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CARDIOPROTECTIVE EFFECTS OF ANTHOCEPHALUS CADAMBA BARK EXTRACT AND PYRIDOXINE IN STZ-INDUCED DIABETIC RATS

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ABSTRACT
Background: Oxidative stress driven by hyperglycaemia and hyperlipidaemia plays a major role in diabetic cardiomyopathy. Although *Anthocephalus cadamba* bark and pyridoxine have traditional uses, their combined efficacy against diabetic cardiomyopathy remains underexplored. In this work, streptozotocin (STZ)-induced type 2 diabetic rats were used to investigate the preventive benefits of an ethanolic bark extract of *A. cadamba* given either with or without pyridoxine. **Methods:** Diabetes was induced in Wistar rats with STZ (45 mg/kg, i.p.). Animals were allocated to eight groups and treated with metformin, *A. cadamba* extract (200 or 400 mg/kg), pyridoxine (100 mg/kg), or combinations of these agents for eight weeks. Biochemical markers (CK-MB, LDH, AST), lipid profile, and oxidative stress enzymes were assessed, along with heart histopathology. **Results:** Diabetic rats showed marked elevations in CK-MB, LDH, and AST, which were significantly reduced by *A. cadamba* (200–400 mg/kg) (e.g., CK-MB, LDH, and AST decreased by approximately 25-33%). ($p < 0.001$). Combining pyridoxine with other therapies produced the strongest effect, lowering CK-MB, LDH, and AST by 30–41% ($p < 0.001$). Treatment with *A. cadamba* extract alone and in combination with pyridoxine significantly improved dyslipidaemia, with HDL rising from ~42 to 52% ($p < 0.01$-0.001) and decreasing serum-lipoproteins concentration (total Cholesterol, triglyceride, LDL, and VLDL) by ~40 to 55% ($p < 0.01$). Antioxidant defenses were also restored, with SOD, CAT, and GSH levels rising by 70–80% ($p < 0.05$–0.001). Histology confirmed reduced necrosis and fibre degeneration, most notably in the combination-treated groups. **Conclusion:** *A. cadamba* extract and pyridoxine, particularly in combination, mitigate oxidative stress, hyperlipidaemia, and cardiac injury in STZ-induced diabetic rats, suggesting therapeutic potential against diabetic cardiomyopathy.

INTRODUCTION

Diabetes mellitus (DM) is one of the most important global health concerns of the 21st century, and the prevalence of this condition is increasing at an alarming rate. In addition to being characterised by persistent hyperglycemia and metabolic changes in carbohydrates, proteins, and lipids, it is also associated with a wide range of chronic complications of diabetes [1]. Among these, cardiovascular illnesses continue to

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be the most destructive, since they contribute a disproportionate amount to the morbidity and death that are associated with diabetes. Specifically, diabetic cardiomyopathy (DCM) has been recognized as a distinct clinical entity [2]. It is characterised by structural and functional abnormalities of the myocardium, and it is different from other cardiovascular risk factors such as hypertension and coronary artery disease. As the burden of DCM continues to increase, there is an urgent need for innovative preventative and treatment techniques that are specifically suited to diabetic populations [3].

Experimental induction of diabetes through streptozotocin (STZ) provides a valuable model for investigating the underlying pathophysiology of diabetic cardiomyopathy. STZ-induced diabetes mimics several metabolic and oxidative stress disturbances observed in human disease, offering a reproducible platform for preclinical testing of cardioprotective interventions [4]. Elevated oxidative stress, resulting from both increased reactive oxygen species (ROS) production and impaired antioxidant defenses, plays a pivotal role in the progression of DCM [5]. This imbalance not only accelerates myocardial cell damage and fibrosis but also amplifies inflammatory cascades and mitochondrial dysfunction, leading to impaired contractility and eventual heart failure [6].

In recent years, the therapeutic potential of bioactive natural compounds has drawn significant attention as alternatives or adjuncts to conventional treatments. Phytochemicals, including flavonoids, alkaloids, and triterpenes, are recognized for their antioxidant, anti-inflammatory, and lipid-lowering effects [7]. *Anthocephalus cadamba*, a traditional medicinal plant, is particularly noteworthy for its diverse pharmacological activities, including antidiabetic, antimicrobial, and cardioprotective properties [8]. The bark of this plant is rich in alkaloids, including cadambine and isocadambine, compounds reported to scavenge free radicals and modulate oxidative stress pathways. Despite these promising attributes, scientific evidence supporting its role in mitigating STZ-induced diabetic cardiomyopathy remains scarce, warranting further systematic investigation [9].

In parallel, micronutrients like pyridoxine (vitamin B6) have gained recognition for their role in maintaining redox homeostasis. Beyond its classical coenzyme functions in amino acid and neurotransmitter metabolism, pyridoxine acts as a potent cardioprotective and antioxidant, neutralizing free radicals and inhibiting the formation of advanced glycation end products (AGEs) [10]. By attenuating glyco-oxidative stress, pyridoxine may protect cardiovascular tissues exposed to hyperglycemic insults. Notably, the potential synergistic action between pyridoxine and phytochemicals from *A. cadamba* has not been extensively explored, despite the strong theoretical rationale [11]. In light of this, the present study assesses the cardioprotective efficacy of Anthocephalus cadamba bark extract, administered alone or in combination with pyridoxine, in a STZ-induced type 2 diabetes mellitus model [12]. By assessing biochemical markers, oxidative stress indices, lipid profiles, and histopathological alterations, this work aims to provide mechanistic insights into how these agents may ameliorate diabetic cardiomyopathy [13]. The findings are expected to contribute to the growing body of knowledge supporting the integration of natural products and essential micronutrients for the management of diabetes-related cardiovascular complications [14].

MATERIALS AND METHODS

Plant Collection

Fresh bark of *Anthocephalus cadamba* (Roxb.) Miq., a member of the family Rubiaceae, was collected in bulk from Mathura district, Uttar Pradesh, India. The plant was taxonomically identified and authenticated by Talever Singh at the National Institute of Science Communication and Information Resources (NISCAIR), Ghaziabad, where a voucher specimen was deposited for future reference.

The bark samples were carefully selected to ensure uniformity and absence of physical damage or fungal contamination. After collection, the bark was thoroughly washed with distilled water to remove adhering dust and soil particles. The clean material was shade-dried at ambient temperature (25–28°C) for 10–15 days to preserve thermolabile phytoconstituents and to prevent decomposition. Dried bark was then coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve to ensure uniform particle size. The powdered material was stored in airtight containers, protected from light and moisture, until further extraction and phytochemical analysis.

A. cadamba bark is rich in diverse bioactive phytoconstituents, including flavonoids, triterpenes, glycosides, saponins, and indole alkaloids (e.g., cadambine, cadamine, isocadambine, and isodihydrocadambine), which have been implicated in its antioxidant, anti-inflammatory, and antidiabetic effects. Thus,

the authenticated and processed bark was selected as the plant material for subsequent extraction and evaluation in this study.

***Anthocephalus cadamba* bark extract preparation**

In a Soxhlet apparatus, 200–250 g of coarsely powdered shade-dried bark was extracted using 50% ethanol over the course of two to three hours. After vacuum-drying to yield a dark brown crystal, the extract was stored at 40°C and examined for signs of streptozotocin-induced diabetic cardiomyopathy. The dried extract was reconstituted in 1% CMC before administration, and a homogeneous suspension was administered orally to animals via a gastric cannula for subsequent assessment of cardioprotective effects in STZ-induced diabetic rats [15].

Animals

Wistar male rats, weighing between 180 and 211 grammes, were purchased from Rajiv Academy for Pharmacy in Mathura. These rats were housed in plastic cages and maintained on a 12-hour light/dark cycle. Additionally, the temperature was maintained at 25 ± 2 °C. It was made available to rats fed a standard pellet diet ad libitum. This research project was approved by the Research Ethics Committee at the Rajiv Academy of Pharmacy in Mathura, Uttar Pradesh, India, giving it the designation RAP/7537/IAEC/2023/06.

Acute toxicity

To determine whether the ethanolic bark extract of *Anthocephalus cadamba* was safe for consumption, the limit test was used. This was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guidelines for acute oral toxicity [16]. When prior evidence indicates that the drug being tested is expected to be non-toxic or have minimal toxicity, this procedure is commonly regarded as the most appropriate approach [17]. In the study, 12 healthy Wistar rats, with an equal number of males and females, were used. The animals were allowed to go without food and water for an entire night before medication administration to establish a baseline physiological state consistent throughout the experiment [18]. The rats were randomly assigned to two groups (n = 6 per group). The control group received only the vehicle (0.5% carboxymethyl cellulose [CMC] in normal saline). In comparison, the treatment group was administered the test suspension containing 4000 mg/kg of *A. cadamba* extract in a 0.5% CMC solution via oral gastric gavage. Administration was performed using a suitable oral cannula to ensure accurate dosing and minimize the risk of aspiration. Following dosing, food was withheld for three hours to avoid possible interference with absorption and to maintain consistency with OECD recommendations. Post-dosing, the animals were closely observed under standardized laboratory conditions. Continuous monitoring was carried out for the first 30 minutes, followed by half-hourly intervals during the initial 4-hour period to detect any immediate clinical or behavioral abnormalities. Special attention was given to signs of altered locomotor activity, tremors, convulsions, salivation, piloerection, lacrimation, and other indicators of neurological or systemic toxicity. Thereafter, the animals were observed periodically at regular intervals for up to 72 hours to record any delayed effects or fatalities. Mortality, morbidity, and any remarkable behavioral changes were carefully documented.

Throughout the observation period, no mortality was recorded in either sex, and no severe behavioral or motor impairments were detected, suggesting that the median lethal dose (LD_{50}) of the extract is greater than 4000 mg/kg. Minor, transient changes such as reduced locomotion or grooming were noted in a few animals but resolved spontaneously without intervention. The absence of significant toxic manifestations indicates that the extract is relatively safe under the conditions tested. These findings, consistent with the report by Adekunle et al. (2023), confirm that the ethanolic bark extract of *A. cadamba* does not produce acute toxicity at the limit dose of 4000 mg/kg and can be classified as practically non-toxic under the OECD Globally Harmonized System of Classification.

Diabetes induction

Streptozotocin (STZ), a nitrosourea derivative that preferentially damages pancreatic β-cells, was used to induce diabetes mellitus in Wistar rats for the experiment. I.P. administration of a freshly made STZ solution at a dosage of 45 mg/kg body weight was carried out in a citrate buffer with a concentration of 0.1 M and a pH of 4.5. To minimize variation in glucose response, the animals were allowed to drink water ad libitum throughout the night (12–14 hours) before the injection. Rats were given a 5% glucose solution ad libitum for the first 24 hours after the injection [19]. This was done to reduce the risk of acute hypoglycaemia mortality after β-cell necrosis. Following induction, animals were returned to a standard pellet diet and observed for 72 h, during which time their general health, activity, and food intake were closely monitored. Fasting blood

glucose (FBG) was measured from the tail vein using a glucometer (Accu-Check Active, Roche Diagnostics) at 72 h post-STZ administration. Animals exhibiting FBG > 250 mg/dL were considered diabetic and included in the study, while those with lower values were excluded [20]. Body weights and fasting glucose levels were further monitored weekly throughout the experimental period to ensure stability of the diabetic condition. This model closely mimics the insulin-deficient, hyperglycemic state observed in human diabetes and is widely used to investigate clinical manifestations, including cardiomyopathy and oxidative stress [21].

Experimental design and animal treatment

Following induction of diabetes mellitus with streptozotocin (STZ), the animals were randomized and distributed into eight experimental groups (n = 8 per group). Allocation was based on body weight to minimize inter-group variation. Animals were housed under controlled environmental conditions (12 h light/dark cycle, 25 ± 2 °C, relative humidity 50–60%), with free access to a standard pellet diet and water ad libitum throughout the study period **(Table 1**. All treatments were administered orally via an intragastric cannula once daily for eight consecutive weeks.

Table 1: Experimental design and treatment protocol of STZ-induced diabetic Wistar rats, showing groups, administered agents, doses, route, and study duration.

Group	Condition / Treatment	Dose & Route	No. of Rats	Duration
1	Normal control (no induction)	Vehicle only	8	8 weeks
2	Diabetic control	STZ 45 mg/kg, i.p.	8	8 weeks
3	Diabetic + Metformin	100 mg/kg, p.o.	8	8 weeks
4	Diabetic + *A. cadamba* extract	200 mg/kg, p.o.	8	8 weeks
5	Diabetic + *A. cadamba* extract	400 mg/kg, p.o.	8	8 weeks
6	Diabetic + Pyridoxine	100 mg/kg, p.o.	8	8 weeks
7	Diabetic + *A. cadamba* extract + Pyridoxine	200 mg/kg + 100 mg/kg, p.o.	8	8 weeks
8	Diabetic + *A. cadamba* extract + Pyridoxine	400 mg/kg + 100 mg/kg, p.o.	8	8 weeks

All animals were carefully monitored for clinical signs, body weight changes, and feed/water intake during the treatment period. At the end of eight weeks, animals were fasted overnight and anesthetized with diethyl ether for blood collection via retro-orbital puncture. Serum was separated for biochemical analysis of cardiac enzymes, lipid profiles, and oxidative stress biomarkers. Thereafter, animals were humanely sacrificed, and the hearts were excised for histopathological examination and antioxidant enzyme assays. All drugs and plant extracts were freshly prepared in 1% CMC before administration to maintain stability. Animals were weighed weekly, and doses were adjusted accordingly.

Dosing Justification

The dose of pyridoxine (100 mg/kg, p.o.) was selected based on previous experimental studies demonstrating its cardioprotective, antioxidant, and antiglycation efficacy in STZ-induced diabetic rats without producing toxicity. Earlier investigations have shown that pyridoxine in the range of 50–150 mg/kg effectively attenuates oxidative stress, improves cardiac biomarkers, and modulates metabolic dysfunction in diabetic or high-fat–diet models, with 100 mg/kg identified as an optimal therapeutic dose producing maximal antioxidant benefit while remaining well below reported toxicity thresholds (Mutavdzin Krneta et al., 2024; D'Haese et al., 2024). Therefore, the 100 mg/kg dose used in this study aligns with established preclinical literature and ensures both safety and pharmacological relevance. Throughout the study, rats were housed under controlled environmental conditions (22–25 °C, 50–60% relative humidity, and a 12 h light/dark cycle) with free access to a standard pellet diet and water ad libitum. Clinical signs, body weight, and mortality were recorded daily. At the end of the 8-week treatment, rats were fasted overnight and subjected to retro-orbital blood collection under light ether anesthesia. Serum was separated for the analysis of cardiac injury markers, oxidative stress biomarkers, and lipid profiles. Animals were then humanely sacrificed, and their hearts were excised for histopathological evaluation and antioxidant enzyme assays. This experimental design enabled a direct comparison of untreated diabetic controls, standard antidiabetic therapy (metformin), individual interventions (plant extract or pyridoxine), and their combined administration, thereby allowing assessment of both independent and synergistic cardioprotective effects.

Serum Cardiac Markers

At the end of the treatment period, blood samples were collected from the retro-orbital plexus of anesthetized rats using diethyl ether. Serum was immediately separated by centrifugation and analyzed for key cardiac marker enzymes, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase-MB isoenzyme (CK-MB) using commercially available diagnostic reagent kits on an automated biochemical analyzer [22]. These biomarkers are clinically and experimentally recognized indicators of cardiac injury, as they are released into the circulation following cardiomyocyte damage or necrosis.

- **AST**: Although primarily a hepatic enzyme, elevated AST activity in serum reflects leakage from damaged myocardial tissue, and is frequently employed in experimental cardiotoxicity and diabetic cardiomyopathy studies [23].
- **LDH**: A cytoplasmic enzyme released during loss of membrane integrity. Increased serum LDH activity indicates cellular necrosis and impaired myocardial metabolism [24].
- **CK-MB**: A cardiac-specific isoform of creatine kinase and the most reliable biomarker for myocardial damage. Its rise in circulation strongly correlates with myocardial infarction and diabetic cardiomyopathy [25].

In this study, the combined evaluation of CK-MB, LDH, and AST provided a comprehensive assessment of cardiac injury and functional impairment in STZ-induced diabetic rats. Elevated levels in diabetic control animals confirmed myocardial injury, while significant reductions in treatment groups indicated cardioprotective effects of *Anthocephalus cadamba* bark extract, pyridoxine, and their combination [26].

Biochemical estimation of markers of oxidative stress

At the end of the treatment period, the animals were humanely euthanized following institutional ethical guidelines, and their hearts were immediately excised for assessment of endogenous antioxidant enzymes. Each heart was carefully rinsed with ice-cold saline to remove excess blood and then minced into small fragments using sterile scissors [27]. The tissue fragments were gently blotted on filter paper to remove residual fluid and subsequently immersed in a chilled 0.25 M sucrose solution to preserve enzymatic activity and stabilize subcellular structures during homogenization [28]. To prevent protein denaturation, the minced cardiac tissue was homogenised in a 10% (w/v) ice-cold Tris-HCl buffer (10 mM, pH 7.4) using a Remi tissue homogeniser (Remi Motors, India). The cold temperatures were maintained throughout the process. Using an Eppendorf 5810-R high-speed chilled centrifuge, the homogenates were centrifuged at 0°C for 15 min at 12,000 rpm [29]. For the purpose of determining the levels of major antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), which are essential markers of oxidative stress and cellular defence against free radical damage, the clear supernatant that was produced was collected and taken into consideration [30].

The non-enzymatic antioxidant GSH was quantified using the colorimetric method described by C. J. Van Noorden et al. [31]. This method is based on the interaction of free sulfhydryl groups with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), resulting in the formation of a yellow-coloured chromophore that can be measured at 412 nm. Based on the enzyme's capacity to block the reduction of nitro blue tetrazolium (NBT) by superoxide anions formed in the phenazine methosulfate-NADH system, the activity of superoxide dismutase (SOD) was determined using the technique developed by C J Van Noorden. According to this approach, SOD activity was determined. A wavelength of 560 nm was used to measure absorbance. To evaluate catalase (CAT) activity, the technique developed by Mahmoud Hadwanb et al. [32] was used.

This approach examines the rate of decomposition of hydrogen peroxide (H_2O_2). The reduction in hydrogen peroxide absorbance was measured spectrophotometrically at 240 nm [33]. The decomposition of hydrogen peroxide was measured in terms of μmoles per minute per milligram of protein. These biochemical assays provided quantitative measures of oxidative stress levels and antioxidant defense capacity in cardiac tissues. Alterations in SOD, CAT, and GSH activities reflect the degree of oxidative insult induced by hyperglycemia and the effectiveness of therapeutic interventions in restoring redox homeostasis [34].

Assessment of serum lipids and lipoproteins

The lipid profile of serum samples was assessed to evaluate the effects of treatments on dyslipidaemia associated with streptozotocin (STZ)-induced diabetes. Serum was separated by centrifugation of collected blood and stored under appropriate conditions until biochemical analysis. Commercially available

diagnostic reagent kits (manufacturer-certified) were employed to determine serum concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) [35]. These assays were performed using an automated biochemical analyzer, following the manufacturer's instructions to ensure accuracy and reproducibility. Low-density lipoprotein cholesterol (LDL-C) was not directly measured; instead, it was calculated using Friedewald's formula, a widely accepted method in both clinical and experimental research [36].

Low-density lipoprotein cholesterol (LDL-C) was calculated using **Friedewald's formula** [37]:
(LDL-C = Total Cholesterol - HDL-C - (Triglycerides/5))

$$\text{LDL} - \text{C} = \text{Total Cholesterol} - \text{HDL} - \text{C} - \left(\frac{\text{Triglycerides}}{5}\right)$$

The standard equation estimated very-low-density lipoprotein cholesterol (VLDL-C)

$$\text{VLDL-C (mg/dL)} = \frac{\text{TG}}{5}$$

This comprehensive lipid profile assessment enabled evaluation of both atherogenic lipoproteins (LDL-C, VLDL-C, TG) and the protective fraction (HDL-C), thereby providing an overall picture of lipid metabolism in diabetic and treated animals. Alterations in these parameters are critical indicators of diabetes-induced dyslipidaemia and serve as important biomarkers for the progression of cardiovascular complications such as cardiomyopathy and atherosclerosis. The calculated indices also permitted comparison between treated groups, diabetic controls, and normal controls to assess the hypolipidaemic and cardioprotective potential of the interventions [38].

Histopathology of Heart

The animals were sacrificed at the conclusion of the experiment, and the hearts that were removed were meticulously washed in ice-cold saline to eliminate any blood that was left behind. To ensure full fixation and prevent autolysis or microbial degradation, the organs were immediately submerged in 10% neutral-buffered formalin for at least 24 hours. To facilitate subsequent staining and microscopy, this fixation method preserved tissue architecture and protein integrity [39].

After being fixed, cardiac tissues were processed according to standard histological procedures. These procedures included dehydration via a series of graded alcohols, clarifying in xylene, and embedding in paraffin wax to permit microtomy. To prevent separation during staining, thin slices approximately 3–5 µm thick were obtained using a rotary microtome. These sections were then mounted on clean glass slides that had been pre-coated with adhesive [40]. Sections were stained with haematoxylin and eosin (H&E) for general histological examination. To demonstrate nuclear morphology and chromatin condensation, haematoxylin selectively stained the nuclei dark blue to purple. On the other hand, eosin counterstained the cytoplasm and extracellular matrix in a variety of pink tones. Using this contrasted colouration, it was possible to clearly visualise cellular and subcellular structures, fibre orientation, and pathological abnormalities such as necrosis, inflammation, and fibrosis [41]. The stained slides were then air-dried, mounted with coverslips using distyrene phthalate xylene (DPX) as a permanent mounting medium, and allowed to cure. This ensured long-term preservation of the specimens without distortion or fading of the staining [42]. Prepared sections were subsequently examined under a light microscope at various magnifications, and representative photomicrographs were captured using a life science microscopy cell imaging system [43]. These digital images were analyzed to assess myocardial architecture, the presence of necrosis, inflammatory cell infiltration, interstitial edema, and the degree of fiber disorganization. Such histopathological evaluations provided qualitative confirmation of the biochemical and antioxidant data, offering insight into the protective effects of Anthocephalus cadamba extract and pyridoxine against STZ-induced myocardial injury [44].

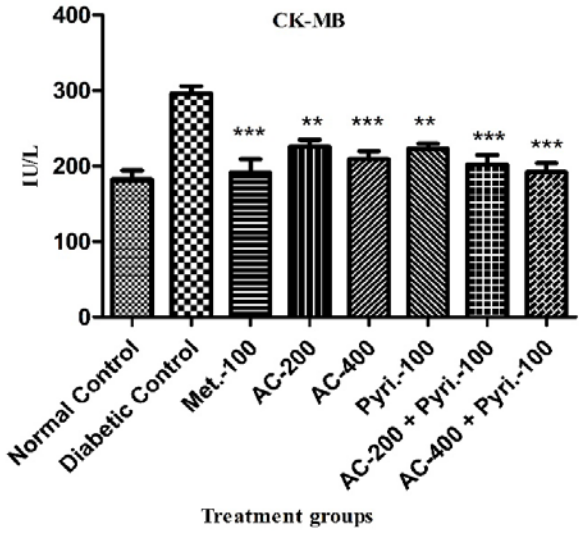
Statistical Analysis

Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Data are presented as Mean ± SEM. P values less than 0.05 were considered statistically significant

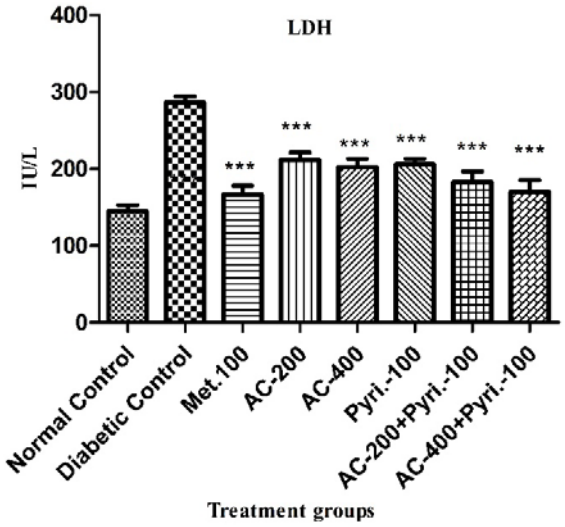
RESULT

Effects of *A. cadamba* Extract on Cardiac Injury Markers

STZ-induced diabetic rats showed significant elevations in serum CK-MB, LDH, and AST compared to normal controls, confirming myocardial injury. Treatment with *A. cadamba* extract (200 and 400 mg/kg) significantly reduced these markers in a dose-dependent manner ($p < 0.05$). Pyridoxine (100 mg/kg) also lowered enzyme levels, whereas *A. cadamba* with pyridoxine produced the greatest reduction ($p < 0.001$), demonstrating superior cardioprotection compared with individual treatments (Figure 1).



(A)



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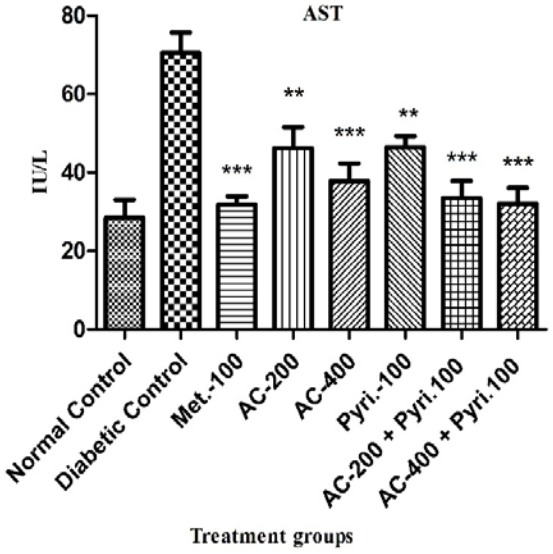


(C)

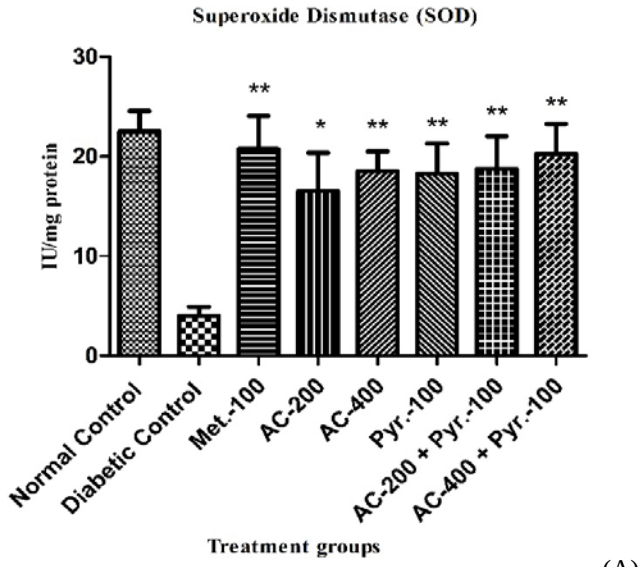
Figure 1: Serum levels of CK-MB, LDH, and AST. Treatment groups were compared with the diabetic control group. Values are expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Dunnett's test.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Effects of *A. cadamba* Extract on Cardiac Oxidative Stress

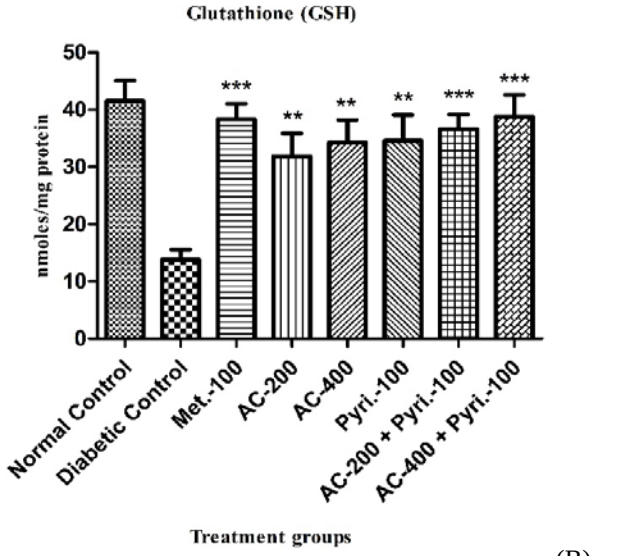
Oxidative stress is a critical contributor to the pathogenesis of diabetic cardiomyopathy, arising from an imbalance between excessive reactive oxygen species (ROS) generation and impaired endogenous antioxidant defense mechanisms. Persistent hyperglycemia in streptozotocin (STZ)-induced diabetes enhances oxidative load, thereby aggravating myocardial damage and functional decline. The antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)—play pivotal roles in neutralizing ROS and maintaining redox homeostasis. Hence, assessing these markers provides insight into the protective efficacy of therapeutic interventions against oxidative injury in cardiac tissue. In the present study, the hearts of diabetic control rats exhibited significantly altered levels of SOD, CAT, and GSH ($p < 0.05$) compared with normal control rats, indicating oxidative imbalance and impaired cardiac defense mechanisms (**Figure 2**). Treatment with pyridoxine (100 mg/kg) and ethanolic bark extract of *A. cadamba* (200 and 400 mg/kg) for eight weeks markedly improved the antioxidant profile. Specifically, a significant ($p < 0.05$) restoration of SOD, CAT, and GSH activities was observed in treated groups compared to untreated diabetic controls, suggesting attenuation of oxidative stress.

Moreover, combination therapy with A. cadamba bark extract and pyridoxine produced a synergistic effect compared with either agent alone, highlighting their potential to reinforce antioxidant defenses. Co-administration not only improved enzymatic activity but also conferred better protection against ROS-induced lipid peroxidation and mitochondrial dysfunction in cardiac cells.

These findings support the hypothesis that *A. cadamba* bark extract, rich in flavonoids and alkaloids with free radical scavenging properties, together with pyridoxine, a potent micronutrient antioxidant, can effectively restore redox balance and limit oxidative tissue injury. Thus, the combination therapy of *A. cadamba* bark extract with pyridoxine offers promising cardioprotective benefits in the management of diabetic cardiomyopathy by mitigating oxidative stress-induced myocardial damage



(A)



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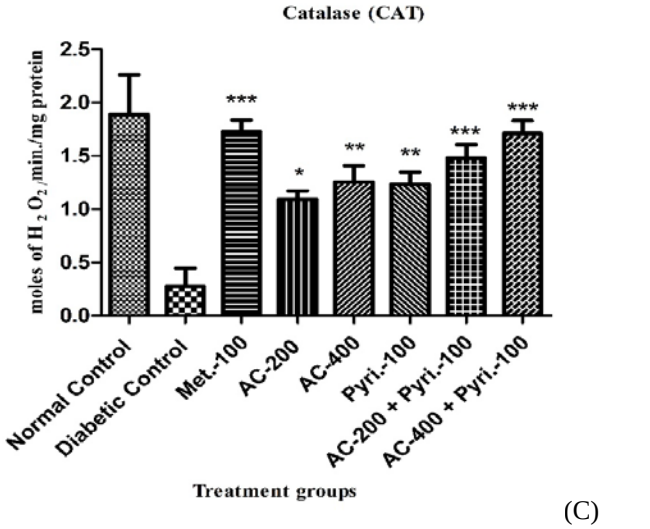

(C)

Figure 2: Cardiac antioxidant enzyme activities. Levels of (A) superoxide dismutase (SOD), (B) reduced glutathione (GSH), and (C) catalase (CAT) in heart tissue. Treatment groups were compared with the diabetic control group. Values are expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Dunnett's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Effects of *A. cadamba* Extract on Hyperlipidaemia

Hyperlipidaemia is one of the most common metabolic disturbances associated with diabetes mellitus, and it plays a pivotal role in the development of secondary cardiovascular complications such as cardiomyopathies, coronary artery disease, and heart failure. In the present study, a marked alteration in the lipid profile was observed in diabetic control rats when compared with normal controls. Specifically, serum concentrations of total cholesterol (TC), triglycerides (TG), very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) were significantly elevated ($p < 0.05$). In contrast, high-density lipoprotein (HDL) levels were markedly reduced. This pattern of dyslipidaemia confirms the atherogenic profile typically associated with STZ-induced diabetes and corroborates earlier findings that persistent hyperglycaemia enhances hepatic lipogenesis while impairing lipoprotein metabolism.

Administration of ethanolic bark extract of *A. cadamba* at both 200 mg/kg and 400 mg/kg for eight weeks produced a significant improvement in lipid metabolism. Treated groups exhibited considerable reductions in TC, TG, VLDL, and LDL levels, alongside a significant increase in HDL concentrations, compared with untreated diabetic controls. These changes indicate that the extract not only reduced circulating lipids but also promoted a more favorable lipoprotein balance, thereby potentially mitigating the risk of atherosclerosis and related cardiovascular complications. The lipid-lowering effects may be attributed to the bioactive alkaloids and flavonoids present in *A. cadamba*, which are known to modulate hepatic cholesterol biosynthesis, enhance LDL clearance, and improve reverse cholesterol transport.

Treatment with pyridoxine (100 mg/kg) alone produced similar improvements in lipid parameters, supporting its established role as an antioxidant and regulator of lipid metabolism. More importantly, when the bark extract was co-administered with pyridoxine, a synergistic effect was observed. The combined therapy produced the most pronounced reductions in TC, TG, VLDL, and LDL levels while substantially elevating HDL

concentrations compared to individual treatments. This suggests that pyridoxine may potentiate the hypolipidemic activity of *A. cadamba* through complementary mechanisms, including inhibition of lipid peroxidation and prevention of advanced glycation end-product (AGE) formation. Taken together, these findings demonstrate that ethanolic bark extract of *A. cadamba*, either alone or in combination with pyridoxine, exerts significant hypolipidaemic activity in STZ-induced diabetic rats. The correction of lipid imbalance observed in this study indicates a protective role against diabetes-related dyslipidaemia and associated cardiovascular complications (Table 2). Further supports these observations by highlighting statistically significant improvements across all measured lipid parameters in the treated groups compared with the diabetic controls.

Table 2: Serum lipid profile in STZ-induced diabetic rats. Treatment groups were compared with the diabetic control group. Values are expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Dunnett's test. * p < 0.05; ** p < 0.01; *** p < 0.001.

SNo.	Treatment Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1.	Normal Control	104.4 ± 4.46	84.40 ± 4.95	61.40 ± 4.68	19.86 ± 2.50	17.00 ± 2.60
2.	Diabetic Control (STZ 45 mg/kg)	190.3 ± 6.00	180.0 ± 9.187	24.76± 2.52	56.80 ± 5.42	51.20 ± 3.26
3.	Standard Metformin (100 mg/kg kg)	107.1 ± 3.317***	88.52 ± 3.51***	55.52 ± 2.28***	22.40 ± 4.06***	23.00 ± 3.03***
4.	Ethanol extract *of A. cadamba* (200 mg/kg)	124.2 ± 4.24***	103.0 ± 6.96***	42.32 ± 2.19*	35.20 ± 4.17*	29.38 ± 4.79**
5.	Ethanol extract of *A. cadamba* (400 mg/kg)	116.3 ± 2.83***	97.56 ± 5.57***	45.56 ± 4.11*	31.96 ± 4.33**	27.40 ± 4.77**
6.	Pyridoxine (100 mg/kg)	118.6 ± 2.60***	99.08 ± 5.86***	41.32 ± 2.40**	30.80 ± 4.12**	26. 82 ± 4.59**
7.	Ethanol extract of *A. cadamba* (200 mg/kg) + Pyridoxine (100 mg/kg)	113.6 ± 5.20***	94.28 ± 4.65***	49.68 ± 4.98**	27.20 ± 5.78***	23.60 ± 5.19***
8.	Ethanol extract of *A. cadamba* (400 mg/kg) + Pyridoxine (100 mg/kg)	110.2± 3.22***	91.56 ± 6.04***	50.00 ± 6.22**	25.44 ± 4.56***	22.58 ± 3.15***

Treatment groups were compared with the diabetic control group. Values are expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Dunnett's test. * p < 0.05, ** p < 0.01, *** p < 0.001.

Effects of *A. cadamba* Extract on Cardiac Histopathology

Hematoxylin and eosin (H&E) staining was performed on cardiac sections from all experimental groups to evaluate structural alterations, including myocardial shrinkage, interstitial inflammation, and necrosis. In the normal control rats, the myocardium exhibited preserved architecture with intact fibers, absence of inflammatory infiltrates, and no evidence of necrosis or degeneration. In contrast, diabetic control rats exhibited marked pathological changes, including pronounced cellular necrosis, interstitial inflammatory infiltration, myocardial atrophy, and fiber disorganization, confirming the deleterious effects of hyperglycemia on cardiac tissue integrity. Treatment with ethanolic bark extract of *Anthocephalus cadamba* alone resulted in partial attenuation of myocardial damage, with moderate improvements in fiber arrangement and a reduction in necrotic lesions compared with diabetic controls. Pyridoxine supplementation also conferred cardioprotection, as evidenced by decreased inflammatory cell infiltration and reduced myocardial degeneration.

Notably, co-administration of *A. cadamba* extract with pyridoxine produced the most pronounced protective effects. Histological sections from these groups revealed substantially preserved myocardial fibers, reduced interstitial inflammation, and minimal necrosis, indicating a synergistic benefit of the combined therapy. Collectively, the histopathological observations corroborate the biochemical and antioxidant findings, suggesting that the combination of *A. cadamba* bark extract and pyridoxine exerts superior cardioprotective effects against streptozotocin-induced diabetic cardiomyopathy compared with either treatment alone (Figure 3).

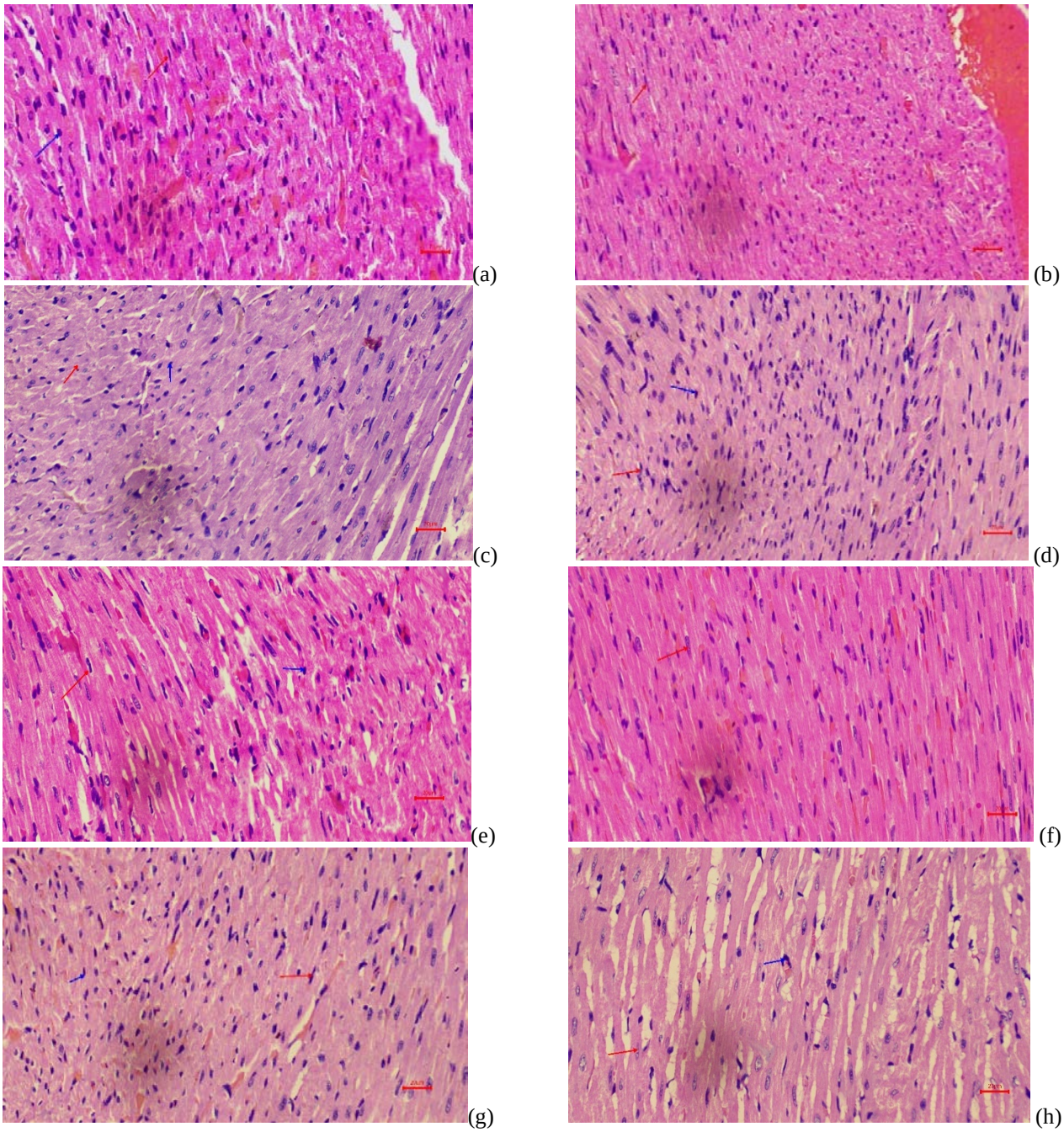

Figure 3: Histopathological examination of cardiac tissue (H&E staining, 400× magnification).
(A) Normal control; (B) Diabetic control; (C) Metformin (100 mg/kg); (D) *A. cadamba* extract (200 mg/kg); (E) *A. cadamba* extract (400 mg/kg); (F) Pyridoxine (100 mg/kg); (G) *A. cadamba* (200 mg/kg) + Pyridoxine (100 mg/kg); (H) *A. cadamba* (400 mg/kg) + Pyridoxine (100 mg/kg)

DISCUSSION

Diabetes is a chronic metabolic illness characterized by persistently elevated blood glucose levels. Among other diabetes-related problems, researchers have shown that those with consistently elevated blood sugar levels are more susceptible to diabetic retinopathy and cardiomyopathy. Due to a condition called cardiomyopathy, heart failure is common among diabetics. The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which result in glucotoxicity due to an imbalance, and STZ-induced persistent hyperglycemia are the main causes of diabetes and its consequences. A better understanding of molecular pharmacology is necessary to improve prediction and therapeutic approaches for cardiovascular failure associated with diabetes. Diabetic cardiomyopathy, or DCM, is infamously challenging to treat and control. Due to compromised

myocardial function, recent research shows that people with cardiomyopathy have higher levels of cardiac markers such as LDH, AST, and CK-MB. This study showed that long-term use of pyridoxine and ethanol bark extract from *A. cadamba* reduced elevated cardiac markers to within the reference range.

Compared with healthy individuals, individuals with diabetes have higher levels of reactive oxygen species (ROS). According to several studies, the generation of reactive oxygen species (ROS) in the heart muscle is the root cause of diabetic cardiomyopathy and exacerbates the condition. Recent research indicates that the formation of reactive oxygen species (ROS) and disruption of the ROS pathway are both associated with superoxide-induced damage and dysfunction. Antioxidants may protect the diabetic heart from oxidative damage. Previous results were reinforced by the fact that the control diabetic rats in our investigation had higher levels of glutathione, superoxide dismutase, and catalase. In the cardiac tissue of rats that were administered pyridoxine in conjunction with an ethanol extract of *A. cadamba*, the levels of GSH, SOD, and CAT were found to be much greater. The findings of this research showed this.

Higher blood glucose levels, lipid accumulation in the heart, and increased indicators of myocardial injury have all been seen in rats with STZ-induced diabetic cardiomyopathy. Multiple aberrant pathways drive the development of cardiac disease. It is thought that the biochemical, structural, and functional abnormalities seen in diabetic hearts are caused by hyperglycemia and the accumulation of lipids in the heart. Due to changes in blood lipid balance, people with diabetes are more vulnerable to myocardial infarction, coronary insufficiency, and early atherosclerosis. Rats with diabetes showed a greater ability to absorb fatty acids from the breakdown of adipose tissue in the liver, produced more triglycerides in the liver, and produced more VLDL particles. Consequently, the plasma triglyceride levels increased. These elements contribute to increased blood total triglyceride levels. A raised HDL level may indicate that the extract has a substantial role in protecting against cardiovascular disease (CHD). Low HDL levels are associated with an increased risk of coronary heart disease. High HDL levels, on the other hand, might suggest that pyridoxine and *A. cadamba's* ethanol bark extract could help prevent coronary heart disease (CHD). The risk of atherosclerosis and other serious cardiovascular diseases is strongly correlated with blood LDL cholesterol levels. According to the study, pyridoxine and *A. cadamba* ethanol bark extract significantly affected blood cholesterol and lipoprotein levels. By lowering cholesterol, triglycerides, VLDL, and LDL, and raising HDL, for example, they may help prevent cardiovascular disease. The histological study showed that pyridoxine and the ethanol bark extract of A. cadamba may promote the healing of damaged heart tissue. This further supports the hypothesis that pyridoxine and ethanol bark extracts of *A. cadamba* may help prevent diabetic cardiomyopathy. Other chemical tests conducted in this study may be responsible for this.

CONCLUSION

This study demonstrates that streptozotocin-induced diabetes in rats leads to substantial oxidative stress, dyslipidemia, and myocardial injury, hallmarks of diabetic cardiomyopathy. Treatment with pyridoxine and the ethanolic bark extract of *A. cadamba*, whether administered separately or in combination, markedly restored antioxidant enzyme activity, improved lipid profiles, and mitigated cardiac histopathological changes. The combined therapy produced the most pronounced cardioprotective effects, suggesting a synergistic effect between the phytoconstituents of *A. cadamba* and the antioxidant properties of pyridoxine.

Despite these promising findings, the study has certain limitations. Because this is an animal-based model, the results may not fully generalize to human diabetic cardiomyopathy, and the precise molecular pathways underlying the observed effects were not investigated. Additionally, the study relied on biochemical and histological assessments but did not examine long-term functional cardiac outcomes.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Talever Singh conceptualized and designed the study, conducted the experimental work, performed data acquisition and analysis, and drafted the manuscript. Saravanan K provided supervision throughout the research process, contributed to the study design and data interpretation, and critically revised the manuscript for important intellectual content. Both authors read and approved the final version of the manuscript.

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