

MODULATION OF KETAMINE-INDUCED MYOCARDIAL INFLAMMATION BY METHANOLIC EXTRACT AND BIOACTIVE FRACTIONS OF *DACRYODES EDULIS* IN WISTAR RATS: AN IMMUNOHISTOCHEMICAL EVALUATION OF TNF- α AND IL-6 EXPRESSION

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ABSTRACT

Objective: Ketamine, a widely used anesthetic and recreational agent, is increasingly associated with dose-dependent cardiotoxicity, including inflammation and myocardial injury. This study evaluated the cardioprotective effects of *Dacryodes edulis* methanolic extract and its bioactive fractions (flavonoid, saponin, alkaloid) against ketamine-induced myocardial inflammation in adult Wistar rats, focusing on the immunohistochemical expression of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α).

Methods: Forty-eight adult male Wistar rats (≈ 0.2 kg) were divided into eight groups. Myocardial inflammation was induced through intraperitoneal administration of ketamine at 100, 150, and 200 mg/kg/day for 21 days. Treatment groups received oral administration of "*D. edulis*" methanolic extract (142 mg/kg/day), saponin fraction (142 mg/kg/day), alkaloid fraction (192 mg/kg/day), or flavonoid fraction (164 mg/kg/day) for 21 consecutive days. Hearts were harvested for histological and immunohistochemical analysis, and IL-6 and TNF- α expressions were assessed semiquantitatively.

Results: Ketamine caused dose-dependent upregulation of IL-6 and TNF- α , with the highest expression observed at 200 mg/kg. Administration of the methanolic extract moderately reduced cytokine expression. The saponin fraction completely suppressed IL-6 and TNF- α , whereas the flavonoid fraction significantly attenuated expression, showing mild positivity. The alkaloid fraction failed to reduce cytokine levels and displayed marked expression similar to high-dose ketamine.

Conclusion: Ketamine induces dose-dependent myocardial inflammation through upregulation of IL-6 and TNF- α . The flavonoid and saponin fractions of *D. edulis* exert potent immunomodulatory and cardioprotective effects, with flavonoids showing the most consistent attenuation. These findings highlight the therapeutic potential of *D. edulis* phytochemicals in mitigating drug-induced cardiotoxicity and provide a basis for future studies exploring plant-based cardioprotective interventions.

Keywords: *Dacryodes edulis*, Ketamine, Cardiotoxicity, Flavonoids, Saponins, Interleukin-6, TNF-alpha, Myocardial inflammation, Immunohistochemistry, Wistar rats.

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INTRODUCTION

Ketamine is a phencyclidine derivative that functions as a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist. It is widely used as a general anesthetic and analgesic in both clinical and veterinary settings due to its rapid onset and preservation of airway reflexes [1,2]. More recently, ketamine has garnered renewed attention for its rapid antidepressant effects, particularly in treatment-resistant depression and suicidal ideation [3,4]. Despite its therapeutic promise, ketamine's toxicity profile – particularly with repeated or high-dose use – has raised significant concerns. Reports indicate that chronic or high-dose ketamine administration is associated with multi-organ toxicity, including neurotoxicity, hepatotoxicity, nephrotoxicity, and cardiotoxicity [5-10].

Emerging data reveal that the heart is a vulnerable target of ketamine toxicity. Several experimental and clinical reports have described dose-dependent alterations in cardiac morphology, oxidative stress, pro-inflammatory cytokine release, and myocardial apoptosis following ketamine exposure [11,12]. In rodent models, ketamine has been shown to induce myocardial injury, manifested by vacuolization of cardiomyocytes, interstitial edema, degeneration of myocardial fibers,

and perturbations in cardiac biomarkers, such as troponins, creatine kinase-MB, and lactate dehydrogenase [13].

A key mechanism implicated in ketamine-induced myocardial damage is the activation of inflammatory pathways, notably involving tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6). TNF- α plays a pivotal role in mediating cardiac remodeling, apoptosis, and contractile dysfunction, while IL-6 promotes myocardial inflammation, vascular endothelial activation, and neutrophil infiltration [14,15]. Elevated levels of these cytokines in cardiac tissue are not only markers of inflammation but are also active drivers of disease progression in drug-induced cardiomyopathies. However, despite increasing awareness, immunohistochemical evidence of TNF- α and IL-6 expression in ketamine-induced myocardial injury remains limited, thus warranting further investigation.

There is growing interest in the use of natural products and medicinal plants to counteract drug-induced toxicities, particularly due to their multi-target mechanisms of action, safety profile, and affordability [16,17]. Phytochemicals, such as flavonoids, saponins, and alkaloids have demonstrated significant antioxidant, anti-inflammatory, anti-apoptotic, and cardioprotective effects in pre-clinical studies [18].

Flavonoids, in particular, have shown the ability to suppress nuclear factor kappa B (NF- κ B) activation, reduce the release of pro-inflammatory cytokines, and scavenge reactive oxygen species, making them potent agents in mitigating inflammatory cardiac damage [19,20].

Several studies have reported the protective roles of phytochemical-rich plant extracts against ketamine-induced organ toxicity. For instance, *Bryophyllum pinnatum* extract has shown efficacy in reversing ketamine-induced nephrotoxicity [6], and curcumin was shown to attenuate ketamine-induced neuroinflammation in postnatal rats [7]. Similarly, *Vigna unguiculata* and *Tetrahydropalmatine* have demonstrated neuroprotective and cognitive-enhancing effects in ketamine-induced memory impairment models [21,22]. Nonetheless, studies focusing on ketamine-induced cardiac toxicity and the therapeutic roles of plant-derived bioactive fractions are scarce.

Dacryodes edulis, commonly known as the African pear or safou, is an evergreen tree of the family Burseraceae, traditionally used in West and Central Africa for treating a wide range of ailments, including gastrointestinal disturbances, wounds, and infections [23]. Phytochemical analyses of *D. edulis* leaves have revealed a rich profile of flavonoids, alkaloids, saponins, and phenolic compounds [24-27]. These constituents are believed to mediate the plant's antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory activities [28].

While prior investigations have explored the neuroprotective effects of *D. edulis* in ketamine-induced hippocampal toxicity [29], there is no known study that has assessed its cardioprotective efficacy, especially in relation to pro-inflammatory cytokine expression in myocardial tissue. More importantly, there are no comparative studies evaluating the relative anti-inflammatory efficacy of isolated bioactive fractions (flavonoid, alkaloid, and saponin) of *D. edulis* in ketamine-induced cardiotoxicity models. While ketamine-induced neurotoxicity in the hippocampus is characterized by oxidative stress-driven cytoarchitectural disruption, our myocardial model emphasizes the primacy of cytokine-mediated inflammation in driving tissue injury and remodeling. In the hippocampal study, *D. edulis* ethanolic leaf extract mitigated neuronal degeneration largely in parallel with reduced lipid peroxidation (lower malondialdehyde [MDA], preserved glutathione), indicating antioxidant-dominated protection. By contrast, myocardial damage evolves through an early surge in TNF- α with downstream IL-6 amplification that coordinates leukocyte recruitment, endothelial activation, and profibrotic signaling – processes insufficiently captured by oxidative stress markers alone [30]. Accordingly, we designate TNF- α and IL-6 as primary endpoints to directly assay the inflammatory axis most responsible for cardiac dysfunction and adverse remodeling, while retaining oxidative stress readouts as contextual secondary measures. This framework allows bioactive extract effects to be differentiated into true immunomodulation (attenuation of TNF- α /IL-6) versus general antioxidant actions, providing a more mechanistically specific and clinically translatable assessment of cardioprotection than has been applied in the hippocampal paradigm.

TNF- α and IL-6 are widely recognized as central mediators of myocardial inflammation and remodeling. TNF- α , a master pro-inflammatory cytokine, is rapidly upregulated in response to cardiac injury and promotes cardiomyocyte apoptosis, endothelial activation, and leukocyte infiltration into myocardial tissue [31]. Elevated TNF- α levels have been consistently associated with contractile dysfunction and adverse cardiac remodeling in both experimental and clinical settings. IL-6, which is induced downstream of TNF- α signaling, amplifies the inflammatory response by stimulating acute-phase protein production, sustaining immune activation, and promoting fibrotic changes that contribute to ventricular hypertrophy and failure [32,33]. While other inflammatory mediators, such as IL-1 β or transcription factors, such as NF- κ B, play important roles, TNF- α and IL-6 provide more direct, measurable, and clinically validated indicators of myocardial injury severity. Similarly, oxidative stress markers, such as superoxide dismutase (SOD) and MDA, reflect redox imbalance but do not fully capture the immune-driven mechanisms underlying myocardial

inflammation. In the context of ketamine-induced myocardial injury, where oxidative stress and inflammatory signaling act synergistically to exacerbate cardiac dysfunction, the measurement of TNF- α and IL-6 offers a reliable means to evaluate both the initiation and propagation of inflammation. Moreover, given that many bioactive compound-rich extracts exert cardioprotective effects through combined antioxidant and anti-inflammatory mechanisms, reductions in TNF- α and IL-6 levels serve as robust mechanistic evidence of their therapeutic efficacy [34,35]. Thus, the selection of TNF- α and IL-6 as primary endpoints provides a pathophysiologically relevant and translationally meaningful strategy for assessing the cardioprotective potential of natural compounds in ketamine-induced myocardial injury.

Given the limitations in present pharmacologic strategies for managing drug-induced cardiac inflammation and the potential of *D. edulis* as a source of anti-inflammatory agents, the present study was designed to investigate the cardioprotective potential of the methanolic extract and major phytochemical fractions (flavonoid, saponin, and alkaloid) of *D. edulis* leaves in ketamine-induced myocardial injury in adult Wistar rats. The study specifically focused on the immunohistochemical expression of TNF- α and IL-6 as markers of myocardial inflammation and damage.

Furthermore, the investigation aimed to determine which phytochemical fraction exhibits the greatest anti-inflammatory efficacy, thereby guiding future drug development and ethnopharmacological applications. The novelty of this study lies in its integrative approach combining immunohistochemical assessments and its evaluation of phytochemical-specific therapeutic responses within the context of ketamine-induced cardiotoxicity.

METHODS

Chemicals and reagents

Ketamine hydrochloride (Rotexmedica, Germany) was used as the cardiotoxic agent. Solvents used included methanol (analytical grade), n-hexane, ethyl acetate, and distilled water (Sigma-Aldrich, USA). Antibodies for TNF- α and IL-6 were obtained from Abcam (UK), along with secondary antibodies and a DAB chromogen kit from Thermo Fisher Scientific.

Plant collection and authentication

Fresh leaves of *D. edulis* were harvested from Akamkpa, Cross River State, Nigeria, and authenticated at the Department of Botany, University of Calabar. A voucher specimen was deposited in the institutional herbarium (Voucher No. UC/PCG/DE-002).

Preparation of methanolic extract

Fresh leaves of *D. edulis* were collected, washed with distilled water to remove debris, air-dried at room temperature, and pulverized into fine powder. Approximately 500 g of the powdered material was macerated in 80% methanol (1:5 w/v) for 72 h with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator. The resulting crude methanolic extract was stored at 4°C in airtight containers until use. A portion of the crude extract was set aside for experimental groups receiving crude extract, while the remaining quantity was subjected to phytochemical fractionation.

From 500 g of powdered leaves, approximately 50–75 g of crude extract (10–15% w/w) is typically recovered, depending on solvent penetration and plant composition.

Phytochemical fractionation

The crude methanolic extract was subjected to solvent-solvent partitioning and classical phytochemical isolation methods to obtain distinct fractions:

Saponin fraction: Isolated using butanol partitioning and precipitation techniques as previously described by Runner RT, Majinda [36].

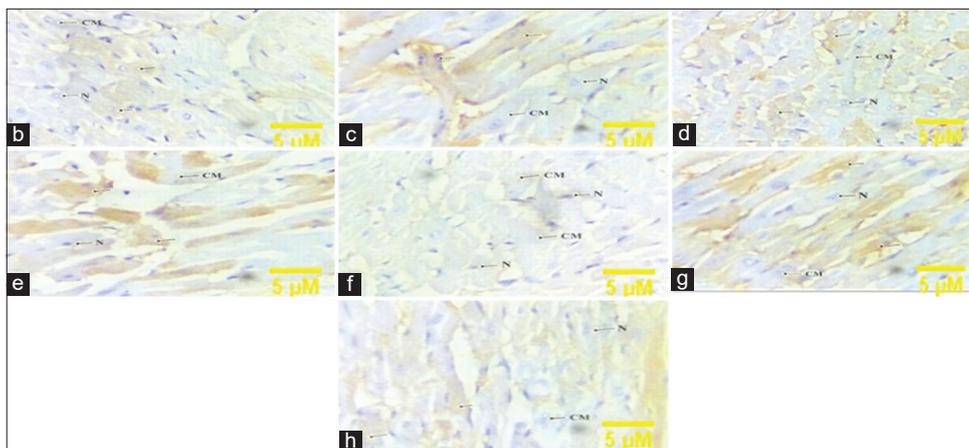


Fig. 1: Interleukin-6; Control and induction with different doses of ketamine. (a) Plate 1: Control group - Section of cardiac muscles shows no expression of interleukin-6 MAG: $\times 400$. (b) Plate 2: 100 mg Ketamine treated Group - Section of cardiac muscles shows moderate expression of interleukin-6 MAG: $\times 400$. (c) Plate 3: Ketamine (150 mg) treated Group - Section of cardiac muscles shows moderate expression of interleukin-6. MAG: $\times 400$. (d) Plate 4: Ketamine (200 mg) treated Group - Section of cardiac muscles show marked expression of interleukin-6. MAG: $\times 400$. (e) Plate 5: Methanolic extract treated Group - Section of cardiac muscles shows moderate expression of interleukin-6. MAG: $\times 400$. (f) Plate 6: Saponin treated Group - Section of cardiac muscles shows no expression of interleukin-6. MAG: $\times 400$. (g) Plate 7: Alkaloid treated Group - Section of cardiac muscles shows marked expression of interleukin-6. MAG: $\times 400$. (h) Plate 8: Flavonoid treated Group - Section of cardiac muscles shows mild expression of interleukin-6. MAG: $\times 400$

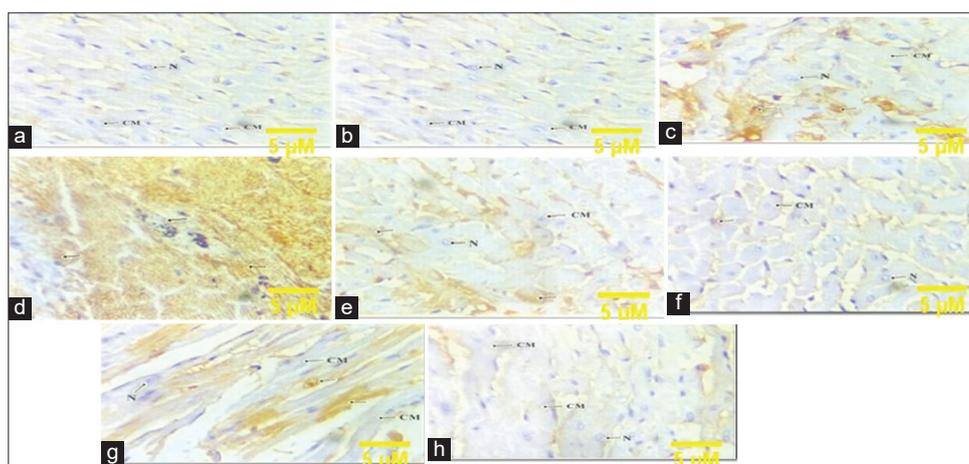


Fig. 2: Tumor necrotic factor alpha (TNF-alpha); control and induction with different doses of ketamine. Plate 9: Control group shows no expression of TNF-alpha. MAG: $\times 400$. (b) Plate 10: Induction with Ketamine shows mild expression of TNF-alpha. MAG: $\times 400$. (c) Plate 11: Induction with Ketamine 150 mg shows moderate expression of TNF- alpha. MAG: $\times 400$. (d) Plate 12: Induction with Ketamine 200 mg. Show marked expression of TNF- alpha. MAG: $\times 400$. (e) Plate 13: Methanolic extract treated Group - Section of cardiac muscles shows moderate expression of TNF. MAG: $\times 400$. (f) Plate 14: Saponin treated Group - Section of cardiac muscles shows no expression of TNF. MAG: $\times 400$. (g) Plate 15: Alkaloid treated Group - Section of cardiac muscles shows marked expression of TNF. MAG: $\times 400$. (h) Plate 16: Flavonoid treated group. Section of cardiac muscles shows mild expression of TNF. MAG: $\times 400$

Alkaloid fraction: Extracted by acid-base extraction followed by pH adjustment according to the method of Tubin [37].

Flavonoid fraction: Separated by ethyl acetate partitioning and column chromatography following the procedure of Khaerunnisa [38].

The fractions were concentrated under reduced pressure, dried, and stored at 4°C until use.

From the crude extract, saponins (30–40%), alkaloids (10–15%), and flavonoids (20–25%) constitute the major phytochemical fractions, with minor components comprising the balance.

The following dosages were prepared and administered:

- Methanolic extract: 28.47 mg (0.56 mL)
- Saponin fraction: 28.47 mg (0.54 mL)

- Alkaloid fraction: 38.37 mg (0.76 mL)
- Flavonoid fraction: 32.83 mg (0.65 mL).

All doses were administered orally once daily for 21 days.

Experimental animals

A total of 48 adult male Wistar rats (180–220 g) were used. The animals were housed under standard laboratory conditions with 12-h light/dark cycles, constant temperature (25 \pm 2°C), and access to standard chow and clean water *ad libitum*. The study protocol was approved by the University of Calabar Research Ethics Committee (Approval No. UC/AREC/025/2024).

Experimental design

The rats were randomly assigned into the following groups:

Group	Treatment
I	Normal control (saline only)
II	Ketamine 100 mg/kg only (i.p.)
III	Ketamine 150 mg/kg only (i.p.)
IV	Ketamine 200 mg/kg only (i.p.)
V	Ketamine 200 mg/kg+Methanolic extract of <i>Dacryodes edulis</i> (142 mg/kg b.w)
VI	Ketamine 200 mg/kg+Saponin fraction (142 mg/kg b.w)
VII	Ketamine 200 mg/kg+Alkaloid fraction (192 mg/kg b.w)
VIII	Ketamine 200 mg/kg+Flavonoid fraction (164 mg/kg b.w)

The oral doses for the crude methanolic extract (≈ 142 mg/kg) and the phytochemical fractions (≈ 142 – 192 mg/kg) were chosen based on established literature where similar ranges (100–200 mg/kg) have demonstrated safety and pharmacological efficacy in Wistar rats. Previous studies on methanolic plant extracts and isolated fractions have consistently employed doses within this range [39,40]. Thus, the selected doses are appropriate for evaluating biological activity without inducing systemic toxicity.

Ketamine was administered intraperitoneally for 21 days and the treatment groups received the plant extract/fractions orally for 21 consecutive days simultaneously.

The treatment duration of 21 consecutive days was selected based on previous studies demonstrating that repeated daily administration over 3 weeks is sufficient to induce myocardial inflammation and oxidative stress in rodents, and to allow assessment of the protective effects of plant extracts [9].

Immunohistochemistry

Sections underwent deparaffinization, rehydration, antigen retrieval in citrate buffer (pH 6.0), blocking, and incubation with:

- Anti-TNF- α (1:100 dilution, overnight)
- Anti-IL-6 (1:100 dilution, overnight).

After secondary antibody incubation, detection was done using DAB chromogen, and slides were counterstained with hematoxylin.

Ethical approval

All animal experiments were conducted in accordance with the approved institutional and natural guidelines for the care and use of animals. Ethical clearance was obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS), Faculty of Basic Medical Sciences, University of Calabar, Calabar, with an issued number: 112ANA1822.

RESULTS

Immunohistochemical expression of IL-6 in cardiac tissue

The control group (Plate 1) showed no detectable expression of IL-6 in cardiac muscle. Following ketamine induction, moderate IL-6 expression was observed in the 100 mg/kg (Plate 2) and 150 mg/kg (Plate 3) groups, while the 200 mg/kg ketamine group (Plate 4) exhibited marked IL-6 expression, indicating a dose-dependent pro-inflammatory response. Treatment with the methanolic extract of *D. edulis* (Plate 5) resulted in moderate IL-6 expression, suggesting partial attenuation. Interestingly, the saponin-treated group (Plate 6) showed no IL-6 expression, indicating strong anti-inflammatory potential. The alkaloid-treated group (Plate 7) showed marked IL-6 expression, similar to the highest ketamine dose, implying poor modulation. In contrast, the flavonoid-treated group (Plate 8) exhibited mild IL-6 expression, suggesting effective reduction of inflammation compared to other treatments.

TNF- α

No TNF- α expression was observed in the control group (Plate 9). Ketamine induction resulted in mild (100 mg/kg, Plate 10), moderate

(150 mg/kg, Plate 11), and marked (200 mg/kg, Plate 12) TNF- α expression, indicating dose-dependent inflammatory activation. Treatment with the methanolic extract (Plate 13) reduced expression to moderate levels, while the saponin fraction (Plate 14) completely suppressed TNF- α expression. The alkaloid fraction (Plate 15) failed to mitigate TNF- α activity, showing marked expression. Conversely, the flavonoid fraction (Plate 16) significantly attenuated TNF- α levels, with only mild expression observed.

Figures 1 and 2. Immunohistochemical expression of IL-6 and TNF- α in cardiac muscle sections of experimental rats following ketamine induction and treatment with *D. edulis* extract and its phytochemical fractions.

- Plates 1-8: IL-6 expression in cardiac muscle
- Plate 1: Control group showing no IL-6 expression
- Plate 2: Ketamine (100 mg/kg) group showing moderate IL-6 expression
- Plate 3: Ketamine (150 mg/kg) group with moderate IL-6 expression
- Plate 4: Ketamine (200 mg/kg) group displaying marked IL-6 expression
- Plate 5: Methanolic extract-treated group showing moderate IL-6 expression
- Plate 6: Saponin-treated group showing no IL-6 expression
- Plate 7: Alkaloid-treated group with marked IL-6 expression
- Plate 8: Flavonoid-treated group showing mild IL-6 expression
- Plates 9-16: TNF- α expression in cardiac muscle
- Plate 9: Control group showing no TNF- α expression
- Plate 10: Ketamine (100 mg/kg) group with mild TNF- α expression
- Plate 11: Ketamine (150 mg/kg) group showing moderate TNF- α expression
- Plate 12: Ketamine (200 mg/kg) group exhibiting marked TNF- α expression
- Plate 13: Methanolic extract-treated group with moderate TNF- α expression
- Plate 14: Saponin-treated group showing no TNF- α expression
- Plate 15: Alkaloid-treated group with marked TNF- α expression
- Plate 16: Flavonoid-treated group displaying mild TNF- α expression
- Magnification: $\times 400$
- Staining: DAB immunohistochemistry for IL-6 and TNF- α .

DISCUSSION

This study investigated the protective effects of the methanolic extract and phytochemical fractions (flavonoid, saponin, and alkaloid) of *D. edulis* against ketamine-induced myocardial inflammation in adult Wistar rats, focusing on the immunohistochemical expression of the pro-inflammatory cytokines IL-6 and TNF- α . The findings demonstrate a dose-dependent increase in myocardial expression of IL-6 and TNF- α following ketamine administration, with attenuation by specific phytochemical fractions, particularly flavonoids and saponins.

Plates 1 to 4 show progressive immunorexpression of IL-6 from the control to increasing ketamine doses (100–200 mg/kg), indicating a dose-dependent inflammatory response. This is consistent with previous studies, which reported that high-dose or repeated ketamine administration can trigger systemic and organ-specific inflammation, including the heart [9,7,11,41]. The marked IL-6 and TNF- α expression observed in the 200 mg/kg ketamine group (Plates 4 and 12) suggests significant activation of cytokine-mediated signaling cascades that may contribute to cardiomyocyte damage, mitochondrial dysfunction, and oxidative stress.

Ketamine's action on NMDA receptors and its disruption of calcium homeostasis may initiate cellular stress responses, leading to the activation of NF- κ B, which drives transcription of IL-6 and TNF- α [42,43]. The implication is that prolonged or high-dose ketamine exposure, whether for recreational or therapeutic purposes, poses a substantial risk for inflammatory cardiomyopathy.

The methanolic extract-treated group (Plates 5 and 13) exhibited moderate expression of both IL-6 and TNF- α , indicating partial

attenuation of ketamine-induced inflammation. This suggests that the whole extract possesses anti-inflammatory bioactivity, likely due to the synergy of multiple phytoconstituents, such as flavonoids, phenolics, alkaloids, and saponins.

However, the moderate cytokine expression implies that the crude extract is less potent than the most effective isolated fractions, supporting the hypothesis that specific bioactive compounds mediate the bulk of the anti-inflammatory activity [16,45] and that concentration or standardization may be necessary for therapeutic applications.

Notably, the saponin-treated group showed no detectable expression of IL-6 or TNF- α (Plates 6 and 14), indicating complete suppression of ketamine-induced inflammatory signaling. Saponins are known to inhibit NF- κ B activation, downregulate cytokine production, and stabilize cellular membranes, thereby preventing inflammatory cascades [46]. Their amphipathic structure also allows them to interact with cholesterol-rich membranes, modulating immune responses at the cellular level.

This strong anti-inflammatory effect may make saponins promising candidates for further development into cardioprotective agents against drug-induced toxicity. However, their hemolytic potential in high concentrations or chronic use warrants additional toxicological screening.

Interestingly, the alkaloid-treated group displayed marked expression of both cytokines (Plates 7 and 15), similar to the high-dose ketamine-only group. This suggests that the alkaloid fraction may be ineffective or potentially pro-inflammatory in this context. While some alkaloids (e.g., berberine) exhibit anti-inflammatory effects [47], others may induce cytotoxic or pro-oxidant activity, especially in cardiac tissues [48].

The presence of bio-incompatible or stimulatory alkaloids in *D. edulis* could explain the exaggerated inflammatory response observed. This highlights the importance of characterizing and purifying individual alkaloid constituents before therapeutic application and underscores the limitations of using crude alkaloid mixtures.

Among all treatment groups, the flavonoid-treated rats showed only mild expression of IL-6 and TNF- α (Plates 8 and 16), demonstrating superior attenuation of ketamine-induced myocardial inflammation. Flavonoids are known to scavenge free radicals, inhibit inflammatory gene transcription, and modulate cell signaling pathways, such as MAPKs and NF- κ B [20]. They also upregulate endogenous antioxidant defenses, such as SOD, CAT, and GPx, reducing ROS-mediated cytokine release [49].

This finding is in agreement with earlier studies showing that flavonoid-rich plant extracts reduce inflammation and oxidative damage in ketamine-induced models of neurotoxicity and nephrotoxicity [16,45]. The mild cytokine expression observed implies that the flavonoid fraction interferes with upstream inflammatory signaling, potentially stabilizing cardiac tissue integrity even under high-dose ketamine challenge.

The immunohistochemical evidence presented in this study highlights the differential anti-inflammatory efficacy of bioactive fractions of *D. edulis* against ketamine-induced myocardial damage. The significant downregulation of IL-6 and TNF- α by flavonoids and saponins, particularly at high ketamine doses, suggests their potential as natural cardioprotective agents in clinical or veterinary medicine.

These findings also expand the pharmacological profile of *D. edulis*, adding cardioprotection and anti-cytokine modulation to its known antioxidant, antimicrobial, and hepatoprotective effects. From a mechanistic perspective, this study supports the hypothesis that protection against ketamine-induced toxicity involves modulation of the inflammatory microenvironment, a target amenable to phytochemical intervention.

The pronounced pro-inflammatory response observed in the alkaloid-treated group may be attributed to the heterogeneous nature of alkaloid mixtures, which can contain both anti-inflammatory and stimulatory or cytotoxic constituents. Certain alkaloids, such as isoquinoline or pyrrolizidine derivatives, are known to induce oxidative stress, disrupt calcium homeostasis, or activate pro-inflammatory signaling pathways, potentially counteracting the anti-inflammatory effects of other alkaloids present [50,51]. The co-existence of such stimulatory alkaloids within the crude fraction could therefore amplify TNF- α and IL-6 expression, exacerbating myocardial inflammation in the ketamine-exposed rats. These findings underscore the importance of detailed phytochemical characterization and purification of individual alkaloid components to identify which compounds contribute to inflammation versus cardioprotection. Future studies employing techniques, such as Liquid chromatography-mass spectrometry (MS)/MS or NMR spectroscopy could clarify the specific alkaloids responsible and facilitate targeted therapeutic development.

While the present study demonstrates the cardioprotective and anti-inflammatory potential of *D. edulis* fractions in a rodent model, several limitations must be acknowledged when extrapolating these findings to humans. First, rodent doses do not directly translate to human therapeutic doses, due to interspecies differences in metabolism, absorption, distribution, and clearance [52]. The mg/kg doses used here (\approx 142–192 mg/kg) would require careful allometric scaling and safety evaluation before human application. Second, the interaction between these phytochemical fractions and ketamine's therapeutic effects, such as NMDA receptor modulation in depression or analgesia, is unknown. While flavonoids and saponins attenuated inflammatory cytokine expression in the myocardium, it is possible that their antioxidant or signaling-modulating properties could interfere with or enhance ketamine's clinical efficacy in neuropsychiatric indications. Therefore, further studies assessing pharmacokinetic interactions, dose optimization, and safety in models more closely aligned with human physiology are essential before considering clinical translation. These investigations would also clarify whether *D. edulis* fractions could serve as adjunct cardioprotective agents during ketamine therapy without compromising its intended therapeutic outcomes.

CONCLUSION

This study demonstrates that ketamine induces dose-dependent myocardial inflammation, evidenced by increased IL-6 and TNF- α expression, and that the flavonoid and saponin fractions of *D. edulis* provide significant anti-inflammatory and cardioprotective effects. Flavonoids exhibited the most consistent attenuation, highlighting their potential as lead compounds for developing botanical therapeutics against cardiotoxic agents, such as ketamine. In contrast, the alkaloid fraction appeared to exacerbate inflammation, emphasizing the need for detailed characterization of individual alkaloid constituents. While these findings provide novel insights into the modulation of myocardial cytokine expression by plant-derived compounds, caution is warranted in translating rodent doses to humans, and further studies are required to assess potential interactions with ketamine's therapeutic applications, including depression and analgesia. Overall, *D. edulis* fractions, particularly flavonoids and saponins, hold promise as adjunct cardioprotective agents in drug-induced myocardial inflammation.

Inclusion criteria

Adult male Wistar rats weighing 180–220 g, in good health with normal behavior, activity, and food and water intake, were included. All animals were acclimatized to laboratory conditions for at least 7 days before the experiment.

Exclusion criteria

Rats showing signs of illness, injury, abnormal behavior, or weight outside the 180–220 g range were excluded. Animals that did not survive initial ketamine administration or exhibited severe distress requiring humane euthanasia were also excluded.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception, design, execution, data analysis, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

This study was carried out accordingly without any conflict of interest.

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