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AMMANNIA BACCIFERA CONFERS CARDIOPROTECTION AGAINST ISOPROTERENOL-INDUCED TOXICITY VIA NRF2/HO-1 PATHWAY ACTIVATION

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ABSTRACT
Myocardial infarction remains a serious global health issue; thus, innovative therapeutic strategies are warranted. This study assessed whether isoproterenol-induced cardiac damage in Wistar rats was mitigated by ethanolic extract of *Ammannia baccifera* (EAB). *In vitro* and *in vivo* studies were conducted to evaluate the cardioprotective properties of EAB, and LC-MS was used to identify its bioactive constituents. Experimental rats were used to evaluate the effects of EAB (50 and 100 mg/kg) on cardiac biomarkers, lipid profiles, oxidative stress indicators, inflammatory cytokines, the Nrf2/Keap1/HO-1 pathway, and cardiac histology of the heart. LC-MS analysis revealed the presence of bioactive phytochemicals, including phenolic acids, flavonoids, coumarins, terpenoids, and sesquiterpenoids. (-)-Epigallocatechin gallate, curcumin, oxyresveratrol, and eugenol sulfate, known antioxidants that activate the Nrf2/HO-1 pathway, were identified. EAB markedly decreased the levels of cardiac biomarkers, such as cardiac troponin T, lactate dehydrogenase, and creatine kinase-MB. EAB also reduced malondialdehyde levels and increased antioxidant enzyme activity in the heart. EAB increased the levels of the anti-inflammatory interleukin-10 and decreased the levels of inflammatory mediators TNF-α, IL-1β, IL-6, NF-κB, and C-reactive protein. Gene expression analysis revealed that EAB restored redox homeostasis by activating the Nrf2/HO-1 antioxidant axis and suppressing Keap1 expression in a dose-dependent manner. The preservation of cardiac architecture in the EAB-treated groups was confirmed by histopathological investigation. By reducing oxidative stress, inflammation, reactivating Nrf2 signalling, and minimizing myocardial damage in ISO-induced MI in Wistar rats, these results support the development of EAB as a natural cardioprotective agent and provide a rationale for additional research.

KEYWORDS: Myocardial infarction, *Ammannia baccifera*, Isoproterenol, Oxidative stress, Inflammation.

INTRODUCTION
Cardiovascular disease (CVD) is a major cause of early mortality and increasing healthcare costs worldwide. In 2008, approximately 17 million people died globally from CVD, and this number is expected to increase to 23.3 million by 2030. The Global Burden of Disease study group reported that in 2015, there were approximately 422 million cases of CVD worldwide, with South Asia contributing the most(Kalra et al., 2023). Compared to the global situation, the impact of cardiovascular disease in India is significantly greater. For example, the age-standardized mortality rate from CVD in India was 282 deaths per 100,000 people (ranging from 264 to 293), which is higher than the global rate of 233 deaths per 100,000 (ranging from 229 to 236)(Roth et al., 2020). Myocardial infarction (MI) is a potentially fatal illness among CVDs because of its late detection and abrupt or sudden onset of symptoms. At present, India bears the greatest burden of MI and acute coronary syndrome, representing a 138% increase from 1990. MI develops as a result of an acute reduction in coronary blood flow, causing ischemic damage to the cardiac tissue. This acute event can lead to persistent impairments in cardiac function and overall health, frequently leading to complications such as heart failure, arrhythmias, and diminished quality of life (Jan et al., 2024).
In the search for novel therapeutic agents, natural products have long been regarded as essential components because of their varied bioactive components and safety profiles. Among these, the medicinal plant *Ammannia baccifera* has ethnopharmacological relevance as a promising candidate for cardioprotective interventions. *Ammannia baccifera* is an annual aquatic/semi-aquatic herb belonging to the Lythraceae family, commonly grown in tropical and subtropical areas of Asia and Africa, including India(Swilam et al., 2022). The plant possesses a broad spectrum of medicinal, pharmacological, and therapeutic properties and is traditionally employed in Ayurvedic, Unani, and Siddha systems of medicine(Ali Esmail Al-Snafi, 2013). The traditional use of *Ammannia baccifera* involves the use of various parts of the plant to treat various diseases. It is traditionally used as an appetizer and stomachic and to treat diseases such as biliousness, dyspepsia, constipation, and flatulence (Upadhyay, 2019). It is also a strong blood purifier among the tribes and is used in veterinary medicine to suppress sexual libido in animals(Dhanapal et al., 2006). Modern chemical profiling techniques, such as UHPLC-QTOF-MS, have demonstrated that *A. baccifera* is rich in flavonoids, phenolics, terpenoids, and sterols. Recent study employed UHPLC-QTOF-MS and isolated several major constituents from ethanolic extract of *A. baccifera* such as chlorogenic acid, quercetin, protocatechuic acid, lamioside, crocetin, and khayasin C(Goodla et al., 2019). Other

studies have isolated compounds such as betulinic acid, daucosterol, ellagic acid, n-hentriacontane, lupeol, and triacontane-1,30-diol, further establishing the phytochemical richness of this plant (Swilam et al., 2022). *A. baccifera* has been reported to have a wide range of therapeutic and pharmacological properties, including antidiabetic (Swilam et al., 2022), hepatoprotective(Goodla et al., 2019), antioxidant, analgesic, anti-inflammatory(Loganayaki et al., 2012), antitumor(Loganayaki & Manian, 2012), and antisteroidogenic activities (Dhanapal et al., 2006). Most reports on the beneficial effects of *A. baccifera* extract have shown that its secondary metabolites, which are phenolic in nature, are responsible for its array of biological activities. Given its wide spectrum of traditional uses, phytochemical richness, and established safety profile, *Ammannia baccifera* is a promising candidate for evaluating cardioprotective potential in experimental models of myocardial injury. It has been pharmacologically reported to have antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic effects that are mechanistically relevant to myocardial protection.

Considering these features and advantages of Ammannia *baccifera*, the present study aimed to evaluate the cardioprotective activity of ethanolic *Ammannia baccifera* extract (EAB) in a rat model of isoproterenol (ISO)-induced myocardial infarction (MI), a reliable experimental model characterized by oxidative stress, lipid peroxidation, inflammatory infiltration, and necrosis of the myocardium. In addition, the underlying mechanism of action was explored by observing the modulation of the Keap1/Nrf2/HO-1 pathway, a key pathway which aids in the restoration of redox homeostasis and inhibition of oxidative cardiac tissue damage. Furthermore, LC-MS analysis was conducted to characterize the bioactive constituents of EAB.

MATERIAL AND METHODS

Plant collection and extraction

The entire *Ammannia baccifera* plant was collected in January 2024 from a local area and authenticated by a botanist with voucher number 0889. The herbarium was deposited at the Department of Botany, Sri Venkateswara University, Tirupati. The dried plant material was ground and extracted by maceration with 90% alcohol at a 1:4 ratio. The solvent was removed using a rotary evaporator at decreased pressure and 60°C, yielding a dark green ethanolic extract. The extract was stored at 4°C until required. Isoproterenol (CAS number: 5984-95-2) was purchased from Sigma–Aldrich. All chemicals used in this study were of analytical grade and purchased locally.

Total poly phenols and total flavonoids content estimation

The total polyphenol content of EAB was evaluated using the Folin–Ciocalteu technique, and the results were reported as milligrams of gallic acid equivalents per gram of extract. The extract stock solution (1 mg/mL) was combined with Folin–Ciocalteu's reagent, and a 20% (w/v) sodium carbonate solution was added. The absorbance was measured at 765 nm after 30 min of incubation. Similarly, the total flavonoid content was determined using the aluminum chloride colorimetric method, which involved adding 5% sodium nitrite and 0.3 mL of 10% aluminum chloride to EAB (1 mg/mL), incubating for 10 min, and adding 1 M sodium hydroxide. The absorbance of the pink chromogen was measured at 510 nm, and the values were expressed as milligrams of rutin equivalent per gram of extract(Senthil et al., 2015).

Liquid Chromatography- Mass Spectrometry (LC-MS) analysis of EAB

The molecular masses of the compounds in the EAB were tentatively identified using LC-MS (Agilent LC-q-TOF) equipped with an electrospray ionization (ESI) source. Liquid chromatography separation was performed by injecting 10 µL of the sample into a C_{18} column (ACQUITY UPLC HSST3 [100 mm × 2.1 mm, 1.8 µm]) and maintained at 40°C, with a 20-min run time using a graded solvent system consisting of methanol and water. Following the detection of the compounds through the flow cell of the diode array detector, the column EAB at was channelled to an electrospray interface-equipped Q-TOF HRMS. The ESI Source was operated in positive ion mode, scanning a mass range of 50-2000 Da at a rate of 1.014 s. Mass detection was performed under the following specific conditions: gas temperature of 30°C gas temperature, 10-12 Arb/min gas flow, nebulizer pressure of 40 psi, 3500 VCap, 175 fragmentor, 65.0 skimmer 1, and 20 Quadrupole Peak Width. The resulting mass and peak area data were cross-referenced using an in-house database and SciFinder for identification.

In vitro studies

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of EAB was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Briefly, 50 µL of freshly prepared 0.1 mM DPPH solution in methanol was mixed with varying concentrations of EAB and incubated for 30 min in the dark at room temperature. Ascorbic acid was used as a reference standard antioxidant. A methanolic DPPH solution without any antioxidant was used as the control, and methanol was used as the blank. After incubation, the absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu UV-2401PC, Japan). The percentage of radical scavenging activity was calculated relative to that of the control, and the half-maximal inhibitory concentration (IC50) was determined from the dose–response curve. All measurements were performed in triplicate(Nelson et al., 2020).

Cell culture

H9C2 rat cardiomyoblast cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL of penicillin, and 100 µg/mL of streptomycin. Cells were cultured at 37 °C in a humidified atmosphere of 5% CO2. After the confluence reached approximately 80–90 %, the cells were detached using 0.05% trypsin–EDTA and centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and

the cell pellet was resuspended in a freshly prepared RPMI medium. Cell viability was assessed using the trypan blue exclusion method, and a single-cell suspension was prepared at a density of 5.0×10^5 cells/mL for subsequent experiments.

MTT cell viability assay

Cell viability was assessed using the MTT assay. H9C2 cells were seeded into 96-well culture plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h. The cells were then exposed to increasing concentrations of EAB (0, 5, 10, 20, 40, and 100 µg/mL) for the specified incubation period. Following treatment, 20µL of MTT solution was added to each well, and the plates were incubated for 4 h at 37 °C to allow for the formation of formazan crystals. The culture medium was then carefully removed, and 150 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals (Nelson et al. 2020). Absorbance was measured at 570 nm using a SpectraMax i3X microplate reader (Molecular Devices, USA). Cell viability was expressed as a percentage relative to untreated control cells, and the IC_{50} value was calculated from the dose–response curve(Pullaiah et al., 2021).

***In vivo* Experimentation**

Animals

Wistar albino rats of both sexes were kept in polycarbonate cages with bedding made of autoclaved corncobs at 25 ± 2°C and relative humidity of 60–70 %. They had unrestricted access to pellet feed and RO water and were kept under a 12-h light/dark cycle. Before the experiment, the rats were acclimatized to the laboratory conditions for 7 days. The Animals Research Reporting In Vivo Experiments (ARRIVE) guidelines were adhered to in this study, and the experimental design and implementation strictly followed the ethical criteria established by the Committee for Control and Supervision of Experiments on Animals (CCSEA) and the Institutional Animal Ethical Committee (1423/PO/Re/S/11/CCSEA) of P. Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Acute toxicity study of EAB

An acute oral toxicity study was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guideline 423 with minor modifications. Twelve healthy female Wistar albino rats were used in this study and randomly allocated into two groups (n = 6 per group). The control group received 0.5% carboxymethyl cellulose (CMC) at a dose of 10 mL/kg body weight, whereas the treatment group received EAB at a dose of 2000 mg/kg body weight suspended in 0.5% CMC by oral gavage. This study was conducted in two sequential stages. Animals were closely observed individually for clinical signs of toxicity and behavioural changes during the first 24 h following administration, with particular attention given during the initial 4 h post dosing. Thereafter, the animals were monitored daily for a total period of 14 days for signs of morbidity and mortality, and body weight was recorded periodically to assess any treatment-related changes. At the end of the 14-day observation period, all surviving animals were humanely euthanized, and a comprehensive gross necropsy was performed to evaluate macroscopic abnormalities in major organs and tissues.

ISO induced myocardial infarction in experimental rats

In an acute toxicity study, an ethanolic extract of *Ammannia baccifera* was found to be safe at doses up to 2000 mg/kg body weight in rats. Previous 28 days repeated-dose toxicity studies on EAB at four different doses (50, 100, 250, and 500 mg/kg body weight) in rats also revealed no signs of toxicity(Lavanya et al., 2010). Hence, we chose 50 and 100 mg/kg doses of EAB in the present study to investigate its cardioprotective activity.

Twenty-four Wistar albino rats (weighing 150–200 g and of either sex; 6–8 weeks old) were randomly divided into four groups of six animals each. The experimental animals in group 1 received the vehicle (0.5% CMC) and served as the normal control, whereas those in group 2 were administered ISO (85 mg/kg, s. c.) to induce myocardial toxicity for the last two consecutive days of the study and served as disease controls. Groups 3 and 4 were administered EAB at low (50 mg/kg, p.o.) and high (100 mg/kg, p.o.) dosages, respectively, for 28 days, with ISO (85 mg/kg, s. c.) on the last two days of the study.

Cardiac and Inflammatory biomarkers

After the experiment, the retro-orbital plexus was punctured to obtain blood samples under isoflurane anesthesia. The serum was then separated from the blood by centrifuging it for 10 min at 3000 rpm and was then stored for further experimental work at -4°C. Commercial diagnostics and an automated biochemistry analyzer (ERBA EM 360) were used to quantitatively estimate cardiac biomarkers, including troponin (CTnT), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH). ELISA kits (Abclonal, USA) were used to quantify inflammatory markers (IL-1, IL-6, IL-10, TNF-α, CRP, and NF-κB) according to the manufacturer's instructions.

Assessment of oxidative stress indicators

After the experiment, the rats were euthanized, and their hearts were excised, rinsed with ice-cold phosphate-buffered saline (PBS) to remove residual blood, and homogenized using 0.1 M Tris solution (pH 7.4). The homogenate was centrifuged at 4500 rpm, and the supernatant was used to evaluate the levels of tissue antioxidants, including SOD, GSH, catalase, and MDA(Pullaiah et al., 2018)

qRTPCR analysis of gene expression for Nrf2, Keap1, and HO-1 in cardiac tissue

Total RNA was extracted from the cardiac tissue using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA purity and concentration were evaluated using an H1 Synergy multimode microplate reader (Agilent, USA), and RNA integrity was verified by agarose gel electrophoresis. A high-capacity cDNA reverse transcription kit (Bio-Rad, USA) was used to reverse-transcribe 1 µg of RNA into cDNA. SYBR Green Master Mix (Bio-Rad, USA) was used for quantitative real-time PCR using a Bio-Rad Opus 96 Real-Time PCR System (Bio-Rad). Nrf2-, Keap1, and HO-1 gene-specific primers were designed and validated. GAPDH was used as an internal control to normalize the expression data. Relative gene expression levels were calculated using the 2^–ΔΔCt method and expressed as fold-change compared to the normal control group(Pullaiah et al., 2021).

Table 1: RTPCR primer sequences used in the present study

Gene	Primer sequence (5′→3′)
Nrf2	F: TGATTTAAGCAGCATACAGCAG R: GTATTAAGACACTGTAACTCGGG
Keap1	F: TAGATGGCCACATCTACGC R: TCTCGATCTGGCTCATATCTC
HO-1	F: TGCTGACCCATGACACCAAG R: GGGCAGAATCTTGCACTTTGTT
GAPDH	F: AGGTCGGTGTGAACGGATTTG R: GGGGTCGTTGATGGCAACA

Western blot analysis

Western blot analysis of Nrf2/HO-1 expression in cardiac tissue

The protein expression levels of Nrf2 and HO-1 in rat cardiac tissue were assessed using western blotting, as described in our previous study. Cardiac tissues were homogenized in RIPA buffer (50 mM Tris–HCl, pH 8.0; 150 mM NaCl; 0.1% Triton X-100; 0.5% sodium deoxycholate; 0.1% SDS) supplemented with protease and phosphatase inhibitors. The lysates were heated at 70 °C for 5 min and then centrifuged at 12,000 × g for 15 min at 4 °C. Protein concentrations were quantified using a Bio-Rad DC Protein Assay Kit. Identical quantities of proteins were fractionated by SDS-PAGE and subsequently transferred to PVDF membranes. The membranes were incubated overnight at 4 °C with primary antibodies against Nrf2 and HO-1 (1:1000) after blocking with 3% skim milk in TBST. Following incubation with horseradish peroxidase-conjugated secondary antibodies, immunoreactive bands were detected using 3,3′-diaminobenzidine (DAB). Band intensities were assessed by densitometry using ImageJ software and standardized against β-actin(Nemalapalli & Mastanaiah, 2026).

Histopathological examination of cardiac tissue

Cardiac tissues were preserved in 10% neutral-buffered formalin, systematically treated, and embedded in paraffin. Paraffin blocks were sliced to a thickness of 4–5 µm and stained with hematoxylin and eosin (H&E) for histological assessment of the liver. Stained sections were analyzed using a light microscope to evaluate the myocardial structure and morphological changes, including cardiomyocyte integrity and tissue organization. Representative photomicrographs were obtained for comparative analysis between the experimental groups.

Statistical analysis

Data are reported as the mean ± SEM and were compared across control groups using one-way ANOVA, followed by Tukey's post hoc test for each parameter individually using GraphPad Prism software (version 5.0). The threshold for statistical significance was set at $P < 0.05$.

RESULTS

Quantification of total phenolic and flavonoid content, alongside *in vitro* antioxidant activity of EAB

The total phenolic content (TPC) of the ethanolic extract of *A. baccifera* was measured using the Folin–Ciocalteu technique and reported as gallic acid equivalents. EAB demonstrated a phenolic content of 7.47 ± 2.82 mg gallic acid equivalents (GAE) per gram of dry extract. The total flavonoid content (TFC), assessed via the aluminum chloride colorimetric method and represented as rutin equivalents, was 5.36 ± 1.46 mg RE/g dry extract. The antioxidant capacity of EAB was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. EAB demonstrated strong free radical scavenging activity in a concentration-dependent manner, with all evaluated concentrations exhibiting significantly higher radical scavenging capacity than that of the control group.

Evaluation of cytotoxicity of EAB in H9C2 cardiomyoblasts

The cytotoxicity of EAB was evaluated in H9C2 rat cardiomyoblasts using the MTT assay. Cells were exposed to increasing concentrations of EAB (0.78125–200 µg/mL), and cell viability was assessed after incubation. EAB caused a dose-dependent decrease in cell viability (Fig. 1). The half-maximal inhibitory concentration (IC_{50}) was determined to be 82.04 µg/mL, showing low cytotoxicity within the evaluated concentration range and confirming the choice of non-toxic doses for forthcoming in vitro studies.

Acute Oral Toxicity Assessment of EAB

The acute oral toxicity of EAB was evaluated following a single oral administration at a limit dose of 2000 mg/kg. Throughout the observation period, no mortality or treatment-related clinical signs of toxicity or morbidity were observed in any of the animals. Furthermore, no statistically significant differences in body weight gain were observed between the EAB-treated and control groups, indicating the absence of adverse effects on general health. A comprehensive macroscopic examination revealed no abnormalities in the external appearance, natural orifices (anal, urethral, vaginal, and nasal), or internal cavities (thoracic and abdominal cavities) of the animals administered EAB. Gross pathological evaluation of the major organs revealed no detectable pathological changes. Histopathological analysis was not performed because of the absence of treatment-related gross lesions.

Metabolite Profiling of EAB by LC-MS Analysis

Data from LC-MS analysis were processed by removing ion peaks with more than 50% missing values within each group. Missing values were imputed by replacing half of the minimum detected value with the remainder, and the positive ion data were combined into a single matrix containing all the extracted important information for further analysis. Correlation analysis was performed using the amalgamation database as input. LC-MS analysis in positive mode indicated the presence of several physiologically active secondary metabolites with distinct molecular weights. LC-MS analysis of EAB indicated the presence of a wide range of bioactive phytochemicals from various structural classes, including phenolic acids, flavonoids, coumarins, terpenoids, and sesquiterpenoids. The investigation identified chemicals using retention duration (RT), *m/z* values, and elemental formulae, revealing diverse phytochemical compositions in the samples. Fig. 2 and Table 1 show the most intense peaks for the identified compounds.

Dose-Dependent Reduction in Serum Cardiac Biomarkers Induced by EAB

The disease control group demonstrated markedly increased serum concentrations of cardiac injury biomarkers, specifically cardiac troponin T (cTnT), lactate dehydrogenase (LDH), and creatine kinase-MB (CK-MB), compared to the normal control group ($p < 0.05$), signifying major myocardial damage following isoproterenol (ISO) administration. Pretreatment with EAB at doses of 50 and 100 mg/kg significantly decreased the levels of these biomarkers compared to those in the disease control group ($p < 0.05$). The level of decrease was higher in the 100 mg/kg group than in the 50 mg/kg group, illustrating the established dose-dependent cardioprotective effect of EAB against ISO-induced myocardial damage (Fig. 3).

EAB Mitigated Oxidative Stress in ISO-Induced Myocardial Damage

To determine the impact of oxidative stress on ISO-induced cardiac damage and the antioxidant efficacy of EAB, we evaluated the activities of endogenous antioxidant enzymes in cardiac tissue. The disease control group showed a substantial increase in malondialdehyde (MDA) levels compared to the normal control group ($p < 0.05$), indicating increased lipid peroxidation. Simultaneously, the activities of essential antioxidant enzymes, such as superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT), were markedly diminished in the disease control group ($p < 0.05$), indicating compromised antioxidant defense mechanisms (Fig. 4). 4). Pretreatment with EAB at doses of 50 and 100 mg/kg significantly reduced lipid peroxidation, as indicated by a substantial decrease in MDA levels compared to that in the disease control group ($p < 0.05$). Moreover, EAB administration markedly reinstated the activities of SOD, GSH, and CAT to near-normal levels ($p < 0.05$ compared to the disease control), with the higher dosage (100 mg/kg) eliciting a more significant effect. These results demonstrate that EAB substantially alleviates ISO-induced oxidative stress by enhancing endogenous antioxidant defense mechanisms (Fig. 4).

EAB restores Cytokine Balance in ISO-Induced Myocardial Damage

The inflammatory condition of the cardiac tissue was assessed by quantifying the levels of pro-inflammatory and anti-inflammatory cytokines. The disease control group demonstrated markedly increased levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), nuclear factor-kappa B (NF-κB), and C-reactive protein (CRP) relative to the normal control group ($p < 0.05$), signifying a pronounced inflammatory response subsequent to the ISO challenge. Conversely, the concentration of the anti-inflammatory cytokine interleukin-10 (IL-10) was markedly diminished in the disease control group ($p < 0.05$), indicating an imbalance between pro- and anti-inflammatory mediators (Fig. 5). Administration of EAB at 50 and 100 mg/kg markedly reduced the increased concentrations of TNF-α, IL-1β, IL-6, NF-κB, and CRP compared to the disease control group ($p < 0.05$). Concurrently, IL-10 levels were markedly elevated after EAB treatment ($p < 0.05$ compared to the disease control). The results were more significant at the elevated dose of EAB (100 mg/kg), indicating that EAB reinstates cytokine equilibrium in a dose-dependent manner, thus mitigating ISO-induced myocardial inflammation (Fig. 5).

Dose-Dependent Regulation of the Nrf2/Keap1/HO-1 Antioxidant Pathway by EAB

The role of the Nrf2/Keap1/HO-1 signalling pathway in the cardioprotective effects of EAB was examined using quantitative RT-PCR analysis. In the disease control (ISO-treated) group, a notable upregulation of Kelch-like ECH-associated protein 1 (Keap1) expression was detected ($p < 0.05$), along with a significant downregulation of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream target, heme oxygenase-1 (HO-1) ($p < 0.05$ vs. normal control group). These modifications indicate a disturbance in the intrinsic antioxidant defense mechanism and increased vulnerability to oxidative stress following ISO exposure. Pretreatment with EAB at 50 mg/kg led to a considerable elevation in Nrf2 and HO-1 expression ($p < 0.05$ vs. ISO control) and a notable decrease in Keap1 expression ($p < 0.05$

vs. ISO control), indicating partial restoration of redox equilibrium. Treatment with EAB at 100 mg/kg significantly ($p \leq 0.05$) enhanced the expression of Nrf2 and HO-1 ($p < 0.05$ vs. ISO control) and effectively normalized Keap1 levels ($p < 0.05$ vs. ISO control) (Fig 6). 6). These results indicate that EAB mitigates ISO-induced oxidative stress by reactivating the Nrf2 signalling pathway in a dose-dependent manner**.**

EAB stimulates the Nrf2/HO-1 antioxidant pathway in ISO-induced myocardial damage

The protein expression levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream target heme oxygenase-1 (HO-1) in cardiac tissue were assessed using western blot analysis (Fig. X). The disease control group demonstrated a significant decrease in Nrf2 and HO-1 protein expression relative to the normal control group, suggesting the inhibition of endogenous antioxidant signalling after isoproterenol (ISO) treatment. In contrast, EAB treatment led to a dose-dependent increase in Nrf2 and HO-1 expression. Rats administered EAB at 50 mg/kg exhibited a significant elevation in Nrf2 and HO-1 protein levels relative to the illness control group, indicating the partial restoration of antioxidant defense mechanisms. The injection of EAB at 100 mg/kg significantly elevated the expression of both Nrf2 and HO-1, approaching the levels observed in the normal control group. β-actin expression remained consistently stable across all experimental groups, validating the uniform protein loading and transfer efficacy. Densitometric examination of immunoreactive bands corroborated these findings, indicating a notable increase in Nrf2 and HO-1 protein expression in the EAB-treated groups relative to the disease control group. These data suggest that EAB provides cardio protection, at least partially, via the activation of the Nrf2/HO-1 antioxidant signalling pathway.

EAB Mitigates Cardiac Inflammation and Tissue Damage Induced by ISO

Histopathological analysis of the heart tissue confirmed the biochemical and molecular findings. The myocardium of normal control rats displayed a retained myocardial architecture, undamaged cardiomyocytes, and no inflammatory cell infiltration. Conversely, the disease control group exhibited significant myocardial damage, characterized by disruption of tissue architecture, interstitial edema, and pronounced infiltration of inflammatory cells, indicating severe ISO-induced myocardial injury. Treatment with EAB at 50 and 100 mg/kg significantly improved the pathological changes. Hearts from EAB-treated rats exhibited diminished inflammatory cell infiltration, reduced interstitial edema, and minor structural changes compared with those from the disease control group. The protective effect was more pronounced in the 100 mg/kg group, which exhibited a nearly normal cardiac shape with minimal tissue damage (Fig. 7). These histological findings further substantiate the cardioprotective and anti-inflammatory properties of EAB against ISO-induced myocardial damage.

DISCUSSION

Myocardial infarction (MI), a leading cause of morbidity and mortality worldwide, is caused by a cascade of interdependent pathological events characterized by ischemia-induced myocardial necrosis, which disrupts the integrity of cardiac cell membranes, leading to the leakage of intracellular enzymes and proteins into the bloodstream (Hartikainen et al., 2023). Various therapeutic approaches, including the use of medicinal plants with cardioprotective properties, have been explored to address this condition. In this study, we assessed the cardioprotective effects of EAB against ISO-induced MI in rats, which is a well-established experimental model that closely resembles the pathophysiology of human MI.

The medicinal plant *A. baccifera*, which is well-regarded in traditional Indian medicine, has been employed to address a variety of health issues due to its abundant phytochemical content. This plant species is rich in secondary metabolites, such as phenolics, hydroxycinnamic acids, flavonoids, terpenoids, and fatty acids, all of which possess therapeutic properties, including anti-inflammatory, antioxidant, and antitumor effects(C. P. Khare, 2007)

To further understand the bioactive compounds responsible for the biological effects, LC–MS analysis of EAB was performed, revealing the presence of a diverse range of phytochemicals, including phenolic acids (cinnamic acid, ferulaldehyde, and drupanin), furocoumarins (herniarin, scopoletin, scoparone, and bergapten), flavonoids (tamarixetin, delphinidin, and oxyresveratrol), and lignans (secoisolariciresinol). Terpenoids include matricin, cafestol, and akhdardiol, as well as sesquiterpenoids such as nootkatone and bioactive chemicals such as prenyl caffeate, cynaropicrin, crocetin, curcumin, and persenone A. Notable antioxidants include (−)-epigallocatechin gallate, delphinidin and cyanidin 3-O-galactopyranoside, as well as sulfur-containing phenolics such as eugenol sulfate. The presence of these bioactive compounds correlated with the high total phenolic and flavonoid content in EAB, suggesting a strong antioxidant potential.

To investigate the biological effects of EAB, its phytochemical profile was assessed using cytotoxicity and antioxidant activity tests. The MTT assay conducted on H9C2 cells showed a high IC_{50} value of 82.04 µg/mL, suggesting that the extract has low cytotoxicity and is safe even at elevated concentrations. These results are consistent with those of earlier research on similar species, where *A. baccifera* did not negatively impact cell viability(Suman et al., 2024) . The DPPH assay further validated the in vitro antioxidant capacity of EAB, demonstrating its ability to quench radical ions. Previous studies have identified biologically active polyphenols in the alcoholic extract of *A. baccifera*, which enhance its antioxidant properties. (Loganayaki et al., 2012).

The cardiotoxicity model employed in this study was based on ISO, a synthetic catecholamine that stimulates β-adrenergic receptors, leading to increased myocardial oxygen consumption, excessive reactive oxygen species (ROS) production, and intracellular calcium overload(Chen et al., 2023).These effects collectively induce oxidative stress, leading to myocardial cell damage, which is reflected by elevated levels of key cardiac biomarkers, such as CK-MB, LDH, and CTnT, which are hallmark indicators of myocardial injury(Pullaiah et al., 2017) .The present study confirmed a significant increase in these biomarkers in the ISO-treated group, indicating extensive myocardial injury. Furthermore, oxidative stress–induced damage amplifies the pro-inflammatory response by recruiting immune cells, triggering cytokine release,

and exacerbating tissue injury. ISO-treated rats exhibited higher levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, NF-κB, and CRP) and lower levels of the anti-inflammatory cytokine IL-10 than the control rats. This proinflammatory milieu contributes to myocardial fibrosis and adverse tissue remodeling, thereby aggravating cardiac dysfunction (Paulus & Zile, 2021). Pre-treatment with EAB significantly mitigated the adverse effects of ISO and a dose-dependent reduction in CK-MB, LDH, and CTnT levels was observed, with the most pronounced effect observed at 100 mg/kg. Furthermore, EAB treatment effectively suppressed pro-inflammatory cytokines while restoring IL-10 levels, thereby disrupting the cycle of inflammation and oxidative stress. The suppression of NF-κB signaling by EAB highlights its molecular mechanism in modulating inflammation, as NF-κB activation drives the expression of multiple pro-inflammatory cytokines(Muro et al., 2024). The identification of oxyresveratrol (m/z 245.0814) in EAB through LC–MS supports findings which exerts its cardioprotective via anti-inflammatory and antioxidant pathways (Aliyev et al., 2024). Further supporting the cardioprotective potential of EAB, other phytochemicals identified in the extract, such as persenone A (m/z 379.2852), delphinidin (m/z 304.0596), and scoparone (m/z 207.066) (Wang et al., 2017)., have been reported to exhibit prominent anti-inflammatory effects. These compounds may have contributed to the observed reduction in inflammation and restoration of myocardial injury in ISO-treated rats. The anti-inflammatory properties of *Ammannia baccifera* were further validated by Loganayaki et al. where the plant extract was successfully reduced the inflammation and oedema in experimental rats (Loganayaki et al., 2012).

At the cellular level, the body's defense against oxidative stress and inflammation is mediated by antioxidant enzymes such as superoxide dismutase (SOD), glutathione (GSH), and catalase. ISO-induced auto-oxidation generates highly toxic free radicals, including quinones and ROS, which disrupt the balance of antioxidant enzyme activity, resulting in extensive myocardial damage(Pullaiah et al., 2017) . Although previous studies have primarily focused on the *in vitro* antioxidant potential of *A. baccifera*(Loganayaki et al., 2012) . the present study is the first to report its *in vivo* antioxidant efficacy in rats with possible molecular mechanism of action. LC–MS analysis identified potential phytochemical compounds responsible for these protective effects, supporting the role of EAB in attenuating oxidative free radicals and restoring antioxidant enzyme levels in tissues.

The antioxidant potential of EAB was further investigated by modulating the Nrf2/Keap1/HO-1 antioxidant pathway, which is closely linked to its rich phytochemical composition, as determined by LC–MS analysis. EAB contains a diverse array of bioactive compounds, including potent antioxidants such as (−)-epigallocatechin gallate, cyanidin 3-O-galactopyranoside, delphinidin, curcumin, and oxyresveratrol, as well as sulfur-containing phenolics such as eugenol sulfate. Scopoletin, a phenolic coumarin tentatively identified (m/z 193.0496) by LC–MS, has been reported to exert antioxidant activity by upregulating the Nrf2/HO-1 axis in ISO-induced myocardial infarction(Zhang et al., 2025) .Another coumarin derivative, bergaptene (m/z 217.0494), effectively attenuated pro-inflammatory markers, such as IL-6, TNF-α, iNOS, and COX-2, and inhibited LPS-induced inflammation by activating the Janus kinase (JAK)/STAT signaling pathway while reducing intracellular reactive oxygen species (ROS) levels (Singh et al., 2019).Similarly, other phytoconstituents such as (−)-epigallocatechin gallate, cyanidin 3-O-galactopyranoside, and curcumin are known to modulate redox-sensitive signaling cascades, particularly via activation of the Nrf2 pathway (Moratilla-Rivera et al., 2023). Supporting this mechanistic basis, gene expression analysis in the present study revealed that EAB co-treatment significantly restored Nrf2 and HO-1 mRNA levels while downregulating Keap1 expression in ISO-induced myocardial injury in rat hearts. Importantly, these transcriptional changes were corroborated at the protein level by western blot analysis, which demonstrated a marked reduction in Nrf2 and HO-1 protein expression in the ISO-treated group compared to that in the control group. In contrast, EAB treatment resulted in a dose-dependent upregulation of Nrf2 and HO-1 protein levels, with the 100 mg/kg dose producing a more pronounced effect than the 50 mg/kg dose, which approached normal control values. β-actin expression remained unchanged across all experimental groups, confirming equal protein loading and transfer efficiencies. These findings indicate that EAB enhances both transcriptional and translational activation of the Nrf2/HO-1 axis, thereby strengthening endogenous antioxidant defense mechanisms.

Collectively, these findings imply that the cardioprotective effects of EAB are at least partly mediated through phytochemical-induced activation of the Nrf2/Keap1/HO-1 signaling pathway, contributing to the attenuation of oxidative stress and myocardial injury in rats. In addition to the above findings, histopathological analysis provided direct evidence of the cardioprotective effects of EAB. Severe necrosis, inflammation, and myocardial disarray were observed in the ISO-treated group, whereas EAB pre-treatment preserved the myocardial architecture, reduced necrotic damage, and decreased inflammatory cell infiltration. These observations further reinforce the therapeutic potential of EAB in mitigating myocardial injury and preventing progressive cardiac deterioration.

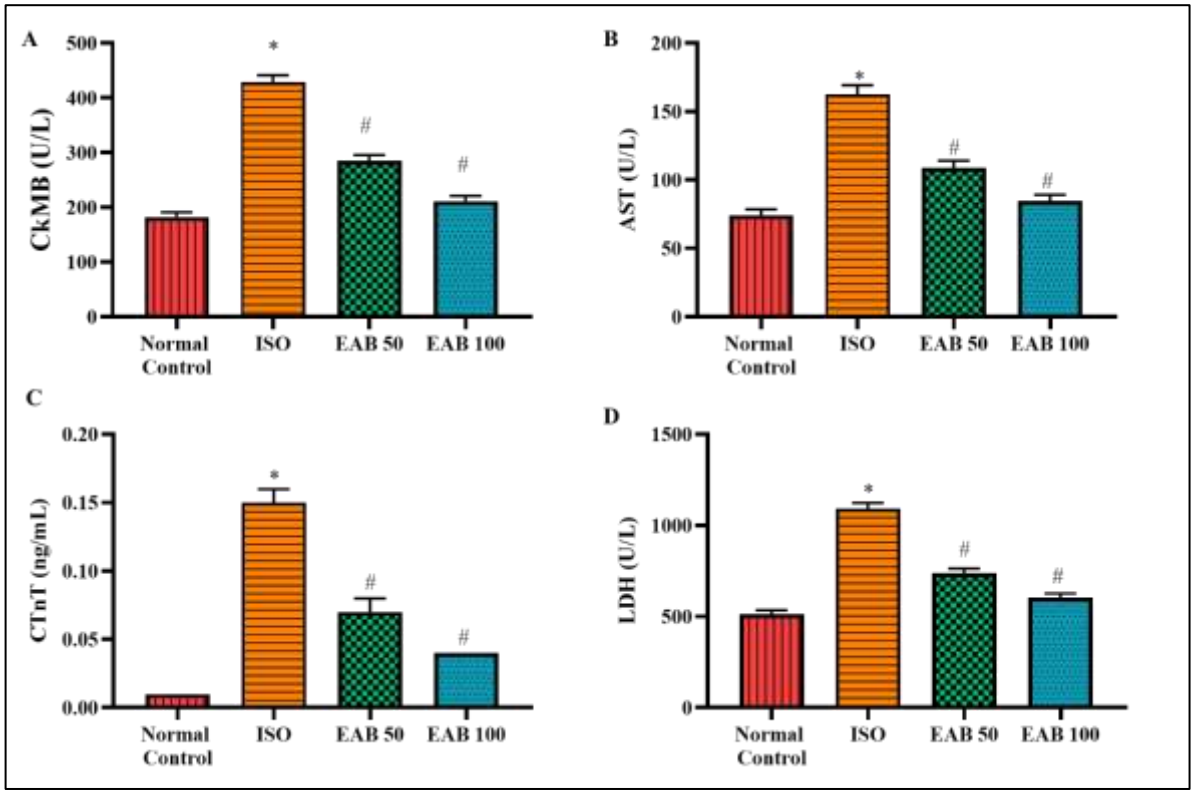

Fig. 3 Effect of EAB on cardiac biomarkers CK-MB, LDH, and CTnT in ISO-induced cardiotoxicity in Wistar albino rats. All data are presented as mean ± SEM (n=6). Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test. *p<0.05 when the disease control was compared to the normal control. #p<0.05 when the test drug-treated groups were compared to the disease control group.

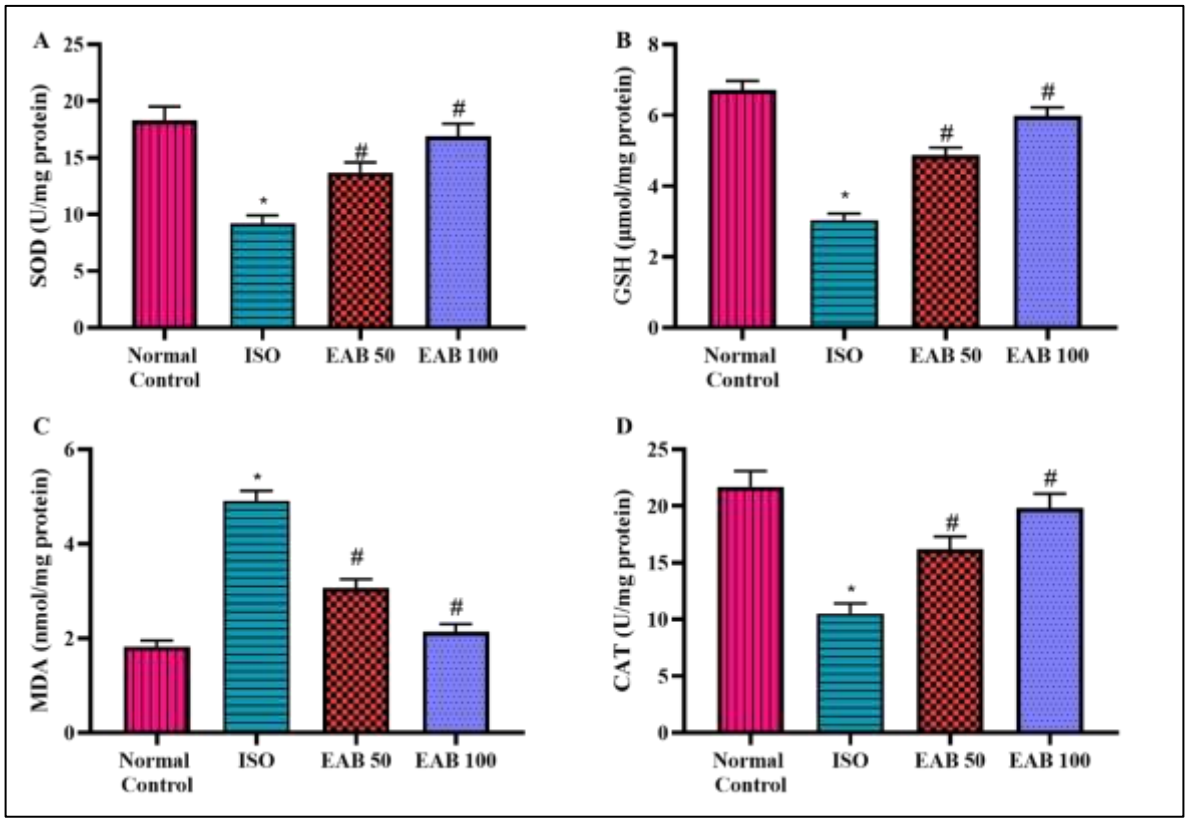

Fig. 4 Effect of EAB on tissue antioxidant enzyme activity against ISO-induced cardiotoxicity in Wistar albino rats. Data are reported as mean ± SEM (n=6) and were analyzed using one-way ANOVA with Tukey's post-hoc test. *p<0.05, vs. normal control; #p<0.05, vs. disease control.

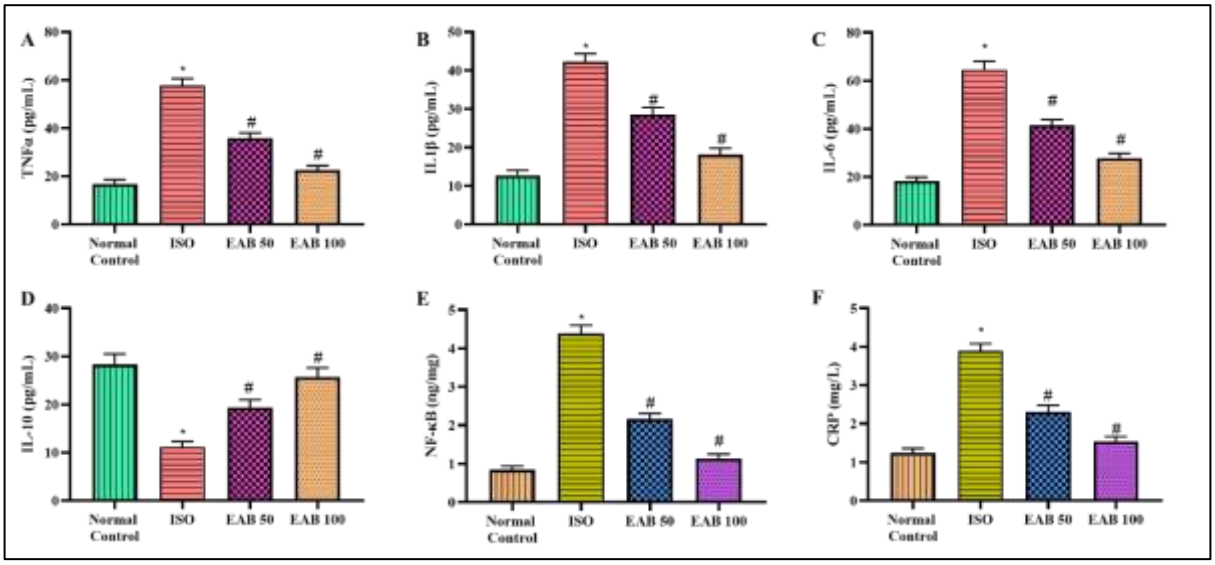

Fig. 5 Effect of EAB on inflammatory cytokine levels in ISO-induced cardiotoxicity in Wistar albino rats. All results are presented as mean ± SEM (n=6). Data were analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test. $^{*}p<0.05$, vs. normal control; $^{\#}p<0.05$, vs. disease control.

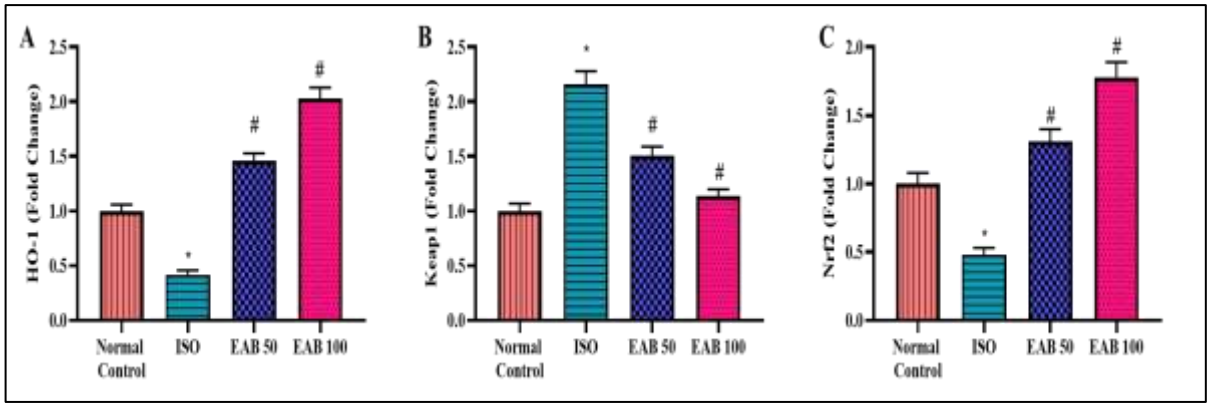

Fig. 6 Effect of EAB on a) Nrf2, b) Keap 1, and c) HO-1 mRNA expression in cardiac tissues. All results are presented as the mean ± SEM (n=6). Data were analyzed using one-way ANOVA with Tukey's post hoc test. $^{*}p<0.05$, vs. normal control; $^{\#}p<0.05$, vs. disease control.

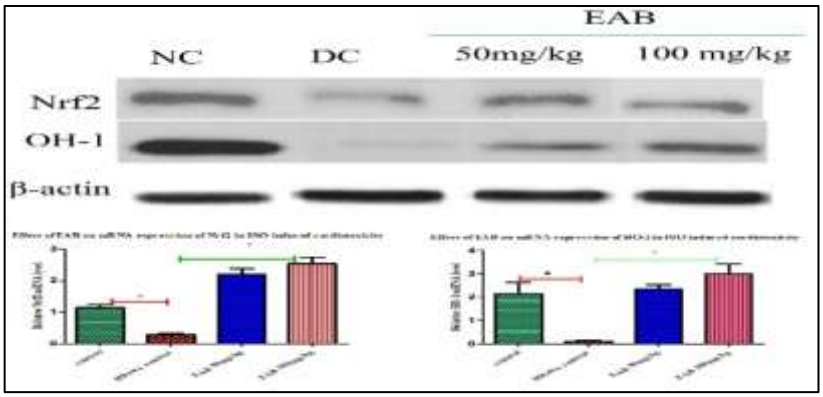

Fig. EAB activates the Nrf2/HO-1 antioxidant signaling pathway in ISO-induced myocardial injury.

Representative Western blot images showing the protein expression of Nrf2 and HO-1 in the cardiac tissue of experimental rats. β-actin was used as the internal loading control. Lane order: Normal control, disease control (ISO), EAB (50 mg/kg), and EAB (100 mg/kg). Densitometric analysis of band intensities was performed using the ImageJ software, and the values were normalized to β-actin. Data are expressed as mean ± SEM. $^{\#}p< 0.05$ compared with normal control; $^{*}p < 0.05$ compared with disease control

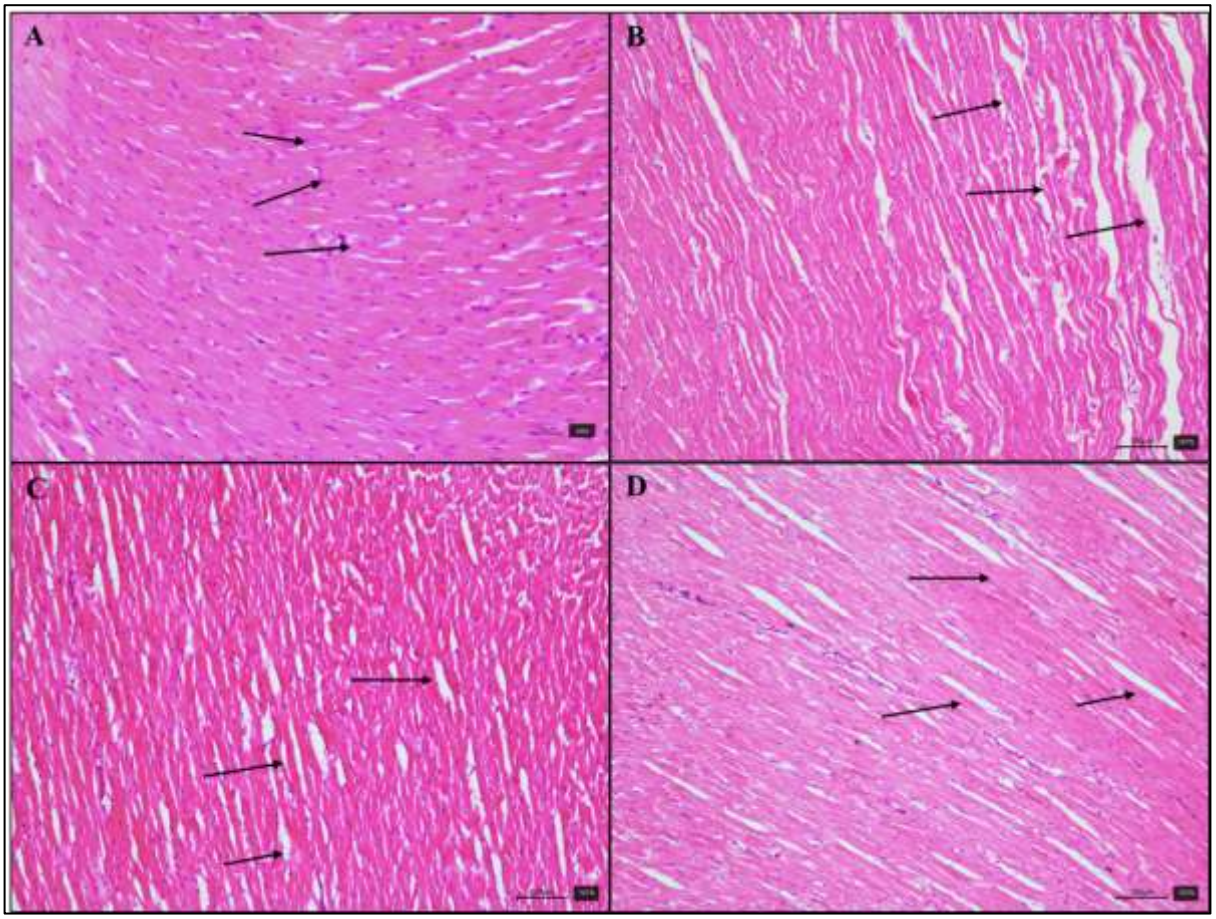

Fig. 7 Histopathology of Heart stained with hematoxylin and eosin observed at 100X. A) Normal Control B) Disease Control C) EAB 50 mg/kg D) EAB 100 mg/kg. The black arrow indicates the infarction region with enucleation, fibrosis, and fluid in (B) and normal architecture or low-level damage in the heart muscle in (A), (C), and (D).

CONCLUSION

The present study demonstrated the cardioprotective efficacy of *A. baccifera* extract (EAB) against isoproterenol-induced myocardial infarction in rats, as evidenced by a marked reduction in elevated cardiac injury biomarkers, suppression of pro-inflammatory cytokines, and improvement in lipid profile parameters, concomitant with enhancement of endogenous antioxidant defenses. Phytochemical profiling using LC–MS analysis led to the tentative identification of biologically active furocoumarins, including scopoleptin and bergapten, along with other putative constituents that may collectively contribute to the observed cardioprotective effects of EAB through modulation of the Keap1/Nrf2/HO-1 signaling pathway. Furthermore, histopathological evaluation corroborated the biochemical and molecular findings by revealing substantial preservation and restoration of myocardial architecture in the EAB-treated groups, underscoring the multifaceted protective effects of the extract at both the cellular and tissue levels. Collectively, these results indicate that *A. baccifera* extract exerts cardioprotective effects by strengthening antioxidant defense mechanisms, regulating inflammatory mediators, and maintaining structural integrity of cardiac tissue. Despite these promising findings, further investigations are warranted to isolate, purify, and structurally characterize the individual bioactive constituents responsible for the observed effects, delineate the precise molecular mechanisms underlying EAB-mediated cardioprotection, and evaluate its therapeutic efficacy in experimental models of cardiovascular and inflammatory disorders. Such studies are essential to substantiate the translational potential of EAB prior to clinical evaluation

Declarations

Author contributions

Nemalapalli Yamini: Methodology, data analysis and drafting original manuscript. Juturu Mastanaiah: Conceptualization, Supervision, Writing - review and editing.

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Competing Interests

The authors declare no conflicts of interest.

Data Availability

The data generated during this study are available upon request.

Ethics approval

The experimental design and implementation strictly followed the ethical criteria established by the Committee for Control and Supervision of Experiments on Animals and the Institutional Animal Ethics Committee of our institution.

REFERENCES

1. Ali Esmail Al-Snafi. (2013). The Chemical Constituents and Pharmacological Effects of Bryophyllum calycinum. A review. *International Journal of Pharma Sciences and Research (IJPSR),* , *5*(12), 171–173.
2. Aliyev, H., Bilgili, S., Toktay, E., Nuriyeva, N., & Bayir, Y. (2024). Protective Effects of Oxyresveratrol in IsoproterenolInduced Myocardial Infarction in Rats: A Stereological Study. *The Eurasian Journal of Medicine*, *1*(0). https://doi.org/10.5152/eurasianjmed.2024.23214
3. Chen, Y., Guo, X., Zeng, Y., Mo, X., Hong, S., He, H., Li, J., Steinmetz, R., & Liu, Q. (2023). Ferroptosis contributes to catecholamine-induced cardiotoxicity and pathological remodeling. *Free Radical Biology and Medicine*, *207*, 227–238. https://doi.org/10.1016/j.freeradbiomed.2023.07.025
4. Dhanapal, R., Kumar, G. S., Bubu, V. L., Chandramohan, K., Kumar, R. A., Gupta, M. A., & Basu, S. K. (2006). Evaluation of antifertility activity of the ethanol extract of Ammania baccifera (L) whole plant in male albino rats. *Journal of Pharmacy & Bioresources*, *3*(2), 89–93. https://doi.org/10.4314/JPB.V3I2.32100
5. Goodla, L., Manubolu, M., Pathakoti, K., Jayakumar, T., Sheu, J.-R., Fraker, M., Tchounwou, P. B., & Poondamalli, P. R. (2019). Protective Effects of Ammannia baccifera Against CCl(4)-Induced Oxidative Stress in Rats. *International Journal of Environmental Research and Public Health*, *16*(8). https://doi.org/10.3390/ijerph16081440
6. Jan, B., Dar, M. I., Choudhary, B., Basist, P., Khan, R., & Alhalmi, A. (2024). Cardiovascular Diseases Among Indian Older Adults: A Comprehensive Review. *Cardiovascular Therapeutics*, *2024*(1), 6894693. https://doi.org/10.1155/2024/6894693
7. Kalra, A., Jose, A. P., Prabhakaran, P., Kumar, A., Agrawal, A., Roy, A., Bhargava, B., Tandon, N., & Prabhakaran, D. (2023). The burgeoning cardiovascular disease epidemic in Indians – perspectives on contextual factors and potential solutions. *The Lancet Regional Health - Southeast Asia*, *12*, 100156. https://doi.org/10.1016/j.lansea.2023.100156
8. Khare, C. P. (2007). Ammannia baccifera Linn. In C. Khare (Ed.), *Indian Medicinal Plants* (pp. 1–1). Springer. https://doi.org/10.1007/978-0-387-70638-2_103
9. Lavanya, G., Manjunath, M., Sivajyothi, R., & Parthasarathy, P. R. (2010). Safety evaluation of the ethanol extract of Ammannia baccifera (Lythraceae): assessment of Acute and Subacute toxicity . *Journal of Pharmacy Research*, *3*(11), 2634–2637.
10. Loganayaki, N., & Manian, S. (2012). Antitumor activity of the methanolic extract of Ammannia baccifera L. against Dalton's ascites lymphoma induced ascitic and solid tumors in mice. *Journal of Ethnopharmacology*, *142*(1), 305–309. https://doi.org/10.1016/J.JEP.2012.05.008
11. Loganayaki, N., Siddhuraju, P., & Manian, S. (2012). Antioxidant, anti-inflammatory and anti-nociceptive effects of Ammannia baccifera L. (Lythracceae), a folklore medicinal plant. *Journal of Ethnopharmacology*, *140*(2), 230–233. https://doi.org/10.1016/J.JEP.2012.01.001
12. Moratilla-Rivera, I., Sánchez, M., Valdés-González, J. A., & Gómez-Serranillos, M. P. (2023). Natural Products as Modulators of Nrf2 Signaling Pathway in Neuroprotection. *International Journal of Molecular Sciences*, *24*(4), 3748. https://doi.org/10.3390/IJMS24043748
13. Muro, P., Zhang, L., Li, S., Zhao, Z., Jin, T., Mao, F., & Mao, Z. (2024). The emerging role of oxidative stress in inflammatory bowel disease. *Frontiers in Endocrinology*, *15*, 1390351. https://doi.org/10.3389/fendo.2024.1390351
14. Nelson, V. kumar, Sahoo, N. K., Sahu, M., Sudhan, H. hara, Pullaiah, C. P., & Muralikrishna, K. S. (2020). In vitro anticancer activity of Eclipta alba whole plant extract on colon cancer cell HCT-116. *BMC Complementary Medicine and Therapies*, *20*(1), 355. https://doi.org/10.1186/s12906-020-03118-9
15. Nemalapalli, Y., & Mastanaiah, J. (2026). Bergapten From Leucas Urticifolia Mitigates Isoproterenol-Induced Cardiotoxicity Through Nrf2/Ho-1 Signalling Pathway. *Asian Journal of Pharmaceutical and Clinical Research*, *19*(3), 144–153. https://doi.org/10.22159/ajpcr.2026v19i3.57402
16. Paulus, W. J., & Zile, M. R. (2021). From Systemic Inflammation to Myocardial Fibrosis: The Heart Failure with Preserved Ejection Fraction Paradigm Revisited. *Circulation Research*, *128*(10), 1451–1467. https://doi.org/10.1161/CIRCRESAHA.121.318159
17. Pullaiah, C. P., Kedam, T., Nelson, V. K., Narasimha Kumar, G. V., & Dayanand Reddy, G. (2018). Supplementation of daucus carota L. Extract prevents urolithiasis in experimental rats. *Indian Journal of Natural Products and Resources*, *9*(3), 253–260.
18. Pullaiah, C. P., Narasimha Kumar, G. V., Jyothsna, K., Thyagaraju, K., Nelson, V. K., & Dayanand Reddy, G. (2017). Rosa damascena Mill. L. attenuates myocardial lysosomal membrane destabilization in isoproterenol induced oxidative stress. *Oriental Pharmacy and Experimental Medicine*, *17*(4), 373–380. https://doi.org/10.1007/s13596-017-0290-x
19. Pullaiah, C. P., Nelson, V. K., Rayapu, S., Narasimha Kumar, G. V., & Kedam, T. (2021). Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: in vivo and in vitro evidence. *BMC Pharmacology and Toxicology*, *22*(1), 43. https://doi.org/10.1186/s40360-021-00510-0
20. Roth, G. A., Mensah, G. A., Johnson, C. O., Addolorato, G., Ammirati, E., Baddour, L. M., Barengo, N. C., Beaton, A., Benjamin, E. J., Benziger, C. P., Bonny, A., Brauer, M., Brodmann, M., Cahill, T. J., Carapetis, J. R., Catapano, A. L., Chugh, S., Cooper, L. T., Coresh, J., … Fuster, V. (2020). Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019: Update From the GBD 2019 Study. *Journal of the American College of Cardiology*, *76*(25), 2982.

https://doi.org/10.1016/J.JACC.2020.11.010
21. Senthil, K., Thirugnanasambantham, P., Oh, T. J., Kim, S. H., & Choi, H. K. (2015). Free radical scavenging activity and comparative metabolic profiling of in vitro cultured and field grown Withania somnifera roots. *PloS One*, *10*(4), e0123360. https://doi.org/10.1371/journal.pone.0123360
22. Singh, G., Kaur, A., Kaur, J., Bhatti, M. S., Singh, P., & Bhatti, R. (2019). Bergapten inhibits chemically induced nociceptive behavior and inflammation in mice by decreasing the expression of spinal PARP, iNOS, COX-2 and inflammatory cytokines. *Inflammopharmacology*, *27*(4), 749–760. https://doi.org/10.1007/S10787-019-00585-6,
23. Suman, T. Y., Jia, H. J., Yin, S., Wei, X. Y., Hu, H., Bu, L. K., Yang, G., & Pei, D. S. (2024). Biofabricated gold nanoparticles from Ammannia baccifera as potential antimicrobial, mosquito larvicidal activity, and alter immune response in zebrafish embryo. *Biomass Conversion and Biorefinery*, *14*(18), 22967–22976. https://doi.org/10.1007/s13399-023-04360-0
24. Swilam, N., Nawwar, M. A. M., Radwan, R. A., & Mostafa, E. S. (2022). Antidiabetic Activity and In Silico Molecular Docking of Polyphenols from Ammannia baccifera L. subsp. Aegyptiaca (Willd.) Koehne Waste: Structure Elucidation of Undescribed Acylated Flavonol Diglucoside. *Plants*, *11*(3), 452–452. https://doi.org/10.3 390/PLANTS1 1030452/S1
25. Upadhyay, H. C. (2019). Medicinal Chemistry of Alternative Therapeutics: Novelty and Hopes with Genus Ammannia. In *Current Topics in Medicinal Chemistry* (Vol. 19, Number 10, pp. 784–794). https://doi.org/10.2174/1568026619666190412101047
26. Wang, C.-H., Zhu, L.-L., Ju, K.-F., Liu, J.-L., & Li, K.-P. (2017). Anti-inflammatory effect of delphinidin on intramedullary spinal pressure in a spinal cord injury rat model. *Experimental and Therapeutic Medicine*, *14*(6), 5583–5588. https://doi.org/10.3892/etm.2017.5206
27. Zhang, J., Yuan, Y., Gao, X., Li, H., Yuan, F., Wu, D., Cui, Q., Piao, G., & Yuan, H. (2025). Scopoletin ameliorates hyperlipidemia and hepatic steatosis via AMPK, Nrf2/HO-1 and NF-κB signaling pathways. *Biochemical Pharmacology*, *231*. https://doi.org/10.1016/j.bcp.2024.116639