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Cardioprotective Potential of *Albizzia lebbeck*: Insights Isoproterenol-Induced Myocardial Infarction in Rats

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Abstract

This study investigates the cardioprotective potential of *Albizzia lebbeck* (Shirish) ethanol leaf extract against Isoproterenol (ISO)-induced myocardial infarction in Wistar albino rats. Cardiovascular Diseases (CVDs) are a major global health concern, contributing significantly to morbidity and mortality. *A. lebbeck*, a medicinal plant with documented pharmacological activities, has not been scientifically studied for its cardioprotective properties. The research utilised a rat model of ISO-induced myocardial infarction, a well-established experimental approach to study preventive effects on myocardial damage. The study included the isolation and preparation of *A. lebbeck* ethanol leaf extract (MEAL) and its administration at different doses (200 mg/kg and 400 mg/kg) to ISO-treated rats. Various parameters, including relative organ weight, cardiac biomarkers (cTnI, LDH, CK-MB), total proteins, oxidative stress markers (SOD, MDA), and histopathological changes, were assessed. The results revealed that ISO administration induced cardiac hypertrophy, increased serum biomarkers, and oxidative stress, indicating myocardial damage. Treatment with MEAL, especially at the higher dose (400 mg/kg), significantly mitigated these effects. MEAL administration reduced the heart-to-body weight ratio, normalised serum biomarkers, restored endogenous antioxidant enzyme levels, and showed a protective effect against structural damage in histopathological examination. In conclusion, *A. lebbeck* ethanol leaf extract demonstrated significant cardioprotective effects against ISO-induced myocardial infarction in rats. These findings suggest the potential of *A. lebbeck* as a natural therapeutic agent for preventing or ameliorating cardiac damage associated with stress-induced conditions. Further research is warranted to elucidate the specific mechanisms underlying its cardioprotective properties and explore its potential clinical applications.

Keywords: Antioxidant Enzymes, Cardiac Biomarkers, CVD Risk Factors, Histopathology, Medicinal Plants, Oxidative Stress

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1. Introduction

Cardiovascular Diseases (CVDs) stand as a leading cause of death and disability globally, contributing to 59% of the 56.5 million recorded fatalities, with chronic conditions like coronary heart disease, cancer, diabetes, and obesity being major contributors (World Health Organization). Coronary heart disease, in particular, is a primary cause of morbidity and mortality worldwide, prompting extensive research into innovative therapeutic approaches[1]. CVDs, encompassing ischemic heart disease and stroke, accounted for 24% of all deaths in 2010, with 80% occurring in low and middle-income countries[2]. In addressing CVDs, modifiable risk factors like cigarette use, physical activity levels, blood pressure, cholesterol, and hyperglycemia are prevalent and amenable to intervention through public health measures and therapeutic interventions[3]. Traditional clinical management targets individual risk factors, but current guidelines globally emphasize the prediction of absolute CVD risk for initiating preventive measures[4]. India, for instance, has implemented the National Program for the Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases, and Stroke (NPCDCS)[5].

Throughout history, medicinal plants have played a crucial role in healing across indigenous cultures, constituting a primary healthcare method for 85% of the world's population[6]. In India, with a rich array of plant resources, there is potential for effective cardioprotective agents. The country has a deep-rooted tradition of using herbal remedies, with ancient texts such as the Rig-Veda and Charak Samhita documenting numerous medicinal plants. India is a significant exporter of plant drugs and derivatives, with plant-derived natural products like flavonoids, steroids, alkaloids, and terpenoids garnering attention for their diverse pharmacological activities[7].

A. lebbeck (Shirish) is a deciduous tree found in India and other parts of Asia and Africa. It has been traditionally used for various medicinal purposes, including treating snake bites. The plant contains phytochemicals like triterpenes and saponins, and its bark extract has demonstrated promising medicinal properties. Studies suggest its potential in activities such as antiprotozoal, hypoglycaemic, antidiabetic, and anticancer effects. The plant extract also shows antioxidant activity, with flavonoids potentially inhibiting various stages of carcinogenesis. This information underscores the need for further exploration of *A. lebbeck* pharmacologically, particularly regarding its isolated compounds[8].

The induction of myocardial infarction in rats through the administration of Isoproterenol (ISO) results in myocardial damage comparable to that observed in patients experiencing acute myocardial infarction. This experimental model is widely utilized to investigate the preventive effects of medications on myocardial damage following an acute myocardial infarction[9,10]. Previous research indicates that oxidative stress induced by the generation of Reactive Oxygen Species (ROS) plays a significant role in the progression of myocardial infarction. This oxidative stress can cause damage to cell membranes, leading to metabolic changes that result in cardiac failure and severe injuries. The increasing prevalence of myocardial infarction contributes to elevated mortality and morbidity in developing countries due to lifestyle changes. Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, induces stress in the myocardium and damages the myocardial membrane when oxidized by free radicals. Medicinal herbs have demonstrated their significance in treating various human disorders, including cardiovascular diseases.

With its diverse pharmacological effects, *A. lebbeck* is an important medicinal plant in India. Many earlier studies have emphasized the biological activity of the *A. lebbeck* plant. However, there have been no scientific findings claiming that *A. lebbeck* leaves have cardioprotective properties. As a result, the current study is required to explore the cardioprotective efficacy of ethanol leaf extract of *A. lebbeck* leaves against ISO-induced myocardial infarction in rats.

2. Materials and Methods

2.1 Animals

Wistar rats of weight 150-200 g were used in the study. The animals were procured from Mahaveera Enterprises, Hyderabad. They were maintained at a temperature of 25°C with a 12/12 h light/dark cycle in polypropylene cages. Animals were kept on a standard pellet diet and water *ad libitum* throughout the experiment. Animals were acclimatized to laboratory conditions for 10 days before initiation of the experimental protocol. The experiment was carried out by standard procedures and Experimental protocols were approved by our Institutional Animal Ethical Committee with approval no: 01/IAEC/SIMS/2023.

2.2 Chemicals

Isoproterenol (ISO) was purchased from the Sigma Aldrich. All other chemicals and reagents used in the experiments were of analytical grade and the same were purchased from the GMP certified companies through local vendors.

2.3 Collection of Plant Materials

Powder of the leaves from the *Albizia lebbeck* plant was collected from Brahma Natural Products of Vijayawada (A.P). The powder was then sieved and fine powder was collected for preparation of methanolic extract.

2.4 Preparation of Plant Extract

The powder (100 g) was subjected to continuous hot extraction with absolute methanol in a soxhelet apparatus at 40°C for 24 h. The residue obtained after the extraction process was dried using a rotary flash evaporator to get the completely dried product. The percentage yield of extract was determined as 4.28 g (4.28% w/w).

2.5 Grouping of Animals

All the animals were randomly assigned to four groups containing 6 each. The treatment of the groups is as follows:

Group I: Vehicle (0.5% CMC)

Group II: Isoproterenol hydrochloride (85 mg/kg body weight, i.p)

Group III: ISO (85 mg/kg; i.p) + MEAL (200 mg/kg; oral) suspended in 0.5% CMC

Group IV: ISO (85 mg/kg; i.p) + MEAL (400 mg/kg; oral) suspended in 0.5% CMC

MEAL dose was selected based on the acute toxicity study reported by Sivakumar *et al.* 2013[11].

2.6 Treatment Procedure

The rats in Group I received 1.0 ml of 0.5% CMC every day via intragastric intubation and served as the untreated control. Group II rats received isoproterenol (85 mg/kg body weight) intraperitoneally twice at an interval of 24 h on the 14th and 15th days. The rats in Group III received MEAL via intragastric intubation at a daily dose of (200 mg/kg body weight suspended in 0.5% CMC) for 15 days. Group IV rats received MEAL at 400 mg/kg suspended in 0.5% CMC for 15 days and at the last of the experimental period on the 14th and 15th days rats received isoproterenol (85 mg/kg body weight) injections intraperitoneally twice at an interval of 24 h. The experiment was terminated after 15 days and all the animals were killed by cervical decapitation after an overnight fast.

2.7 Induction of Myocardial Infarction

The myocardial infarction was induced by intraperitoneal (i.p) injection of isoproterenol hydrochloride (85 mg/kg body weight, dissolved in physiological saline, for two consecutive days (14th and 15th days)12.

2.8 Blood and Tissue Sampling

After 15 days of treatment, the animals were weighed while fasting overnight, and blood samples were taken by retro-orbital puncture. The anaesthesia for the procedure was administered through an intraperitoneal injection of a ketamine-xylazine mixture (ketamine 100 mg/kg and xylazine 10 mg/kg). At the end of the experiment, animals were euthanised under carbon dioxide asphyxiation. Hearts were collected, cleaned using normal saline and stored for further examinations.

2.9 Relative Organ Weight

Heart tissues were weighed and relative organ weight was calculated against body weight. A Sartorius analytical balance was used to weigh the organs moist, and the following equation was used to calculate the organ weight ratios as a proportion of body weight (relative organ weight):

$$\frac{Absolute\ organ\ weight}{Body\ weight\ at\ sacrifice} \times 100$$

2.10 Serum Preparation

Blood was obtained for clinical biochemistry in clot activator tubes. The serum was separated by centrifugation of blood samples approximately at 5000 rpm for 10 mins at 250C. The clear, clean serum was put into plastic tubes with labels and stored in the freezer at -4 °C for further use. Total proteins[13].

2.11 Tissue Homogenate Preparation

The Heart muscle samples from all experimental rats were sliced into pieces and homogenised in ice-cold 0.067 M phosphate buffer using a tissue homogeniser (Dynaken, Malaysia) to give 20% homogenate. After homogenisation, the samples were centrifuged at 4000 rpm for 10 min at 4°C

in a cold centrifuge. The subsequent supernatants were kept at -20 °C and used for Cardiac markers estimations. Cardiac markers like LDH[14], CK-MB (standard assay kit Coral Clinical Systems (Tulip Diagnostics)), and cTnI (ELISA, CAT IT6638, G-Biosciences) were analysed. Oxidative parameters like SOD[15], MDA[16] were also analysed.

2.12 Histopathological Analysis

For histopathological analysis, the pancreas and liver tissues were fixed in 10% formalin, dehydrated on treatment with a series of different concentrations of ethanol, cut into sections and embedded in paraffin wax. Specimen slides with 5 µm thick tissue sections were prepared using a semi-automatic rotatory microtome (M/s Biocraft, India). Hematoxylin and eosin stains were used to stain these slides, and a light microscope (BX 43, Olympus, India) was used to take photomicrographs.

2.13 Statistical Analysis

The statistical analysis was done using SPSS V26 software. All data are expressed as mean ± standard deviation. The statistical significance is calculated via one-way ANOVA, with Tukey's post hoc test for the biochemical data, and Dunnet's multiple comparison tests for cell culture and animal experiment data. The results have been expressed in $p < 0.05$, 0.01, and 0.001 for statistical significance.

3. Results

3.1 Relative Organ Weight

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased relative heart weight compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* eaves to the ISO-treated rats showed a significant decrease in the heart weight at a dose of 400 mg/kg body weight.

3.2 Effect on Cardiac Parameters

3.2.1 Effect of MEAL on Cardiac Troponin I (cTnI)

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased the CtnI levels compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* leaves to the ISO-treated rats showed a significant decrease in the levels of CtnI at a dose of 400 mg/kg body weight (Figure 2(A)).

3.2.2 Effect of MEAL on LDH

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased the LDH compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* eaves to the ISO-treated rats showed a significant decrease in the levels of LDH at both doses of 200 and 400 mg/kg body weight (Figure 2(B)).

3.2.3 Effect of MEAL on Ck-MB

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased the Ck-MB compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* leaves to the ISO-treated rats showed a significant decrease in the levels of Ck-MB at a dose of 400 mg/kg body weight (Figure 2(C)).

3.2.4 Effect of MEAL on Total Proteins

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased the total proteins compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* leaves to the ISO-treated rats showed a significant decrease in the levels of proteins at a dose of 400 mg/kg body weight (Figure 3(A)).

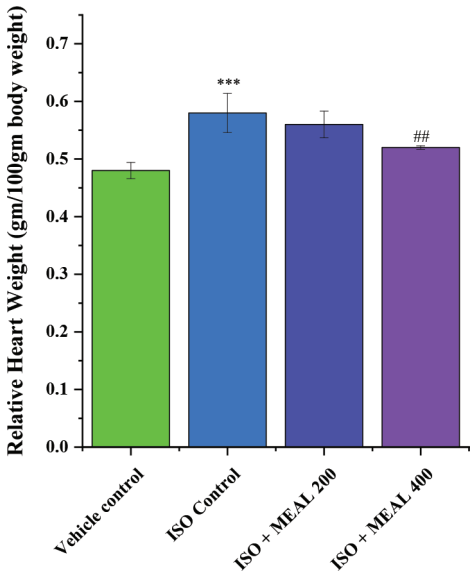


Figure 1. Relative heart weight of Wistar rats. The values were presented as Mean ± SEM (n=6); ONE WAY ANOVA test was done using Tukey's post hoc test and considered P<0.05, 0.01, 0.001 were significant. *** P<0.001 when compared Vehicle Control; ##P<0.01 when compared to ISO control.

3.2.5 Effect of MEAL on Oxidative Stress Markers

3.2.5.1 Effect of MEAL on Superoxide Dismutase (SOD)

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly decreased the SOD compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* leaves to the ISO-treated rats showed a significant increase in the levels of SOD at a dose of 400 mg/kg body weight (Figure 3(B)).

3.2.5.2 Effect of MEAL on Malondialdehyde (MDA)

The study found the animals treated with ISO 85 mg/kg body weight were found to have significantly increased the MDA compared to vehicle control. Treatment of methanolic extract of *A. lebbeck* leaves to the ISO-treated rats showed a significant decrease in the levels of SOD at a dose of 400 mg/kg body weight (Figure 3(C)).

3.3 Effect of MEAL on Gross Pathology of Heart

In the ISO-alone-administered group, a significant anatomical change in the heart shape was observed in comparison to vehicle control group rats. This remodelling of rat heart shape also confirms that cardiotoxicity was successfully induced. The white or pale grey area in cardiac tissues is identified as an infarct area. This infarct area was significantly reduced in MEAL (200 and 400 mg/kg) administered groups as compared to isoproterenol (Figure 4).

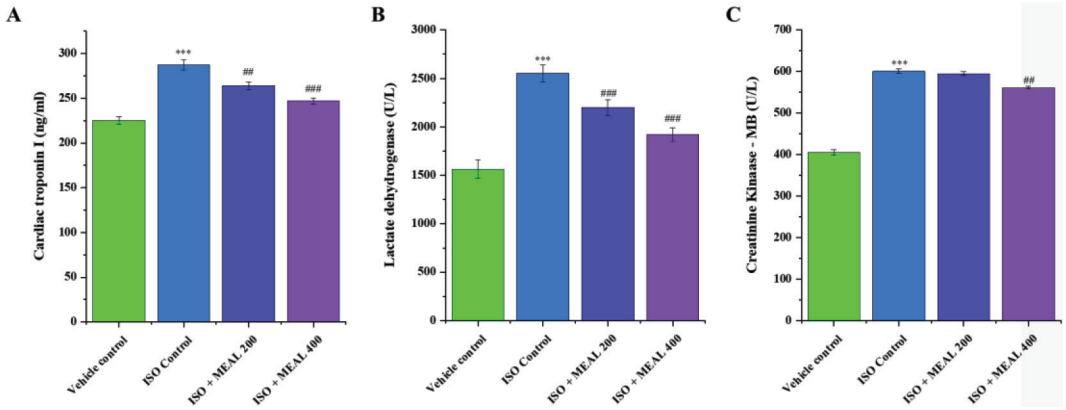


Figure 2. Effect of MEAL on cardiac parameters. **(A)** cTnI, **(B)** LDH, **(C)** Ck-MB.
The values were presented as Mean ± SEM (n = 6); ONE WAY ANOVA test was done using Tukey's post hoc test and considered P<0.05, 0.01, 0.001 were significant. *** P<0.001 when compared Vehicle Control; ##P<0.01, ###P<0.001 when compared to ISO control.

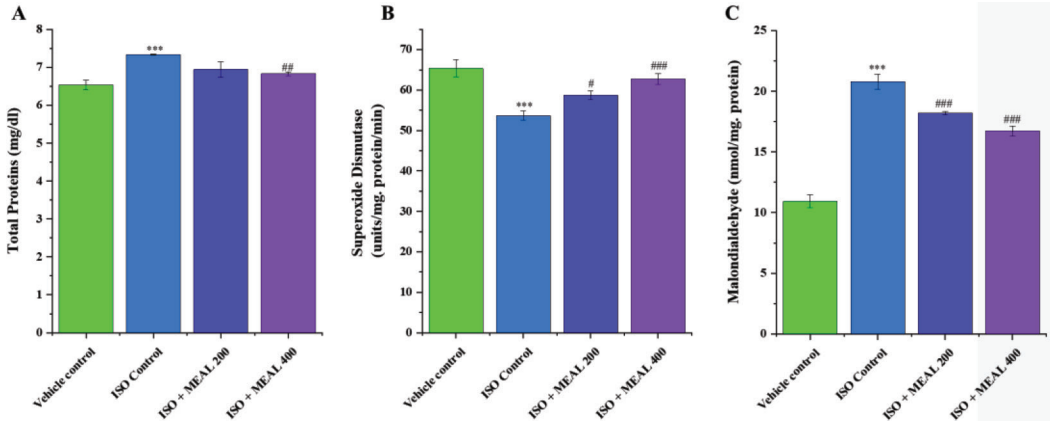


Figure 3. Effect of MEAL on **(A)** Total proteins, **(B)** SOD, **(C)** MDA.
The values were presented as Mean ± SEM (n = 6); ONE WAY ANOVA test was done using Tukey's post hoc test and considered P<0.05, 0.01, 0.001 were significant. *** P<0.001 when compared Vehicle Control; ##P<0.01, ###P<0.001 when compared to ISO control.

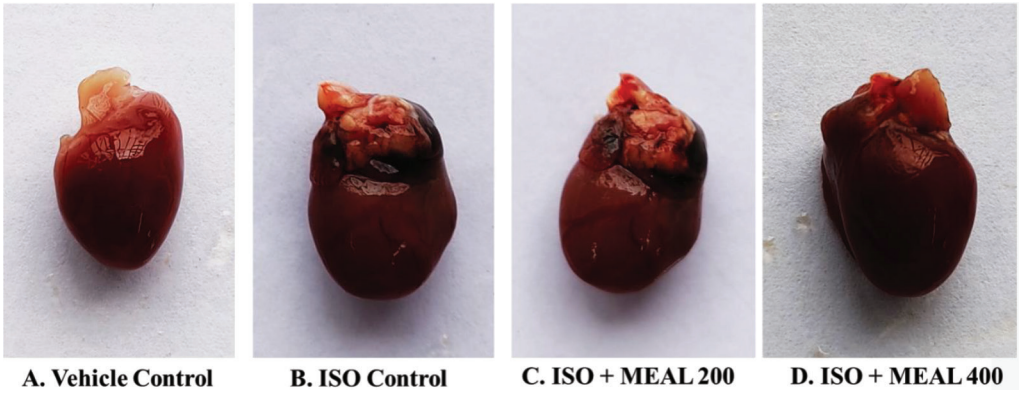


Figure 4. Effect of MEAL on the heart.

3.4 Effect of MEAL on Histopathology of Heart

In isoproterenol-treated rats' hearts, histological changes such as perivascular cuffing of vasa vasorum with intimal fibrosis, disruption of medial elastic fibres with diffuse interstitial fibrosis and myocytolysis were seen. In MEAL (400 mg/kg body weight) and isoproterenol (85 mg/kg body weight) treated rats there was no appreciable change in the heart (Figure 5).

4. Discussion

Isoproterenol (ISO), a β-adrenergic agonist, induces considerable oxidative stress in the heart, leading to infarct-like damage in the myocardium. While various mechanisms have been suggested for ISO-induced myocardial damage, the substantial generation of ROS through the autoxidation of catecholamines stands out as a significant contributing factor[17]. When administered in large doses, ISO triggers morphological and functional changes in the heart, ultimately resulting in myocardial necrosis. This detrimental effect is exacerbated by the excessive production of free radicals during the oxidative metabolism of catecholamines. Mounting evidence supports the notion that the cardiotoxicity of ISO is primarily attributed to the generation of free radicals and the ensuing oxidative stress[18].

In the current investigation, a notable reduction in body weight, coupled with a substantial rise in both heart weight and the heart-to-body weight ratio, was observed in rats treated with isoproterenol. The heightened ratio implies cardiac hypertrophy, likely stemming from factors such as increased ventricular stiffness, elevated water content, oedematous intramuscular space, and extensive necrosis of cardiac muscle. This necrotic process may subsequently lead to the infiltration of damaged tissue by inflammatory cells[19].

Treatment with MEAL significantly decreased the heart/body weight ratio, suggesting that the combination could effectively suppress the stimulus for hypertrophy.

The current research indicates that the application of ISO leads to a significant increase in the concentrations of cardiac serum marker enzymes such as LDH and CK-MB. The findings of this study align with those documented by Kurian *et al.*, in 2005[20]. These enzymes, specific to the cardiovascular system, are released into the bloodstream when the heart experiences damage, typically caused by insufficient oxygen supply or glucose. This damage can lead to increased permeability or rupture of cell membranes, resulting in the release of enzymes into the serum[21].

LDH is a cytosolic enzyme with five isoforms, namely LDH 1 to LDH 5. In cardiac tissue, LDH 1 and LDH 2 are the predominant forms. Consequently, identifying an elevated concentration of these enzymes serves as a conclusive diagnostic indicator for cardiac toxicity. In our current investigation, we noted a rise in the intensity of LDH1 and LDH2 isoenzymes in the serum of rats treated with isoproterenol, possibly attributed to isoproterenol-induced necrosis in the rats[22].

Creatine kinase-MB (CK-MB), an enzyme predominantly found in the heart, serves as a crucial biomarker. Elevated CK-MB levels in the bloodstream indicate myocardial injury, making it valuable in assessing the extent of damage in experimental models. While isoproterenol-induced cardiotoxicity studies often incorporate CK-MB measurements, it's important to consider experimental conditions, including dosage and duration of exposure. CK-MB's utility in these contexts

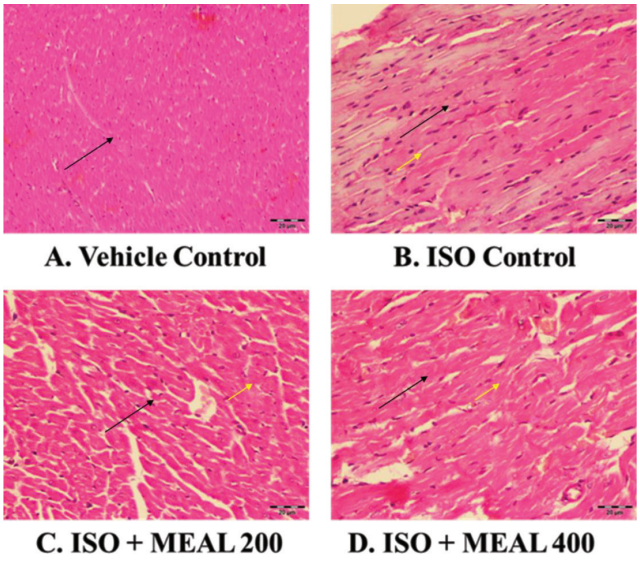


Figure 5. Effect of MEAL on the HE stained sections of heart at 200X magnification. (Black arrow – arrangement of myofibrils, yellow arrow – separation of myofibrils).

underscores its role as a marker for myocardial damage in response to isoproterenol-induced stress[22].

Treatment with MEAL normalized the levels of serum biomarker enzymes compared to MEAL 200+ISO and MEAL 400+ISO treatment groups. This protection might be due to the effect of MEAL on the myocardium, which had reduced the extent of myocardial damage induced by ISO and thereby restricting the leakage of these enzymes from the myocardium, suggesting the membrane stabilizing potential of the combination.

Cardiac troponin, a low molecular weight protein integral to the myofibrillary contractile apparatus of cardiac muscle, serves as a sensitive and specific marker for myocardial cell injury. Normally absent in serum, it is released following myocardial necrosis[23]. In the study, a notable elevation in serum cardiac troponin I (cTnI) levels was observed in rats treated with Isoproterenol (ISO) compared to the control group, indicating ISO-induced cardiac damage. Remarkably, animals pretreated with MEAL 400 mg/kg before ISO administration showed a significant reduction in cTnI levels compared to ISO-treated rats without MEAL pretreatment. This outcome aligns with findings in a prior study conducted by Priscilla and Prince, reinforcing the potential protective effect of MEAL against ISO-induced cardiac damage as evidenced by the reduced levels of cTnI[24]. The assumption is that MEAL could potentially safeguard the structural and functional integrity of the contractile apparatus, thereby preventing cardiac damage and the subsequent release of troponins from the heart into the bloodstream. However, it is imperative to conduct additional research to precisely unravel the mechanisms responsible for the cardioprotective effects attributed to MEAL.

Endogenous antioxidant enzymes, such as Superoxide Dismutase (SOD), are equipped with superoxide anions, playing a crucial role in safeguarding cell membranes against oxidative stress and preventing cellular damage induced by Reactive Oxygen Species (ROS)[25]. In rats subjected to Isoproterenol (ISO) intoxication, a significant rise in lipid peroxidation levels and a noteworthy decline in SOD activities were observed, indicating the involvement of oxidative stress in ISO-induced toxicity. This outcome aligns with findings from prior studies[26]. The heightened levels of lipid peroxides in ISO-treated rats could be attributed to lipid accumulation in the heart and irreversible damage to myocardial membranes. The reduced SOD activity in this study may result from the increased generation of highly cytotoxic free radicals due to the auto-oxidation of catecholamines.

The histological examination of the hearts of rats treated with isoproterenol revealed notable pathological changes, including perivascular cuffing of vasa vasorum with intimal fibrosis, disruption of medial elastic fibres accompanied by diffuse interstitial fibrosis, and myocytolysis. These alterations are indicative of vascular inflammation, structural damage to blood vessels, and myocardial cell destruction. However, in rats concurrently treated with MEAL (at a dose of 400 mg/kg body weight) and isoproterenol, there was a remarkable absence of significant changes in the heart tissue. This suggests a potential protective effect of MEAL against the adverse histological effects induced by isoproterenol. The findings raise intriguing possibilities regarding MEAL's role in mitigating cardiac damage and call for further investigation into the specific mechanisms underlying its cardioprotective properties. If validated, MEAL could emerge as a promising candidate for therapeutic interventions aimed at preventing or ameliorating cardiac conditions associated with stress-induced damage.

5. Conclusion

In conclusion, this study provides compelling evidence of the detrimental impact of Isoproterenol (ISO) on the myocardium, characterized by oxidative stress, infarct-like damage, and myocardial necrosis. Treatment

with MEAL shows promise in reducing hypertrophy, stabilizing membranes, and protecting against structural damage, suggesting its potential as a cardioprotective agent. These findings propose MEAL as a promising candidate for mitigating ISO-induced cardiac damage, calling for further investigations into its precise mechanisms and therapeutic potential in stress-induced cardiac conditions. If validated, MEAL could emerge as a valuable cardioprotective agent.

6. References

1. Kadali VN, Kindangi KR, Pola SR, Ramesh T, Sandeep BV. Cardio-protective plants present in west Godavari district of Andhra Pradesh, India: A short review. Int J Pharmacogn. 2016; 3:19-25.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, *et al*. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380(9859):2095-128. https://doi.org/10.1016/S0140-6736(12)61728-0 PMid:23245604
3. Barnes AS. Emerging modifiable risk factors for cardiovascular disease in women: Obesity, physical activity, and sedentary behavior. Texas Hear Inst J. 2013; 40(3):293-5.
4. Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S. A review on coronary artery disease, its risk factors, and therapeutics. J Cell Physiol. 2019; 234(10):16812-23. https://doi.org/10.1002/jcp.28350. PMid:30790284
5. Pencina MJ, Navar AM, Wojdyla D, Sanchez RJ, Khan I, Elassal J, *et al*. Quantifying importance of major risk factors for coronary heart disease. Circulation. 2019; 139(13):1603-11. https://doi.org/10.1161/CIRCULATIONAHA.117.031855 PMid:30586759 PMCid:PMC6433489
6. Pěsíc M, Stanković S. Development of natural product drugs in a sustainable manner. Brief for United Nations Global Sustainable Development Report; 2015.
7. SS Agarwal. Paridhavi M. Herbal Drug Technology. Vol 9. University Press (India) Private Limited; 2007.
8. Hassan LG, Umar KJ, Atiku I. Nutritional evaluation of *A. lebbeck* (L.) pods as source of feeds for livestock. Am J Food Technol. 2007; 2(5):435-9. https://doi.org/10.3923/ajft.2007.435.439
9. Khalil MI, Ahmmed I, Ahmed R, Tanvir EM, Afroz R, Paul S, *et al*. Amelioration of isoproterenol-induced oxidative damage in rat myocardium by *Withania somnifera* leaf extract. Biomed Res Int. 2015; 2015:1-10. https://doi.org/10.1155/2015/624159 PMid:26539517 PMCid:PMC4619872
10. Huang H, Geng Q, Yao H, Shen Z, Wu Z, Miao X, *et al*. Protective effect of scutellarin on myocardial infarction induced by isoprenaline in rats. Iran J Basic Med Sci. 2018; 21(3):267-76.
11. Sivakumar B, Velmurugan C, Bhargava A, Kumar PL. Diuretic activity of methanolic extract of *Albizia lebbeck*. Res Rev J Pharmacol Toxicol Stud. 2013;1(1):3-5.
12. Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian J Pharmacol. 2011; 43(5):516-19. https://doi.org/10.4103/0253-7613.84957 PMid:22021992 PMCid:PMC3195119
13. Flack CP, Woollen JW. Prevention of interference by dextran with biuret-type assay of serum proteins. Clin Chem. 1984; 30(4):559-61. https://doi.org/10.1093/clinchem/30.4.559 PMid:6200256
14. MacDonald RP. Serum lactic dehydrogenase-a diagnostic aid in myocardial infarction. J Am Med Assoc. 1957; 165(1):35. https://doi.org/10.1001/jama.1957.02980190037009 PMid:13462739
15. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3):469-74. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x PMid:4215654
16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3 PMid:36810
17. Mert H, Yilmaz H, Irak K, Yildirim S, Mert N. Investigation of the protective effect of kefir against isoproterenol induced myocardial infarction in rats. Korean J Food Sci Anim Resour. 2018; 38(2):259-72.
18. Rajadurai M, Prince PSM. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology. 2007; 230(2-3):178-88. https://doi.org/10.1016/j.tox.2006.11.053 PMid:17188415
19. Nirmala C, Puvanakrishnan R. Protective role of curcumin against isoproterenol induced myocardial infarction in rats. Mol Cell Biochem. 1996; 159(2):85-93. https://doi.org/10.1007/BF00420910 PMid:8858558
20. Kurian GA, Philip S, Varghese T. Effect of aqueous extract of the *Desmodium gangeticum* DC root in the severity of myocardial infarction. J Ethnopharmacol. 2005; 97(3):457-61. https://doi.org/10.1016/j.jep.2004.11.028 PMid:15740881
21. Arya DS, Bansal P, Ojha SK, Nandave M, Mohanty I, Gupta SK. Pyruvate provides cardioprotection in the experimental model of myocardial ischemic reperfusion injury. Life Sci.

2006; 79(1):38-44. https://doi.org/10.1016/j.lfs.2005.12.039 PMid:16457854

22. Maghamiour N, Safaie N. High Creatine Kinase (CK)-MB and Lactate dehydrogenase in the absence of myocardial injury or infarction: A case report. J Cardiovasc Thorac Res. 2014; 6(1):69-70.
23. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined- a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol. 2000; 36(3):959-69. https://doi.org/10.1016/S0735-1097(00)00804-4 PMid:10987628
24. Priscilla DH, Prince PSM. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem Biol Interact. 2009; 179(2-3):118-24. https://doi.org/10.1016/j.cbi.2008.12.012 PMid:19146839
25. Wei H, Li H, Wan SP, Zeng Q-T, Cheng L-X, Jiang L-L, *et al.* Cardioprotective effects of malvidin against isoproterenol-induced myocardial infarction in rats: A mechanistic study. Med Sci Monit. 2017; 23:2007-16. https://doi.org/10.12659/MSM.902196 PMid:28445445 PMCid:PMC5414594
26. Rajadurai M, Prince PSM. Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: Biochemical and histopathological evidences. Toxicology. 2006; 228(2-3):259-68. https://doi.org/10.1016/j.tox.2006.09.005 PMid:17084010