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Cardioprotective Effects of *Echium amoenum* in an Isoproterenol-Induced Rat Model of Heart Failure

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ABSTRACT

Echium amoenum possesses anti-inflammatory and antioxidant characteristics and is traditionally recommended in Persian medicine for managing cardiac conditions. In order to investigate its potential cardioprotective benefits, 50 male Wistar rats were assigned to five groups: a control group receiving normal saline, an isoproterenol group serving as negative control, and three treatment groups given aqueous extract of E. amoenum at daily doses of 150, 300, and 450 mg/kg. Except for the control group, all animals received subcutaneous injections of isoproterenol (150 mg/kg) on two successive days to trigger heart failure. Starting from day three through day nine, the treatment groups were orally administered the extract via gavage. Following this, the rats were given a one-week recovery period. On day 17, echocardiography was performed prior to anesthesia; blood was then drawn to assess levels of CK (creatine kinase), LDH (lactate dehydrogenase), troponin, MDA (malondialdehyde), NO (nitric oxide), superoxide dismutase (SOD), and glutathione peroxidase (GPX). Heart tissues were also harvested for histopathological examination. Doses of 150, 300, and 450 mg/kg of E. amoenum extract led to a notable rise in ejection fraction; a significant improvement in fractional shortening was observed solely with the 300 mg/kg dose. The highest dose (450 mg/kg) lowered CK-MB and troponin concentrations. Every dose reduced LDH values. Compared to both control and isoproterenol groups, the 450 mg/kg dose enhanced SOD and GPX activities. Moreover, doses of 300 and 450 mg/kg markedly lowered NO and MDA concentrations in comparison to the isoproterenol group. Administration of E. amoenum at 300 mg/kg diminished the extent of tissue fibrosis. *Echium amoenum* demonstrates substantial cardioprotective activity against heart failure induced by isoproterenol.

Keywords: Cardiotonic agents, *Echium amoenum*, Heart failure, Iranian traditional medicine, Rat

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Introduction

Heart failure constitutes a complex clinical condition characterized by high rates of mortality and reduced quality of life [1-3]. Key pathophysiological elements include dysfunction of the endothelium, activation of neurohormonal pathways, venous congestion, and damage to the myocardium [2, 4]. Furthermore, oxidative stress plays a significant role in the development of heart failure [5-7]. Common symptoms encompass fatigue, shortness of breath, congestion in the lungs and periphery, as well as edema in peripheral tissues [1, 8]. Management strategies typically involve inotropic medications, beta-blockers, anticoagulants, vasodilators, diuretics, surgical procedures, and implantation of pacemakers [9, 10]. These approaches, however, carry high financial burdens and notable adverse reactions [1, 11, 12]; hence, the exploration of alternative options is warranted. The utilization of herbal medicines and natural substances for preventing and treating chronic illnesses dates back centuries. Known in Iranian traditional medicine as “Gol-e-gavzaban,” *Echium amoenum* Fisch. is among the most popular and

valued herbs for supporting cardiac function according to Persian medical traditions [13]. As a member of the Boraginaceae family, E. amoenum displays potent antioxidant and anti-inflammatory effects [14-16]. Therefore, it holds promise for conditions involving inflammation, including heart failure. This research aimed to examine the influence of E. amoenum on experimentally induced cardiac failure in rats.

Materials and Methods

Chemicals

The ketamine-xylazine mixture was obtained from a supplier in Woerden, Netherlands. Isoproterenol hydrochloride was supplied by Sigma Aldrich, located in the USA. Assays for biochemical indicators, including troponin, CK-MB, SGOT, SGPT, ALP, NO, and LDH, were performed using diagnostic kits from Pars Azmoon (Iran). Measurements of GPX, SOD, and MDA were carried out employing assay kits provided by Zell-Bio GmbH, Germany.

Plant material

In 2018, dried petals of E. amoenum were purchased from a local herbal marketplace in Tehran. The specimen was authenticated and archived in the Herbarium of the Traditional Medicine and Materia Medica Research Center (TMRC) at Shahid Beheshti University of Medical Sciences, Tehran, Iran (specimen voucher: 456 HMS).

Extraction

The aqueous extract was prepared by pulverizing 10 grams of E. amoenum petals and infusing them in 100 mL of boiling water for 10 minutes using a water bath, followed by filtration. The filtered solution was then lyophilized in a freeze dryer.

Total anthocyanins content

Quantification of total anthocyanins in E. amoenum petals was conducted using cyanidin 3-O-glucoside chloride as the reference standard [17]. A 20-gram sample of dried, powdered petals was combined with 45 mL of methanol and agitated for 30 minutes on a shaker. The resulting filtrate was diluted to 50 mL with additional methanol. Subsequently, 1 mL of this extract was placed in a 10-mL volumetric flask and brought to volume with 0.1% hydrochloric acid dissolved in methanol. Absorbance readings were taken using a UV spectrophotometer at 528 nm, with a blank consisting of 0.1% hydrochloric acid in methanol. All determinations were done in triplicate.

Dosage determination

Drawing from prior related research, the selected doses for testing the extract's effects were 150, 300, and 450 mg/kg [18].

Animals

The experiment involved 50 male Wistar rats aged 8–10 weeks and weighing 250–300 g. Rats were provided with standard pellet feed and water freely available, kept in individual cages under a controlled environment featuring a 12-hour light-dark cycle, temperature maintained at 21 °C, and relative humidity between 40–60%. Unlimited access to food and water was ensured throughout the study duration.

Experimental protocol

The 50 rats were randomly assigned to five groups of 10 animals each: a control group given normal saline, a negative control group treated with isoproterenol hydrochloride, and three treatment groups receiving daily doses of 150, 300, and 450 mg/kg of E. amoenum aqueous extract, as detailed in **Table 1.**

The entire protocol lasted 17 days. On days 1 and 2, subcutaneous injections of isoproterenol (150 mg/kg) were given to induce cardiac dysfunction (this dosage had been previously verified via echocardiography to be non-lethal). For the following 7 days, the designated treatment groups were dosed orally with the aqueous extract (150, 300, or 450 mg/kg) through gavage, whereas the control group received equivalent volumes of normal saline by the same route. A subsequent one-week resting phase was implemented to permit recovery. On day 17, echocardiography was conducted on all subjects at the veterinary facility.

Echocardiographic assessment

Cardiac function was evaluated using transthoracic echocardiography. All ten animals per group underwent the same standardized imaging procedure. The ultrasound device, equipped with a 10 MHz transducer, was supplied by Kanvas Medical Equipment Company.

On the 17th day, anesthesia was induced intraperitoneally with 10% ketamine at 80 mg/kg combined with 2% xylazine at 10 mg/kg. The thoracic region was depilated using razor blades with animals placed in left lateral decubitus position. Both two-dimensional and M-mode echocardiograms were recorded via a 10 MHz probe attached to the Clear Canvas ultrasound platform (Synaptive Medical, Toronto, Canada).

Measurements of interventricular septal thickness during diastole (IVSd) and systole (IVSs) were utilized to identify left ventricular hypertrophy (LVH), characterized by increased left ventricular wall thickness and serving as an indicator of cardiac pathology. Typically, thicknesses of 1.1-1.3 cm denote mild LVH, 1.4-1.6 cm indicate moderate LVH, and values ≥1.7 cm signify severe LVH. Posterior wall thicknesses in systole (LVPWs) and diastole (LVPWd) were additionally examined to gauge LVH and ventricular remodeling, the latter describing structural adaptations in ventricular size and configuration in response to damage or overload [19].

Ejection fraction (EF) quantifies left ventricular systolic performance by assessing the volume of blood expelled during contraction. Values ≥55% are considered normal, while lower figures suggest impaired systolic function or heart failure. Fractional shortening (FS) similarly evaluates systolic capability through the percentage reduction in ventricular diameter from diastole to systole. Normal FS exceeds 25%, with reduced values pointing to systolic compromise or heart failure [19].

Reference ranges for IVSd and IVSs span 0.6-1.1 cm. Key parameters—maximum short-axis left ventricular internal dimensions in diastole (LVDd) and systole (LVDs), LVPWs, LVPWd, EF, and FS—were extracted from M-mode tracings by a blinded, skilled operator experienced in small-animal cardiac imaging. Averages were calculated across three successive heartbeats.

Blood sampling

On day 17, immediately after echocardiography, thoracotomy was performed to obtain cardiac blood samples for analysis of myocardial injury markers (creatine kinase (CK), lactate dehydrogenase (LDH), troponin) alongside indicators of oxidative stress and lipid peroxidation (malondialdehyde (MDA), nitric oxide (NO)). Levels of protective antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPX), were also quantified. MDA was determined through the thiobarbituric acid reactive substances technique [20]. Nitric oxide was assayed via the Griess reaction [21]. To screen for possible toxicity to liver or kidney, additional assays included alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and creatinine.

Table 1. Time table of animal study steps

Group	Days 1–2	Days 3–9	Days 10–16	Day 17
Isoproterenol	Subcutaneous injection of isoproterenol at a dose of 150 mg/kg to induce heart failure	—	Oral administration of normal saline by gavage	Rats were anesthetized intraperitoneally with ketamine (80 mg/kg) and xylazine (10 mg/kg); echocardiography and blood sampling were performed; animals were then sacrificed, and heart and liver tissues were collected for pathological examination
E. amoenum 150	Induction of heart failure via subcutaneous injection of 150 mg/kg isoproterenol	—	Oral gavage of E. amoenum extract at 150 mg/kg	Same anesthesia, echocardiography, serum collection, sacrifice, and tissue sampling as described above
E. amoenum 300	Induction of heart failure via subcutaneous injection of 150 mg/kg isoproterenol	—	Oral gavage of E. amoenum extract at 300 mg/kg	Same anesthesia, echocardiography, serum collection, sacrifice, and tissue sampling as described above

E. amoenum 450	Induction of heart failure via subcutaneous injection of 150 mg/kg isoproterenol	—	Oral gavage of E. amoenum extract at 450 mg/kg	Same anesthesia, echocardiography, serum collection, sacrifice, and tissue sampling as described above
Normal Saline (Control)	Subcutaneous injection of 1 mL normal saline (0.09%)	Rats received routine care without any intervention (rest period)	Oral gavage of 2 mL normal saline (0.09%)	Same anesthesia, echocardiography, serum collection, sacrifice, and tissue sampling as described above

E. amoenum: *Echium amoenum*

Day-17 serum concentrations of creatine kinase-myocardial band (CK-MB) were quantified colorimetrically employing specialized kits on a Roche Hitachi Modular DP automated analyzer (Germany). Every step complied fully with the provided manufacturer guidelines.

Histological analysis

Paraffin blocks of left ventricular tissue were sectioned transversely (5.0 μm thickness) at the mid-papillary level and stained using Masson's trichrome to highlight fibrotic areas. Photomicrographs were acquired with a Nikon Optiphot binocular microscope (USA) fitted with a Sony DKC5000 camera and processor (USA), followed by quantitative assessment of fibrosis via NIH Image software (NIH, Bethesda, USA).

Statistical analysis

All values were reported as mean ± SEM. Comparisons employed one-way or two-way analysis of variance (ANOVA) with subsequent Tukey's multiple comparison test; statistical significance was set at $p < 0.05$. GraphPad Prism software was used for all computations.

Results and Discussion

The content of total anthocyanins, calculated as equivalents of cyanidin 3-O-glucoside chloride, amounted to 3.81 ± 0.14 mg per 100 g of plant material [17].

Principal echocardiographic variables recorded on day 17 are outlined in **Table 2.** The isoproterenol group exhibited a notable expansion in left ventricular systolic internal dimension (LVDs) relative to the normal saline group. An increase in left ventricular diastolic internal dimension (LVDd) was observed in the isoproterenol group, although it lacked statistical significance.

Regarding fractional shortening (FS), as shown in **Figure 1**, two successive subcutaneous doses of isoproterenol (150 mg/kg) in the ISO group produced a marked decrease in FS. Seven days of oral gavage with E. amoenum aqueous extract significantly restored FS exclusively at the 300 mg/kg level. Elevated FS signifies enhanced ventricular pumping capacity in rats experiencing isoproterenol-triggered heart failure. The other doses (150 mg/kg and 450 mg/kg) did not yield a significant FS improvement. Since FS holds greater diagnostic weight than ejection fraction (EF) in echocardiographic assessments, and significant enhancement was limited to the 300 mg/kg dose, this dosage was identified as optimal for interpreting subsequent outcomes. **Figure 1** also demonstrates that isoproterenol (150 mg/kg subcutaneously over two days) substantially lowered EF in the ISO group. Oral treatment with E. amoenum extract at 150, 300, and 450 mg/kg for 7 days significantly raised EF in all treated groups. Higher EF denotes improved contractile efficiency in this heart failure model induced by isoproterenol. Importantly, no meaningful differences in EF emerged among the three dosage levels.

Both ejection fraction and heart rate (HR) contribute to cardiac output by modulating stroke volume, preload, afterload, and inotropic state. Although increased HR can augment output, it compromises diastolic filling, thereby diminishing stroke volume and EF. The ideal HR optimizes EF to ensure adequate perfusion. Greater EF supports superior pump mechanics and offers clinical advantages in heart failure.

Administration of isoproterenol subcutaneously (150 mg/kg for two days) raised serum indicators of cardiac injury—LDH, CK-MB, and troponin—confirming model induction [22]. All doses of E. amoenum extract (150, 300, and 450 mg/kg) lowered LDH, highlighting protective action against isoproterenol damage. LDH levels showed no notable distinction between the 150 mg/kg and 300 mg/kg groups. Specifically, the 450 mg/kg dose markedly suppressed CK-MB and troponin. Declines in these markers underscore the robust cardioprotective influence of the 450 mg/kg regimen **(Figure 2).**

Assessment of SOD and GPX activities across groups indicated that isoproterenol (150 mg/kg subcutaneously over two days) in the ISO group produced no alterations in either enzyme.

Table 2. Effects of *Echium amoenum* on echocardiographic parameters in isoproterenol-induced heart failure

Parameter	Normal Saline (N=10)	ECH150 (N=10)	Isoproterenol (N=10)	ECH450 (N=10)	ECH300 (N=10)
IVSs (cm)	0.243 ± 0.03	0.262 ± 0.02	0.178 ± 0.02	0.272 ± 0.03	0.292 ± 0.02[b]
IVSd (cm)	0.163 ± 0.02	0.142 ± 0.01	0.151 ± 0.03	0.152 ± 0.01	0.215 ± 0.02
LVDs (cm)	0.202 ± 0.01	0.302 ± 0.01	0.470 ± 0.06[a]	0.328 ± 0.08	0.176 ± 0.03[b]
LVDd (cm)	0.488 ± 0.01	0.543 ± 0.02	0.597 ± 0.04	0.560 ± 0.08	0.482 ± 0.03
LVPWs (cm)	0.346 ± 0.01	0.237 ± 0.02[a]	0.172 ± 0.02[a]	0.262 ± 0.04	0.319 ± 0.01[b]
LVPWd (cm)	0.300 ± 0.03	0.173 ± 0.02[a]	0.196 ± 0.03[a]	0.227 ± 0.03	0.252 ± 0.02
EF (%)	91.33 ± 1.49	81.17 ± 2.66[b]	48.33 ± 7.29[a]	84.33 ± 5.93[b]	93.67 ± 2.60[b]
FS (%)	58.50 ± 3.10	43.90 ± 2.95	22.83 ± 4.88[a]	53.43 ± 8.19[b]	64.50 ± 5.35[b]

a: p<0.05 compared to NS group; b: p<0.05 compared to ISO group; ECH: *Echium amoenum*; IVSs: interventricular septum in systole; IVSd: interventricular septum in diastole; LVDs: left ventricular diameters during systole; LVDd: left ventricular diameters during diastole; LVPWs: left ventricular systolic posterior wall thickness systolic; LVPWd: left ventricular posterior wall thickness diastolic; EF: ejection fraction; FS: fractional shortening; ISO: isoproterenol

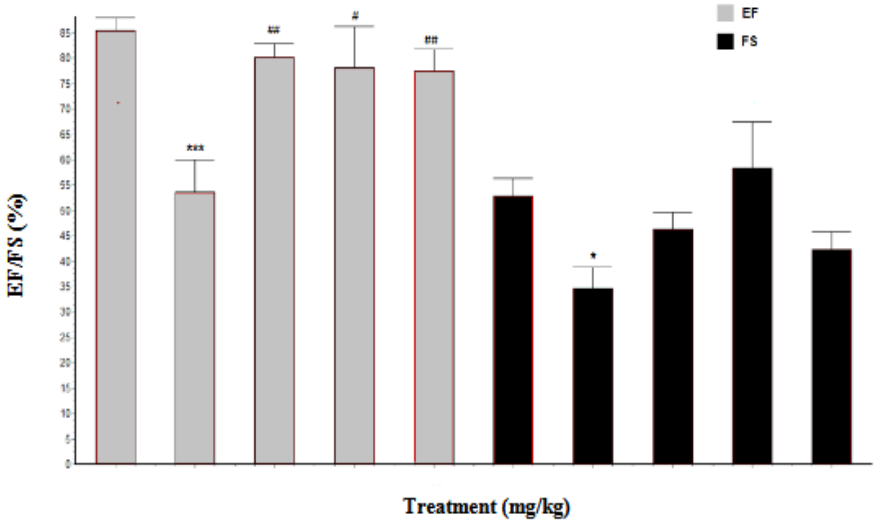


Figure 1. Comparison of ejection fraction (EF) and fractional shortening (FS) in isoproterenol-induced heart failure rats for assessment of *Echium amoenum* aqueous extract (150, 300 and 450 mg/kg); the results of each column represent mean ± mean of standard error; *p <0.05 compared with the Normal saline group; *** p<0.001 compared with the NS group; #p<0.05 compared with the isoproterenol group; ##p<0.01 compared with the ISO group.

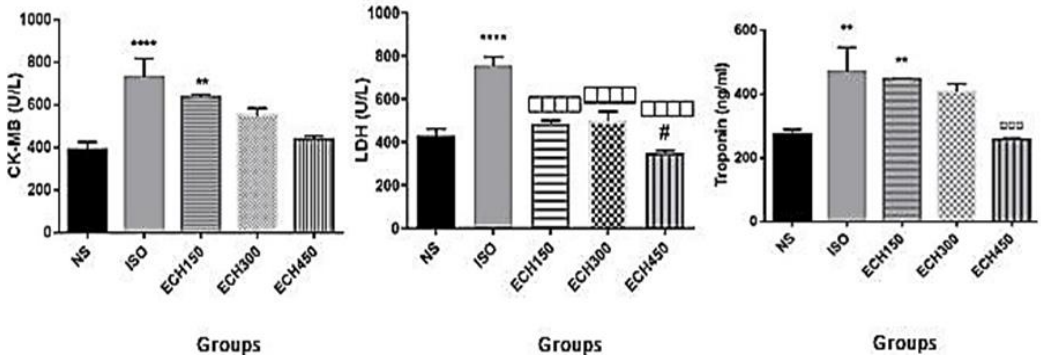


Figure 2. Comparison of myocardial damage markers in isoproterenol-induced heart failure rats; *Echium amoenum* aqueous extract (150, 300 and 450 mg/kg); ** p<0.01 compared with the NS group; **** p<0.0001 compared with the NS group; □□□p<0.001 compared with the ISO group; □□□□p<0.0001 compared with the ISO group; # p<0.05 compared with ECH300 group; ISO: isoproterenol; NS: normal saline; ECH: *Echium amoenum*.

The 450 mg/kg extract dosage boosted SOD and GPX activities when compared to both the control and ISO groups, showing a particular significant enhancement in GPX over the 300 mg/kg dosage. No notable variations in SOD were detected among the other groups. Higher extract doses led to a progressive escalation in both SOD and GPX, highlighting improved antioxidant defenses and protection against oxidative injury in heart failure **(Figure 3).**

Assessment of NO and MDA across the groups indicated that two successive subcutaneous doses of isoproterenol (150 mg/kg) substantially elevated NO and MDA concentrations, reflecting oxidative mechanisms underlying the induced cardiac impairment. Doses of 300 mg/kg and 450 mg/kg of the extract reduced both NO and MDA versus the ISO group. The 450 mg/kg regimen achieved a markedly lower NO level than the 300 mg/kg one. No significant NO difference existed between the 150 mg/kg and 300 mg/kg treatments. MDA concentrations fell in a dose-dependent manner, reinforcing the extract's capacity to counteract oxidative stress and lipid peroxidation linked to heart failure **(Figure 3).**

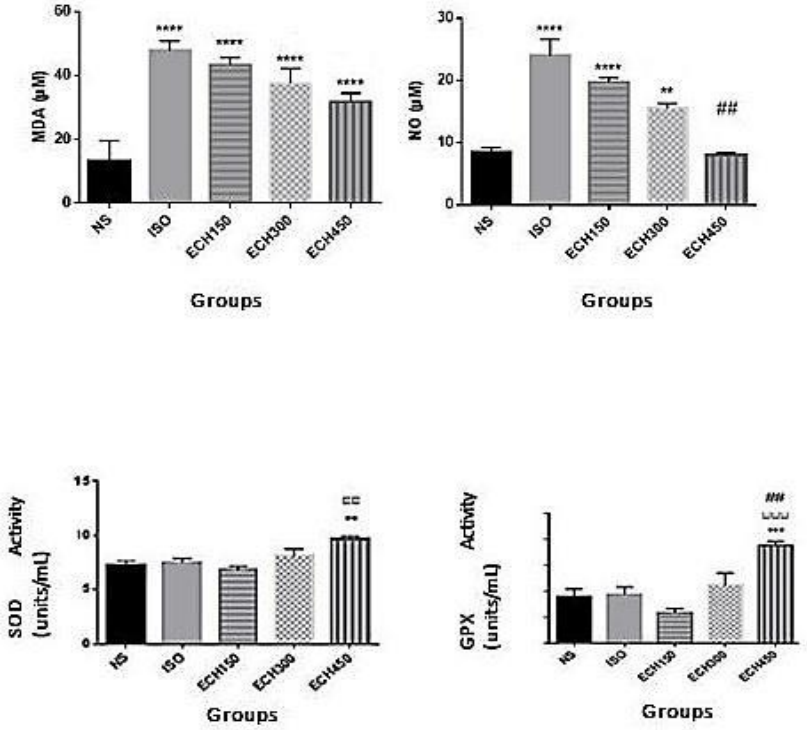


Figure 3. Comparison of markers of oxidative stress and lipid peroxidation and antioxidant enzymes in isoproterenol-induced heart failure rats; E. amoenum aqueous extract (150, 300 and 450 mg/kg); □□p<0.01 compared with the E. amoenum and ISO group; □□□ p<0.001 compared between E. amoenum and ISO group; □□□□ p<0.0001 compared between E. amoenum and ISO group; **** p<0.001 compared between E. amoenum and NS group; *** p<0.001 compared between E. amoenum and NS group; ** p<0.01 compared between E. amoenum and NS group); ## p<0.01 compared with E. amoenum 300 mg/kg and ISO group.

Findings revealed that isoproterenol (150 mg/kg subcutaneously for two days) in the ISO group caused no meaningful changes in SGPT, SGOT, ALP, or creatinine **(Figure 4).** Treatment with 300 mg/kg extract increased SGPT relative to the control and ISO groups, but the elevation stayed under double the control baseline. The 450 mg/kg dosage significantly lowered SGOT versus the control, ISO, and 300 mg/kg groups. At 150 mg/kg, the extract raised ALP significantly only against the control, without differences elsewhere. Creatinine levels stayed below 1 mg/dL in all extract groups, suggesting absence of notable kidney dysfunction.

Examination of myocardial fibrosis across groups detected a sharp rise to 50.5% after isoproterenol induction (150 mg/kg subcutaneously over two days). Every extract dosage (150, 300, and 450 mg/kg) considerably diminished fibrosis percentage, achieving the minimal collagen deposition at 300 mg/kg (14% fibrosis). This marked decline in fibrotic tissue demonstrates the extract's antifibrotic benefits **(Figure 5).**

The current research establishes that E. amoenum mitigates isoproterenol-provoked myocardial fibrosis and cardiac dysfunction in a rat model. This rodent paradigm shares substantial similarities with human heart failure progression [23]. Administration of high isoproterenol doses (85–300 mg/kg) triggers arrhythmias, cardiomyocyte death, fibrosis, ischemia-like changes, and eventual heart failure in rats [24, 25].

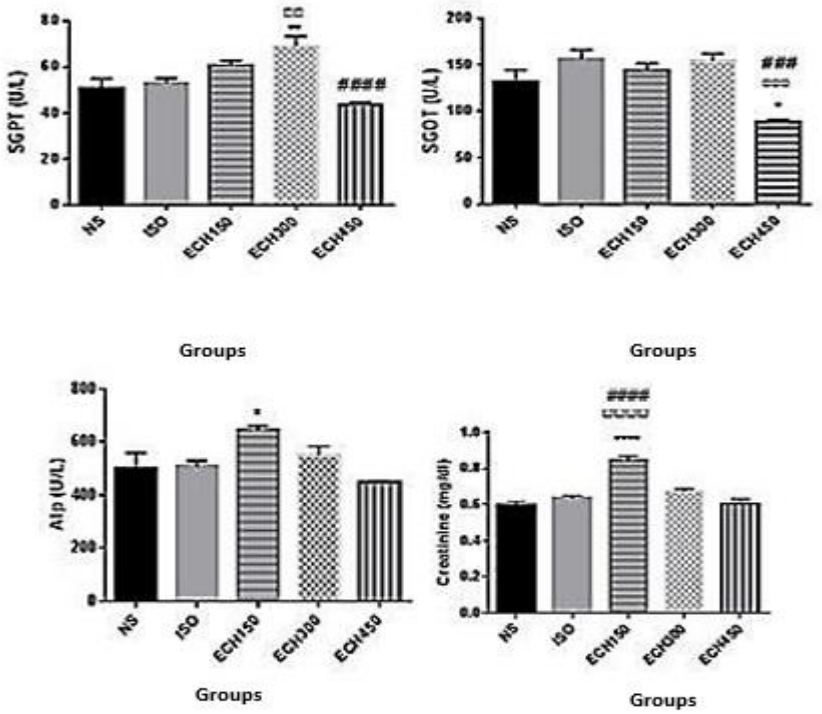
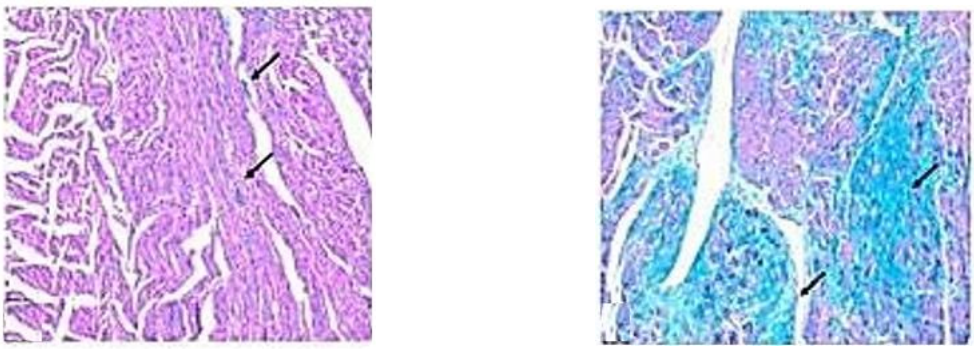
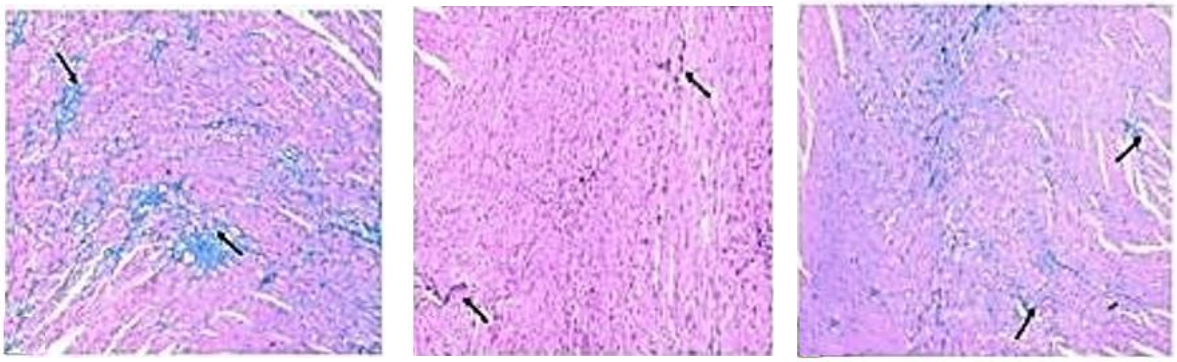


Figure 4. Comparison of SGPT, SGOT, alkaline phosphatase (ALP) and creatinine in isoproterenol induced heart failure rats for assessment of E. amoenum (150, 300 and 450 mg/kg) aqueous extract; * p<0.05 compared with the NS group; ** p<0.01 compared with the NS group; **** p<0.0001 compared with the NS group; □□ p<0.01 compared with the ISO group; □□□ p<0.001 compared with the ISO group; □□□□ p<0.0001 compared with the ISO group; ### p<0.001 compared with the ECH300 group; #### p<0.0001 compared with the ECH300 group.



a) b)



c) d) e)

Figure 5. Hematoxylin and eosin staining and Masson's trichrome staining of myocardial tissues; comparison of collagen distribution in the myocardium in isoproterenol-induced heart failure rats for assessment of *Echium amoenum* (150, 300 and 450 mg/kg) aqueous extract effects; (trichrome × 100); a) heart sections of normal saline group showing normal little collagen distribution in the myocardium (5 % fibrosis); b) heart sections of isoproterenol group showing the highest collagen (50.5 % fibrosis); C: heart sections of 150 mg/kg group showing more collagen (39 % fibrosis); d) heart sections of 300 mg/kg group showing the lowest collagen (14 % fibrosis); e) heart sections of 450 mg/kg group showing small area of collagen (23 % fibrosis)

Administration of isoproterenol at 150 mg/kg produced a marked decline in both EF and FS. Findings from this work indicate that oral delivery of E. amoenum aqueous extract at doses of 150, 300, and 450 mg/kg substantially enhanced EF across the treated groups. Elevated EF reflects superior ventricular pumping efficiency in rats experiencing isoproterenol-triggered heart failure. Significant improvement in FS was achieved exclusively with the 300 mg/kg dose. Greater FS corresponds to better contractile performance in animals affected by isoproterenol-induced cardiac impairment. Two successive subcutaneous injections of isoproterenol (150 mg/kg) caused a notable elevation in serum indicators of cardiac injury, namely LDH, CK-MB, and troponin [22]. The 450 mg/kg extract dosage led to a pronounced reduction in LDH, CK-MB, and troponin concentrations. Diminished levels of these markers highlight the therapeutic potential of the treatment.

Oxidative stress promotes protein oxidation, lipid peroxidation, DNA injury, and cellular disruption, thereby driving myocardial remodeling, hypertrophy, and progression to heart failure in both clinical and preclinical settings [4-6]. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) serve as primary antioxidant defenses critical for combating oxidative insults during heart failure [26].

In the present investigation, activities of these anti-peroxidative enzymes (SOD and GPX) remained largely unchanged in isoproterenol-treated rats relative to controls. Nevertheless, escalating doses of E. amoenum extract elicited a dose-dependent augmentation in SOD and GPX activities, underscoring the extract's beneficial impact on bolstering antioxidant reserves and attenuating oxidative harm associated with heart failure.

Excessive myocardial collagen deposition exerts detrimental effects on cardiac function in heart failure [27]. Histological evaluation here showed that E. amoenum aqueous extract at 300 mg/kg markedly lowered the extent of tissue fibrosis, confirming its protective action. Prior experimental evidence has indicated that E. amoenum lacks hepatotoxicity [28, 29]. Consistent with this, no substantial elevations in liver function tests or creatinine were observed across any treatment group, suggesting absence of toxicity to liver or kidney. In contrast, a 2003 study by Zahedi *et al*. examined the effects of Valeriana officinalis and Borago officinalis on hepatic and renal parameters in rats, reporting modest increases in liver enzymes following seven days of borage extract at 100 and 200 mg/kg, with no impact on kidney markers [30].

Conclusion

This investigation demonstrates that E. amoenum extract exhibits antioxidant and cardiotonic activities in a rat model of isoproterenol-induced heart failure.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: Approval for this research was granted by the Ethics Committee at Shahid Beheshti University of Medical Sciences (ethical code: IR.SBMU.RETECH.REC.1396.531).
During the study period, rats were provided unlimited access to food and water and were cared for in compliance with the National Institutes of Health (NIH) guidelines for laboratory animal handling and use. All laboratory procedures adhered strictly to established ethical standards.

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