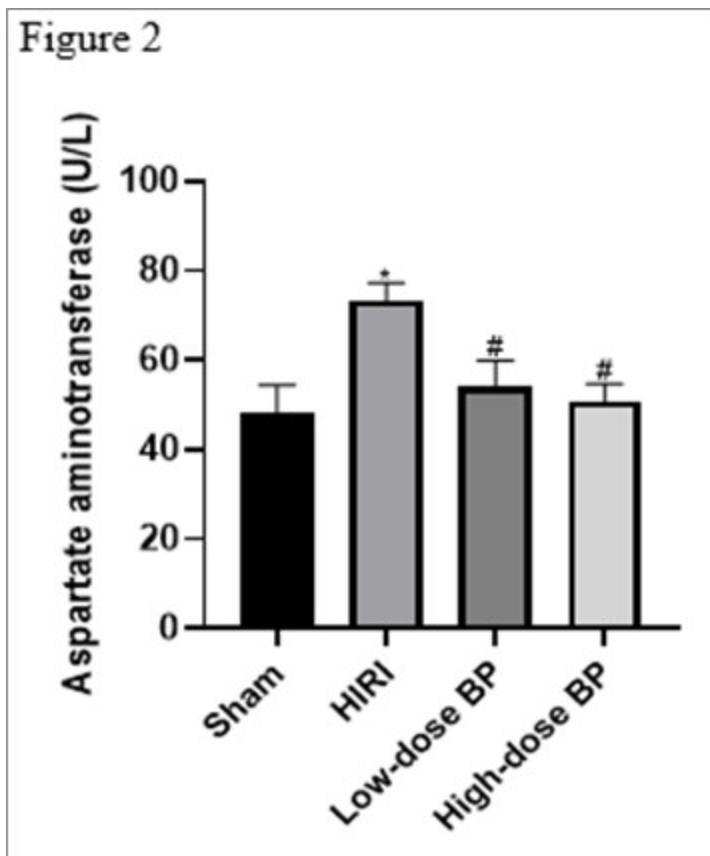


Figure 2

Effect of *Bryophyllum pinnatum* on Aspartate aminotransferase (ALT) concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.01$ and $P<0.001$) respectively vs HIRI group.

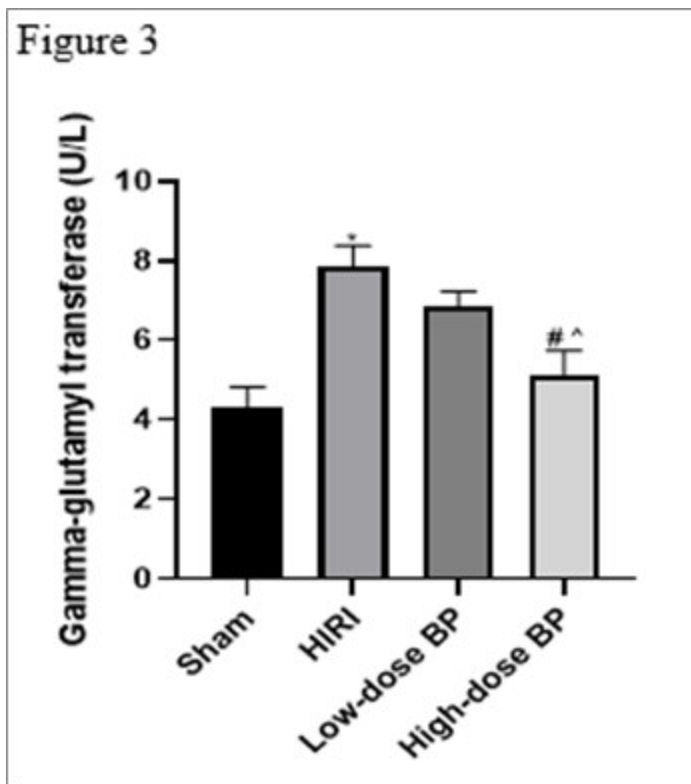


Figure 3

Effect of *Bryophyllum pinnatum* on Gamma-glutamyl transferase (GGT) concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$) vs HIRI group. ^ represents significance at ($P<0.01$) vs low-dose group.

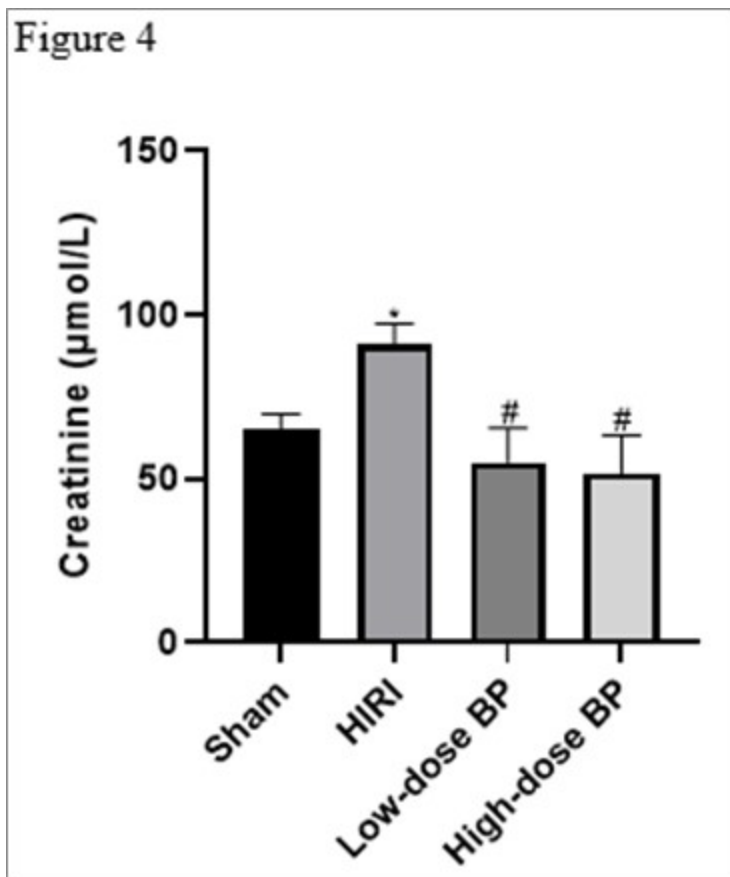


Figure 4

Effect of *Bryophyllum pinnatum* on Creatinine concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.05) vs sham group. # represents significance at (P<0.01 and P<0.001) vs HIRI group.

Figure 5

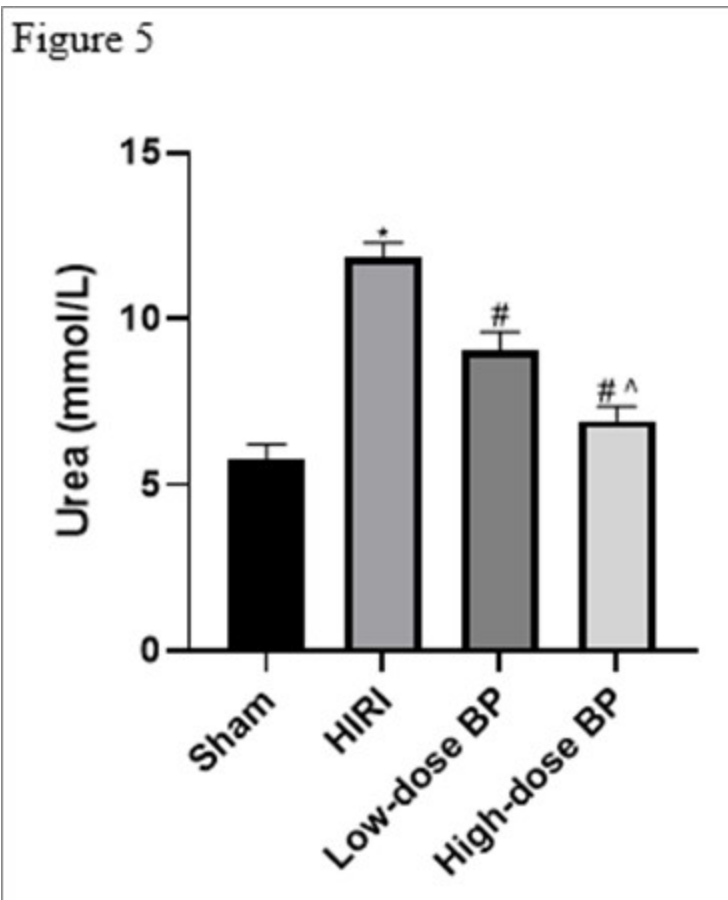


Figure 5

Effect of *Bryophyllum pinnatum* on Urea concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$ and $P<0.001$) vs HIRI group. ^ represents significance at ($P<0.001$) vs low-dose BP group.

Figure 6

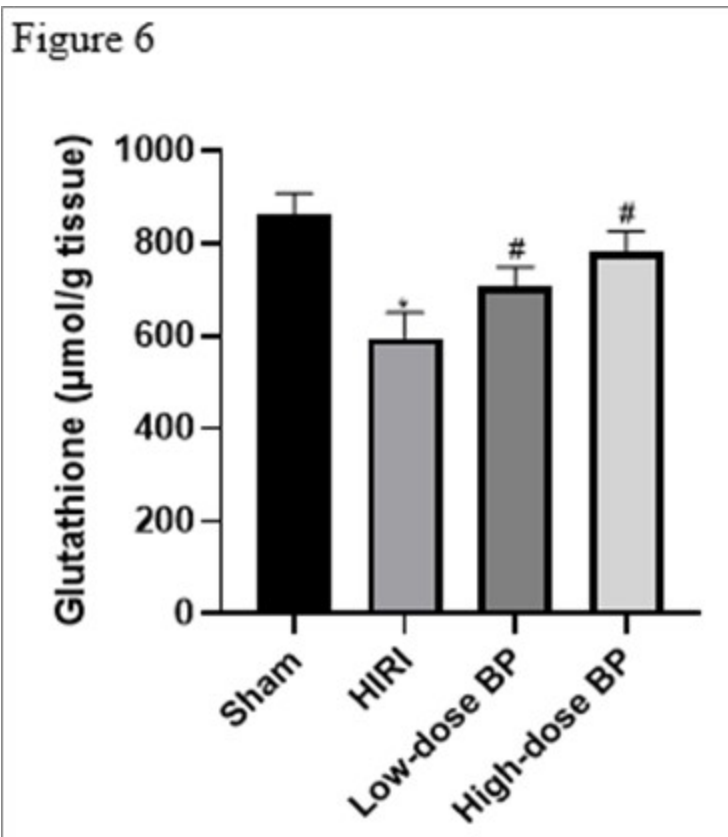


Figure 6

Effect of *Bryophyllum pinnatum* on hepatic GSH concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.01$ and $P<0.001$) vs HIRI group.

Figure 7

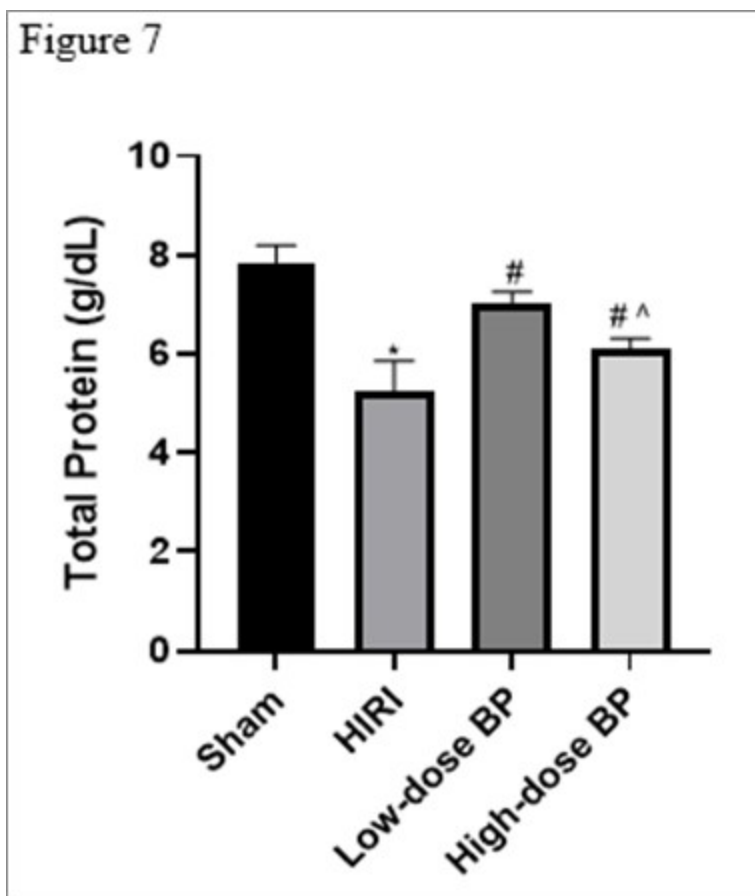


Figure 7

Effect of *Bryophyllum pinnatum* on hepatic total protein concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.001) vs sham group. # represents significance at (P<0.001 and P<0.05) vs HIRI group. ^ represent significance at (P<0.01) vs low-dose BP group.

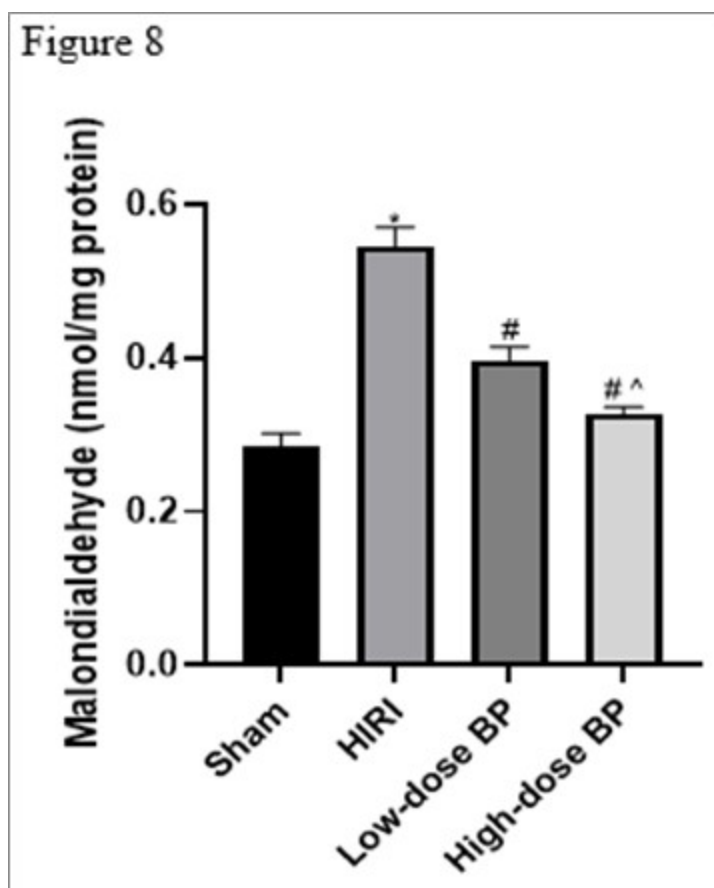


Figure 8

Effect of *Bryophyllum pinnatum* on hepatic MDA concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$ and $P<0.001$) vs HIRI group. ^ represent significance at ($P<0.001$) vs low-dose BP group.

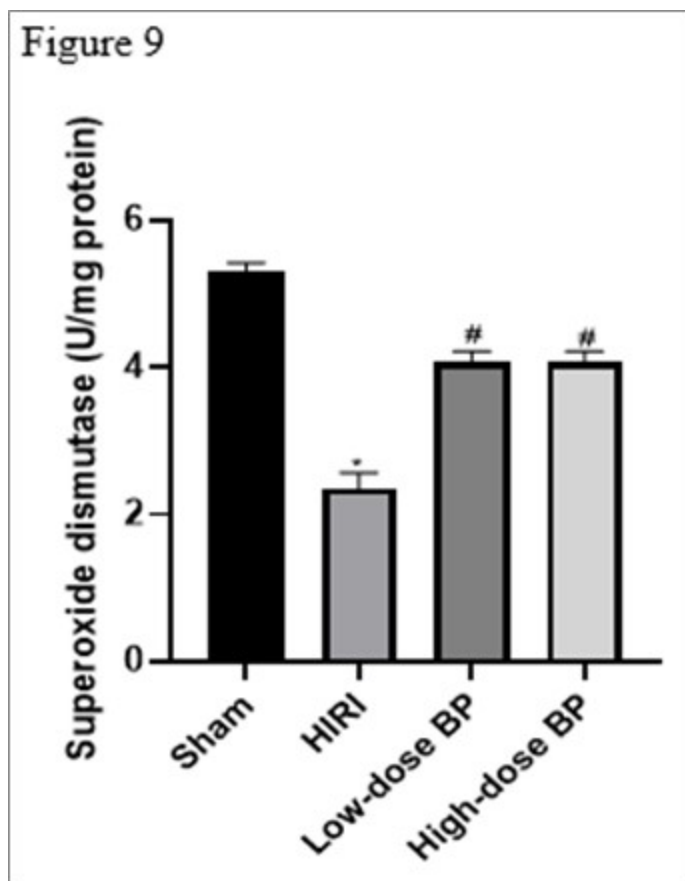


Figure 9

Effect of *Bryophyllum pinnatum* on hepatic SOD concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$ and $P<0.001$) vs HIRI group.

Figure 10

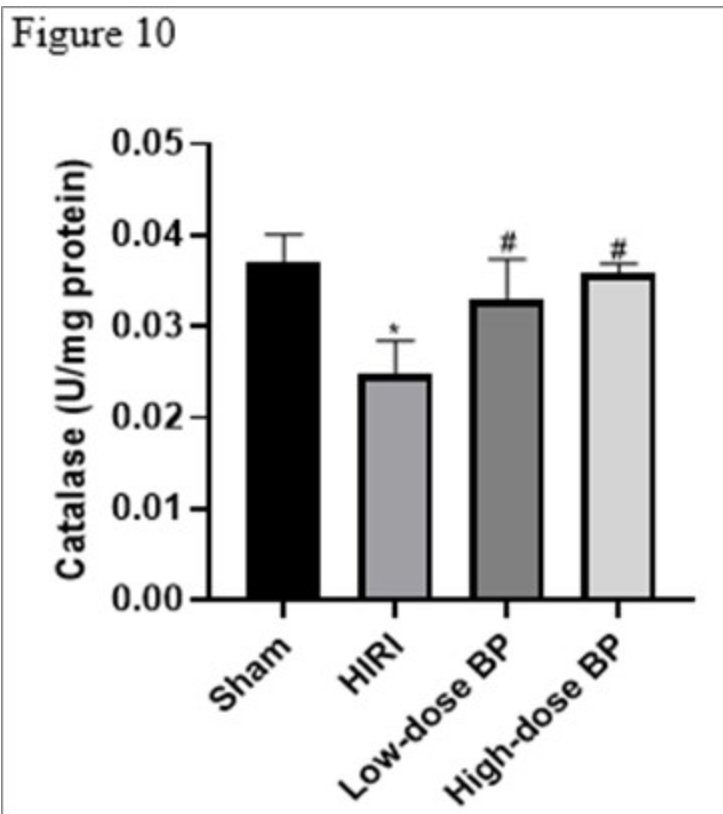


Figure 10

Effect of *Bryophyllum pinnatum* on hepatic Catalase concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.001) vs sham group. # represents significance at (P<0.05 and P<0.01) vs HIRI group.

Figure 11

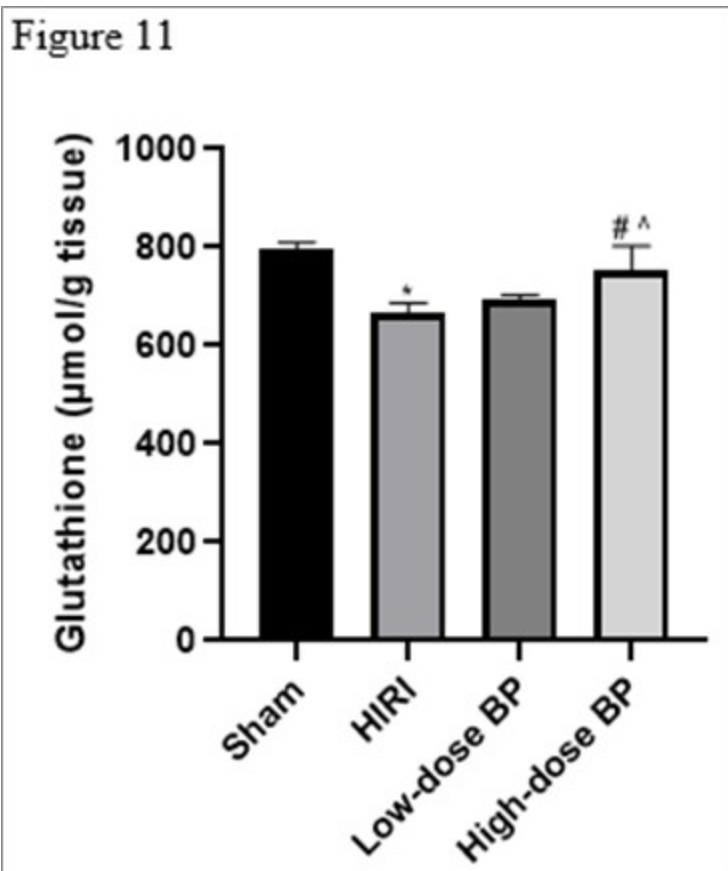


Figure 11

Effect of *Bryophyllum pinnatum* on renal GSH concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.001) vs sham group. # represents significance at (P<0.05) vs HIRI group. ^ represents significance at P<0.05) vs low-dose BP group.

Figure 12

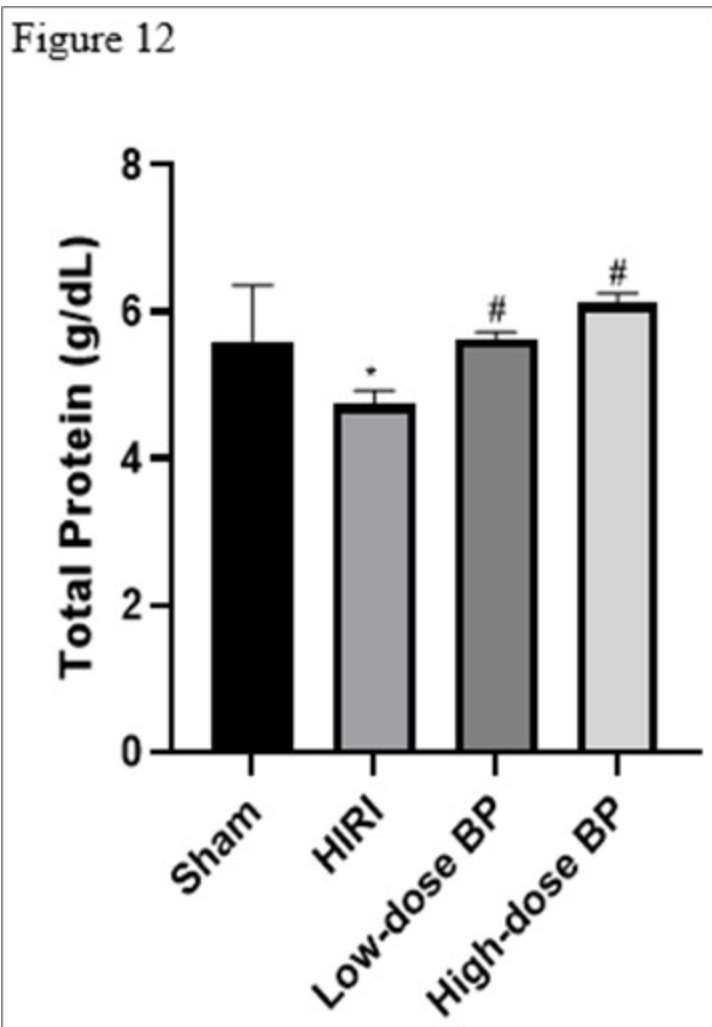


Figure 12

Effect of *Bryophyllum pinnatum* on renal total protein concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.05) vs sham group. # represents significance at (P<0.05 and P<0.001) vs HIRI group.

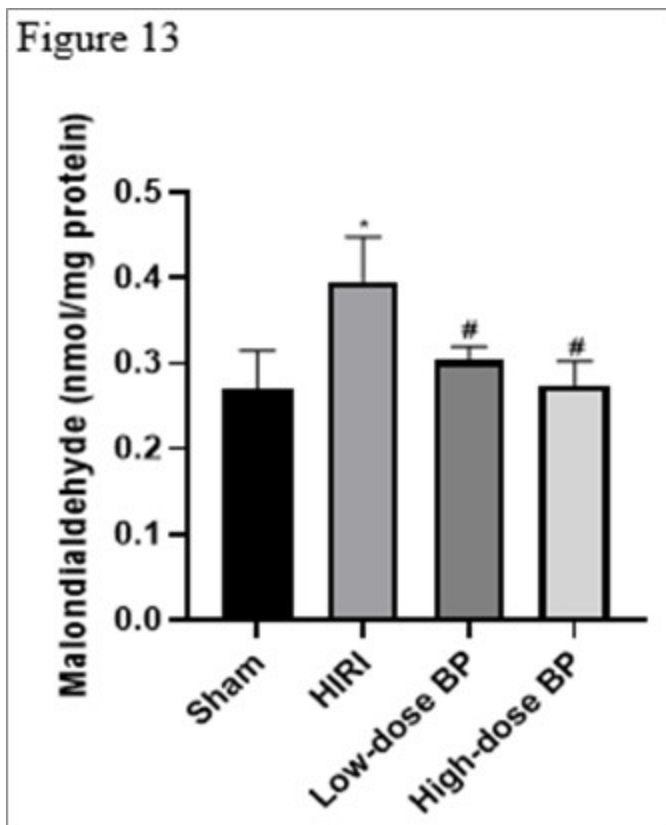


Figure 13

Effect of *Bryophyllum pinnatum* on renal MDA concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.01$) vs sham group. # represents significance at ($P<0.01$ and $P<0.01$) vs HIRI group.

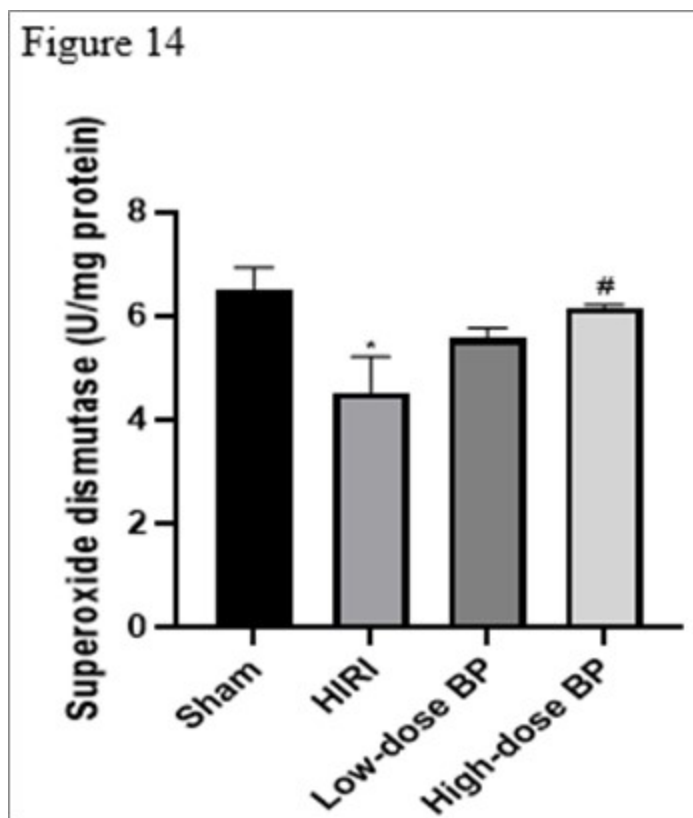


Figure 14

Effect of *Bryophyllum pinnatum* on renal SOD concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.01$) vs HIRI group.

Figure 15

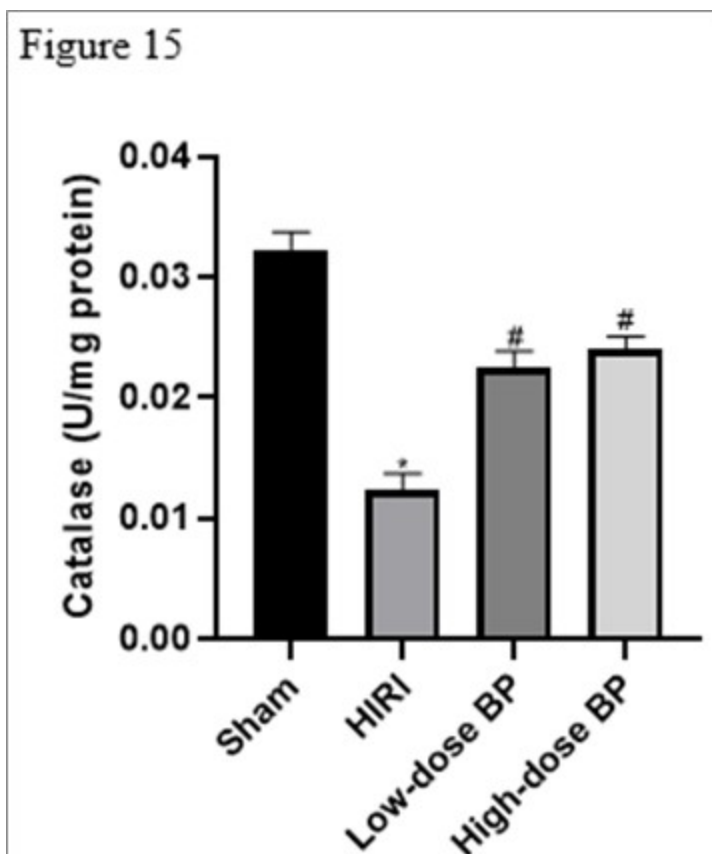


Figure 15

Effect of *Bryophyllum pinnatum* on renal catalase concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$ and $P<0.001$) vs HIRI group.

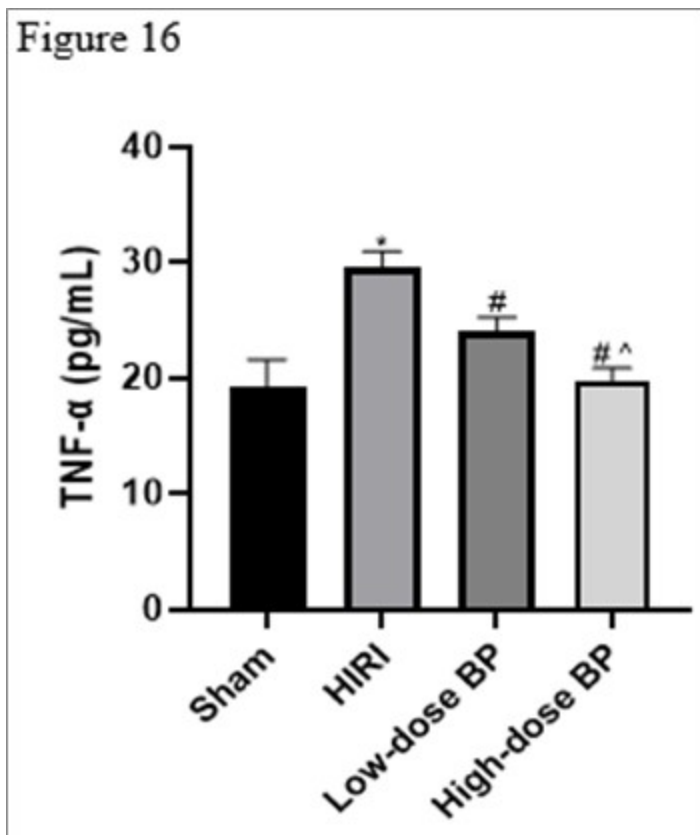


Figure 16

Effect of *Bryophyllum pinnatum* on TNF-α concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P < 0.001$) vs sham group. # represents significance at ($P < 0.001$ and $P < 0.001$) vs HIRI group. ^ represents significance at ($P < 0.01$) vs low-dose BP group.

Figure 17

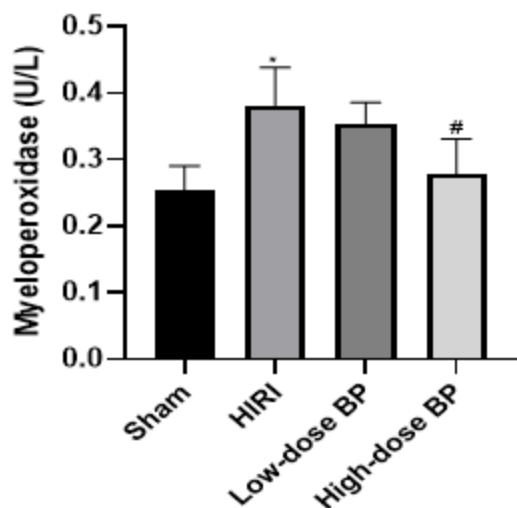


Figure 17

Effect of *Bryophyllum pinnatum* on MPO concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at (P<0.01) vs sham group. # represents significance at (P<0.05) vs HIRI group.

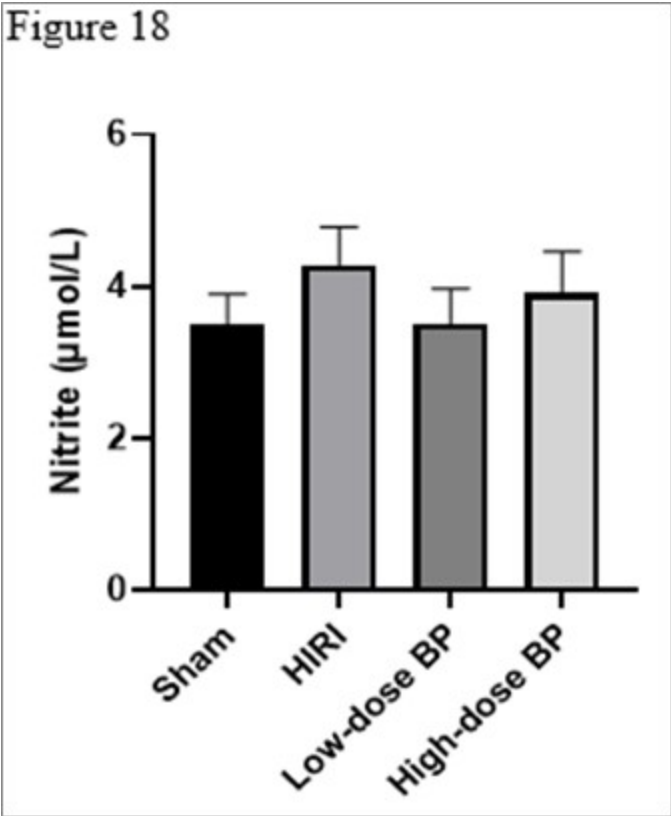


Figure 18

Effect of *Bryophyllum pinnatum* on nitrite concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group).

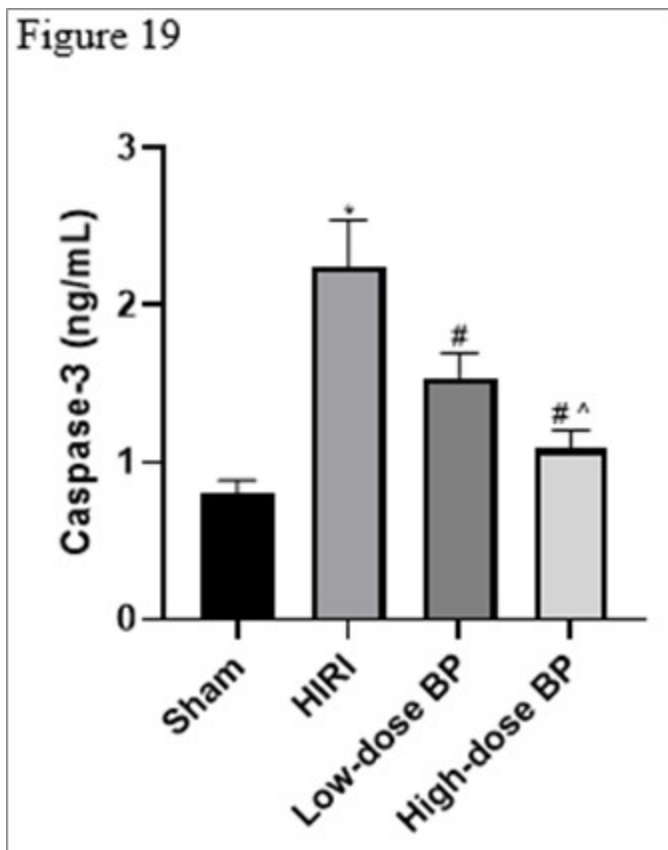


Figure 19

Effect of *Bryophyllum pinnatum* on caspase-3 concentration following HIRI in Male Wistar Rats

Data were presented as mean $\pm$ SEM, n=10 (number of rat per group). * represents significance at ($P<0.001$) vs sham group. # represents significance at ($P<0.001$ and $P<0.001$) vs HIRI group. ^ represents significance at ($P<0.01$) vs low-dose BP group.

Supplementary Files

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