Natural Products Analysis
https://www.sciltp.com/journals/npa

Article

Check for updates

Cardio-Protection by *Tamarindus indica* Leaves: Mitigating Left Ventricular Hypertrophy in Isoproterenol-Induced Mice

Farjana Sharmin [1,2], Farhad Hossain [2,3], Joy Sarker [2], Ibrahim Hossain [2], Rozina Khatun [2], Khadiza Khanam [2], A. B. M. Ashraful [2,4], Medha Islam [2], AHM Khurshid Alam [2] and Mamunur Rashid [2,*]

[1] Department of Pharmacy, Khwaja Yunus Ali University, Sirajganj 6751, Bangladesh
[2] Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh
[3] Department of Pharmacy, State University of Bangladesh, Dhaka 1461, Bangladesh
[4] Department of Pharmacy, R. P. Shaha University, Narayanganj 1400, Bangladesh
* Correspondence: mamun69jp@yahoo.com

How To Cite: Sharmin, F.; Hossain, F.; Sarker, J.; et al. Cardio-Protection by *Tamarindus indica* Leaves: Mitigating Left Ventricular Hypertrophy in Isoproterenol-Induced Mice. *Natural Products Analysis* **2026**, *2*(1), 100013. https://doi.org/10.53941/npa.2026.100013

Received: 23 April 2026
Revised: 22 June 2026
Accepted: 26 June 2026
Published: 30 June 2026

Abstract: Introduction: Cardiovascular diseases (CVDs) are the leading cause of death globally, with left ventricular hypertrophy (LVH) as a key independent risk factor. Objective: This study evaluates the cardioprotective potential of *Tamarindus indica* leaf extract (TILE) in mitigating isoproterenol-induced Left Ventricular Hypertrophy (LVH), a critical risk factor for cardiovascular diseases (CVDs). Methods: The crude extract underwent phytochemical screening followed by total phenolic content, total flavonoid content, DPPH, and OH radical scavenging assays. Swiss albino mice with isoproterenol-induced LVH were treated orally with TILE (three doses) over four weeks. Key parameters, including lipid profiles (TC, TG, HDL, LDL), cardiac biomarkers (Troponin I), oxidative stress markers (SOD, CAT), liver and renal function (SGPT, SGOT, Serum Creatinine), LVW/BW ratio, and histopathology, were measured and compared with atorvastatin, which has been taken as a standard drug. Results: TILE showed considerable phenolic (29.69 ± 2.14 mg/g) and flavonoid contents (145.42 ± 7.58 mg/g) and potent antioxidant activity with IC_{50} values of 10.82 µg/mL (DPPH) and 17.86 µg/mL (OH radical scavenging assay). Isoproterenol elevated TC, TG, LDL cholesterol, Troponin I, SGPT, SGOT, and serum creatinine, while reducing HDL cholesterol, SOD, and catalase levels. TILE treatment reversed these changes significantly, demonstrating antioxidative and antihyperlipidemic properties, and reducing cardiac hypertrophy by improving the LVW/BW ratio. Conclusion: TILE exhibited significant cardioprotective effects against isoproterenol-induced LVH, which were associated with improvements in antioxidant enzyme activities and lipid profile parameters. However, further studies are required to elucidate the precise molecular mechanisms underlying these effects.

Keywords: *Tamarindus indica*, antioxidant, cardioprotective, isoproterenol, hypertrophy

1. Introduction

Cardiovascular disease (CVD) encompasses a wide range of conditions affecting the circulatory system, including the heart and blood vessels. These are typically chronic diseases that develop gradually and often remain asymptomatic for extended periods. Among the key risk factors for CVD is left ventricular hypertrophy (LVH), which is associated with increased cardiovascular morbidity and mortality [1] and is recognized as an independent predictor of cardiovascular events such as coronary artery disease, stroke, and heart failure [2].

LVH refers to the thickening and stiffening of the left ventricle, the heart's primary pumping chamber, leading to compromised cardiac output. It often arises as a compensatory response to chronic pressure overload caused by hypertension or aortic stenosis [3]. A key mechanism underlying this adaptation is the activation of the intracardiac renin-angiotensin system, which increases the production of angiotensin II [4]. Angiotensin II triggers multiple signaling cascades through the activation of phospholipases, generating secondary messengers such as inositol trisphosphate (IP_3) and diacylglycerol (DAG) [5,6]. These messengers further activate the small G-protein Rho via distinct pathways: IP_3 through Ca^{2+} mobilization and DAG via protein kinase C (PKC) [7,8]. Activated Rho subsequently stimulates Rho kinase (ROCK), which drives hypertrophic gene expression and contributes to myocardial remodeling [9].

Another major pathway involved in myocardial hypertrophy is β-adrenergic receptor agonism. Activation of β_1 and β_2 receptors stimulates the G-protein coupled receptor system, elevating intracellular cAMP, which activates protein kinase A (PKA). PKA then modulates downstream targets, including L-type calcium channels, phospholamban, and troponin [10]. Simultaneously, the Gq/G11 pathway activates small G-proteins, such as Ras and Rho, initiating downstream signaling cascades, including the Ras-Raf-MEK1/2-ERK1/2 and RhoA-ROCK pathways, both of which are critical for hypertrophic gene transcription [11−13].

In addition, Gαs-mediated activation of Rac1 leads to stimulation of NADPH oxidase, resulting in the generation of superoxide and the redox-sensitive transcription factor NF-κB, which further regulates genes involved in myocardial hypertrophy [14]. Elevated Rac1 activity and increased oxidative stress have been identified as markers of left ventricular hypertrophy in humans [15]. Therefore, antioxidant compounds may offer therapeutic potential in the management of LVH.

Bangladesh, rich in plant biodiversity, is home to numerous plants with antioxidant properties. One such plant is *Tamarindus indica (T. indica)* Linn., traditionally used in countries such as Bangladesh, India, Sudan, and Nigeria for a variety of ailments. It has been reported to possess multiple pharmacological properties, including antioxidant, anti-diabetic, antimicrobial, antimalarial, hepatoprotective, laxative, anti-asthmatic, and anti-hyperlipidemic activities [16]. Almost every part of the plant, leaves, seeds, pulp, and roots, has been associated with medicinal benefits [17]. In Bangladesh, its fruit pulp is commonly used to manage hypertension. However, this study focused on the leaves of *T. indica*, which are available year-round and are known to contain a high concentration of phenolic compounds (approximately 80%), flavonoids (60–70%), and tannins (50%) [17]. Additionally, polyphenols such as catechin and epicatechin [18], triterpenes like lupeol and lupanone [19], and essential oils including limonene and benzyl benzoate [20] have been identified in its leaves.

Despite the availability of various synthetic agents for CVD management, these drugs often pose limitations in terms of cost, side effects, and long-term tolerability. Therefore, this study aims to explore a natural and affordable alternative for managing LVH. The objective of this investigation is to evaluate the cardioprotective potential of *T. indica* leaf extract in an isoproterenol-induced LVH mouse model, with a focus on its antioxidant-rich phytochemical composition.

2. Materials and Methods

2.1. Chemicals

The USA-based Sigma Chemical Company (Burlington, MA, USA) was the source of DPPH and BHT. We bought gallic acid from Wako Pure Chemicals Ltd. in Japan. The following materials were purchased from Sigma-Aldrich in Germany: sodium carbonate, tri-chloroacetic acid (TCA), ferric chloride (FCR), catechin, ammonium molybdate, 2-Deoxy-D-ribose, ascorbic acid, ethanol, and chloroform. Pyrogallol was acquired from QualiChem in India, while cobalt (II) nitrate hexahydrate and sodium hexa-metaphosphate was purchased from SMART-LAB, Indonesia.

2.2. Medications & Diagnostic Kits

Atorvastatin powder was provided by Square Pharmaceuticals Ltd. Isoprenaline injection (Isolin), and Heparin were bought from Samarth Life Sciences PVT. LTD., Mumbai, India, and PANPHARMA Ltd., Dhaka, Bangladesh, respectively.

The test kits for triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), serum creatinine, troponin I, SGPT, and SGOT were purchased from Human, Germany.

2.3. Collection & Extraction of Plant Material

Mature green *T. indica* leaves were collected from the Rajshahi University campus (latitude: 24.3682° N, longitude: 88.6376° E) in Bangladesh and verified by Dr. A.H.M. Mahbubur Rahman, a specialist in taxonomy from the Department of Botany at the University of Rajshahi (Collection number: FK 121). For extraction, one kilogram of the coarsely ground leaves was soaked in ethanol at room temperature, allowed to stand for seven days with sporadic shaking and stirring. The mixture was then filtered. To ensure complete extraction, the soaking, intermittent stirring, and filtration processes were repeated once using the same plant material. After soaking the powder, the procedure was carried out once more. The collected ethanolic extract was concentrated in a rotary evaporator at a lower pressure after 14 days to obtain the TILE (98.2 g) [21].

2.4. Phytochemical Screening of TILE

TILE was subjected to qualitative phytochemical tests using the standard phytochemical methods described by Islam et al. [22] to identify the presence of various phytochemicals, such as alkaloids, cardiac glycosides, steroids, tannins, saponins, phenolic compounds, terpenoids, and reducing sugar.

2.5. Total Phenolic and Flavonoid Tests

Total phenolic and flavonoid contents were determined following the method of Reza et al. with minor modifications [23]. For total phenolic content (TPC), plant extracts or standard solutions were reacted with diluted Folin–Ciocalteu reagent and 7.5% Na_2CO_3, incubated at 25 °C for 30 min, and the absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE)/g dry weight.

Total flavonoid content (TFC) was assessed using the aluminum chloride colorimetric method. Extracts or standards were mixed sequentially with 5% $NaNO_3$, 10% $AlCl_3$, and 4% NaOH, followed by measurement of absorbance at 510 nm. Flavonoid content was calculated from a catechin standard curve and expressed as mg catechin equivalents (CE)/g dry weight. All measurements were performed in triplicate.

2.6. Antioxidant Activity Tests

2.6.1. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

The DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Free Radical Scavenging Activity of TILE was assessed by a method described by Hossain et al. [24]. The extract was added to the methanol at different concentrations, and the absorbance was recorded at 517 nm. The percentage of scavenging activity of DPPH radicals was calculated from the following equation:

$$\% \text{ I} = \frac{Ac - As}{Ac} \times 100\%$$

where, % I = the percentage of scavenging activity, *Ac* = the absorbance of the control, *As* = the absorbance of the extract/standard.

2.6.2. Hydroxyl (OH) Radical Scavenging Assay

The OH radical scavenging activity of *T. indica* extract was determined using the method described by Gutteridge and Halliwell with slight modifications [25,26]. Test tubes were filled with different concentrations of TILE and standard (Catechin). The reaction mixture was incubated at 37 °C for 60 min, then 1% ice-cool TBA (Thiobarbituric acid) & 10% TCA (Trichloroacetic acid) were added. The absorbance of each tube was measured at 532 nm using Shimadzu UV-1800 UV-Visible spectrophotometer. Using the following formula, the percentage of OH radical scavenging activity was determined:

$$\% \text{ I} = \frac{Ac - As}{Ac} \times 100\%$$

where, % I = the percentage of scavenging activity, *Ac* = the absorbance of the control, *As* = the absorbance of the extract/standard.

2.7. Experimental Animals & Study Design

A total of 42 Swiss Albino female mice, aged 2 months, weighing around 40 g, were purchased from the Department of Biochemistry, Rajshahi University, Bangladesh. Before the beginning of the experiments, all the mice were adjusted to a new environment for two weeks, housed in cages, and monitored for behavioral changes. The experimental protocol was approved (Ethical clearance number: 249) by the Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (IAMEBBC) at the Institute of Biological Science, University of Rajshahi, Bangladesh, on 16 November 2022.

Mice were randomly assigned to 6 groups (Normal Control (NOC), Negative Control (NC), Standard (Atvn-20), Treatment A (TILE-50), Treatment B (TILE-100), Treatment C (TILE-200)), 7 mice in each group. All the experimental groups were hypertrophic groups except the Normal Control. Cardiac hypertrophy was induced by intraperitoneal administration of isoproterenol. The mice received isoproterenol at a dose of 5 mg/kg/day for seven consecutive days [27]. After the induction of cardiac hypertrophy, the standard group was treated with Atorvastatin (0.3 mg/kg BW, oral), and Treatment A, B & C groups were treated orally with TILE at 50, 100 & 200 mg/kg BW, respectively. Repeated dose treatment was performed for four weeks to observe the effects of different doses of TILE on lipid profile, Troponin-I, SOD, catalase, LVW/BW ratio, SGPT, SGOT, and serum creatinine in isoproterenol-induced hypertrophic mice (IIHMs).

2.8. Dose Selection

The doses of 50, 100, and 200 mg/kg body weight used in the present study were selected based on previously published pharmacological investigations of *T. indica* leaf extracts, which demonstrated efficacy and an acceptable safety profile within this dose range and at substantially higher doses [28]. These doses were chosen to evaluate potential dose-dependent cardioprotective effects while remaining well below levels previously reported to be safe in experimental animals.

2.9. Preparation of Doses of Atorvastatin & TILE

Atorvastatin, an amorphous soluble substance, was prepared in doses of 0.3 mg/kg body weight, according to a previously published report [29], since atorvastatin is effective in such a dose in humans. The crude leaf extracts were dissolved in distilled water. Three different dilutions of the extract were made using distilled water to obtain 50 mg/kg, 100 mg/kg, and 200 mg/kg of TILE. 100 µL of each solution was administered by oral gavage to the mice.

2.10. Biochemical Assays

After four weeks of treatment, mice were anesthetized, and the blood was collected from the thoracic artery, and serum was separated for biochemical studies after centrifugation. Heart was collected for histopathological investigation.

2.10.1. Measurement of Lipid Profile

The serum lipid profile was estimated using UV spectrophotometric methods using Cholesterol Liquicolor test kits, TG Liquicolor test kits, HDL Cholesterol (HDL-C) test kits, and LDL Cholesterol (LDL-C) test kits.

2.10.2. Cardiac Troponin I (cTnI) Test

Cardiac cTnI level in serum was determined by using cTnI ELISA test kit. The test is based on the principle of a solid-phase enzyme-linked immunosorbent assay.

2.10.3. Serum Creatinine Test

The serum creatinine test was conducted using a test kit purchased from Human, Germany. The principle of this test is that creatinine forms an orange-red colored complex with picric acid in an alkaline solution. The complex's absorbance is directly correlated with the sample's creatinine concentration.

2.10.4. Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) Test

Serum SGPT & SGOT test was performed using SGPT & SGOT test kits (ELISA kits) (Human, Germany).

2.10.5. Estimation of Superoxide Dismutase (SOD) & Catalase (CAT) Enzyme Levels

The liver was removed from the mice and homogenized in Tris buffer for the investigation. After centrifuging the homogenate, the supernatant was examined for the presence of catalase and superoxide dismutase (SOD). SOD was assayed using pyrogallol auto-oxidation, which produces a brown compound that absorbs UV light [30].

Catalase activity was measured using the Hadwan method [31], which converts cobalt (II) to cobalt (III) by hydrogen peroxide in bicarbonate solution. The 440-nm band was used for catalase activity assessment.

2.10.6. LVW/BW Ratio

During LVH, cardiomyocyte enlargement leads to an increase in left ventricular mass. Consequently, the LVW/BW ratio increases and is widely used as an index of cardiac hypertrophy. Therefore, an elevated LVW/BW ratio is indicative of hypertrophic remodeling of the left ventricle. LVW/BW ratios were determined by weighing isolated left ventricles. After euthanasia, hearts were excised, and the left ventricle was separated from other cardiac tissues under a dissecting microscope, blotted dry, weighed, and normalized to body weight [32].

2.10.7. Histopathology of Left Ventricle

The cross-section was done according to Slaoui & Fiette [33]. The study involved cutting left ventricle tissues, staining them with hematoxylin and eosin, and scanning them at a magnification of about ×400 with an Optoedu microscope to determine the extent of cardiomyocyte hypertrophy after the sacrifice of mice.

2.11. Statistical Analysis

The results were represented as mean ± SEM using the GraphPad Prism 9 computer program. A one-way analysis of variance (ANOVA) was employed, with Dunnett's post-hoc test and students paired or unpaired t-tests as necessary. A description of the statistical technique used in each analysis was included in each figure. Results were considered to be significant when p values were less than 0.05 ($p < 0.05$) for paired or unpaired t-test or less than 0.01 ($p < 0.01$) for one-way analysis of variance (ANOVA).

3. Results

3.1. Phytochemical Analysis

The qualitative phytochemical analysis of the crude ethanolic extract of *T. indica* leaves reported the presence of alkaloids, cardiac glycosides, steroids, tannins, phenolics, flavonoids, and terpenoids (Table 1).

Table 1. Result of qualitative phytochemical Analysis.

Name of the Phytochemical	Result [Present (+)/Absent (−)]
Alkaloids	+
Glycosides	+
Steroids	+
Tannins	+
Saponins	−
Phenolic compounds	+
Flavonoids	+
Terpenoids	+
Reducing sugar	−

3.2. Total Phenolic and Flavonoid Content Tests

The total phenolic content (TPC) of the extract at 100 µg/mL was 29.69 ± 2.14 mg GAE/g dry weight. In contrast, the total flavonoid content (TFC) was substantially higher, reaching 145.42 ± 7.58 mg CAE/g dry weight (Figure 1).

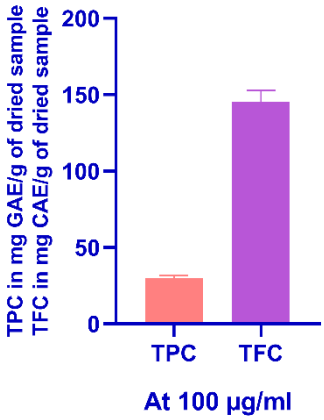

Figure 1. Determination of total phenolic content (TPC) and total flavonoid content (TFC).

3.3. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay and Hydroxyl (OH) Radical Scavenging Assay

The Antioxidant activity of the TILE was evaluated using a range of *in vitro* assays, including the DPPH radical and OH radical scavenging tests. The results, illustrated in Figure 2, demonstrated that TILE exhibited significant radical scavenging activity in a dose-dependent manner. The half-maximal inhibitory concentration (IC_{50}) values for DPPH and hydroxyl radical scavenging were found to be 10.82 µg/mL and 17.86 µg/mL, respectively, indicating potent antioxidant capacity.

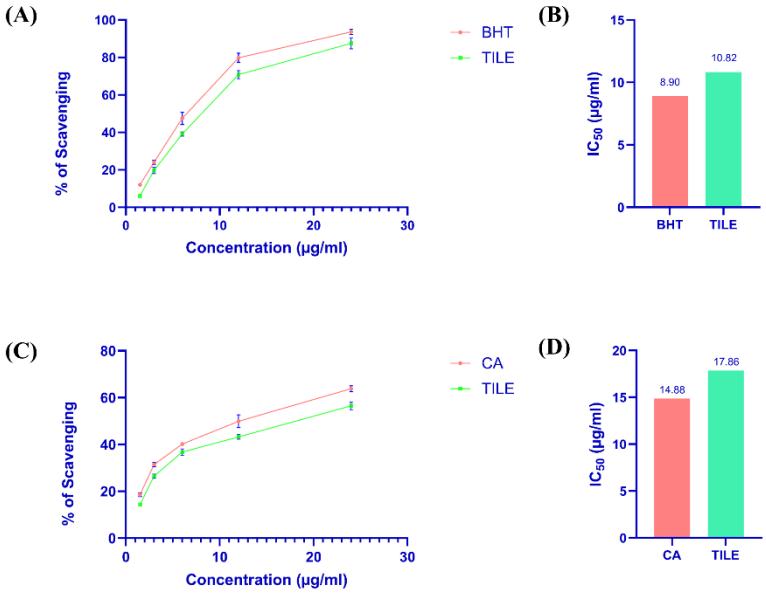

Figure 2. (**A**) Determination of DPPH free radical scavenging activity (**B**) Comparison of IC_{50} values extract with standard for DPPH free radical scavenging activity (**C**) Determination of Hydroxyl (OH) radical scavenging activity (**D**) Comparison of IC_{50} values extract with standard for Hydroxyl (OH) radical scavenging activity.

3.4. In-Vivo Assays

3.4.1. Effect of Atorvastatin and TILE on Lipid Profile

Induction of isoproterenol significantly changes the lipid profile in hypertrophic mice when compared with that of normal mice. To make it clear, we examined TC, TG, LDL-C, and HDL-C levels after four weeks of treatment with atorvastatin and different doses (50, 100, and 200 mg/kg) of TILE in hypertrophic mice.

Mice treated with isoproterenol for seven days exhibited significantly higher TC level (102.19 ± 2.41 mg/dl), TG level (152.59 ± 4.59 mg/dl), and LDL cholesterol level (91.81 ± 0.78 mg/dl), but lowered HDL cholesterol level (22.90 ± 0.90 mg/dl) than normal mice (77.70 ± 4.36 mg/dl). Following four weeks' TILE treatment, it was

found that all three doses of TILE, as well as the standard drug atorvastatin, significantly decreased the TG, TC, and LDL-C levels, whereas increasing the HDL-C levels in comparison to IIHMs given in Figure 3.

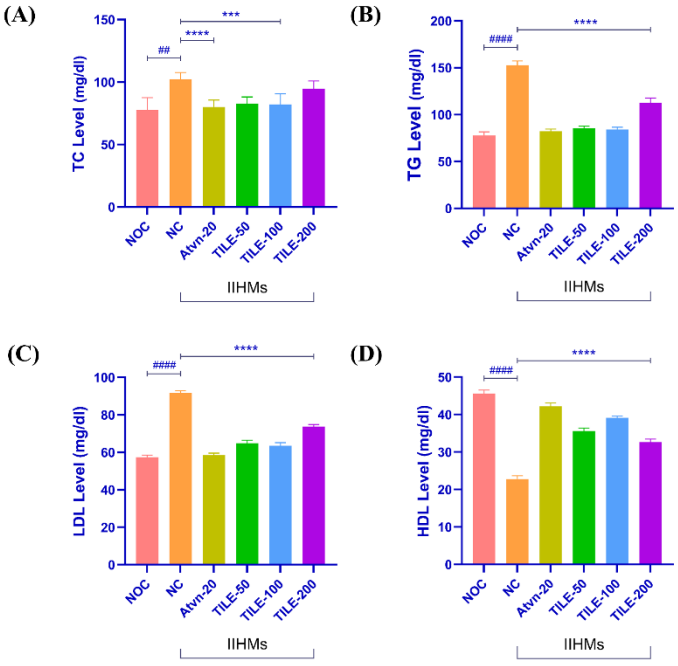

Figure 3. Effects of TILE on (**A**) Total Cholesterol, (**B**) Triglyceride level, (**C**) LDL-C level, (**D**) HDL-C level in IIHMs for four weeks in comparison with IIHMs. Data are represented as mean ± SEM, n = 7 in each group. $^{\#\#}$ $p < 0.01$, $^{\#\#\#\#}$ $p < 0.0001$ vs. normal (t-test) and *** $p < 0.001$, **** $p < 0.0001$ vs. IIHMs (ANOVA followed by Dunnett's test).

3.4.2. Cardiac Troponin I Test

Mice treated with isoproterenol for seven days exhibited significantly higher cTnI levels (0.081 ± 0.004 ng/mL) than normal mice (0.010 ± 0.001 ng/mL). Following the four-weeks' TILE treatment, it was found that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE decreased the cTnI levels to (0.037 ± 0.004 ng/mL), (0.018 ± 0.002 ng/mL), and (0.043 ± 0.003 ng/mL), respectively, in comparison to IIHMs. Additionally, atorvastatin significantly decreased the cTnI level (0.019 ± 0.002 ng/mL) (Figure 4A).

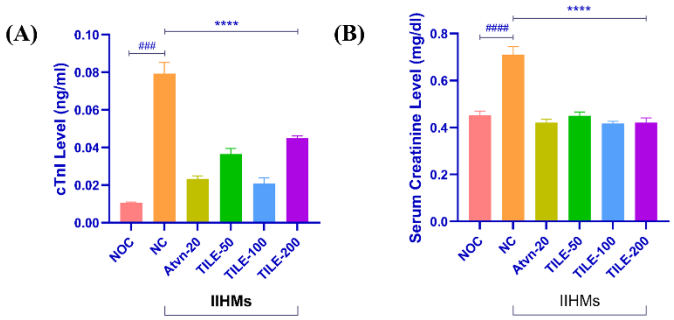

Figure 4. Effects of TILE on (**A**) cTnI level (**B**) serum creatinine level in IIHMs for four weeks in comparison with IIHMs. Data are represented as mean ± SEM, n = 7 in each group. $^{\#\#\#}$ $p < 0.001$, $^{\#\#\#\#}$ $p < 0.0001$ vs normal (*t*-test), and **** $p < 0.0001$ vs IIHMs (ANOVA followed by Dunnett's test).

3.4.3. Serum Creatinine Test

Mice treated with isoproterenol for seven days exhibited significantly higher serum creatinine levels (0.713 ± 0.024 mg/dl) than normal mice (0.453 ± 0.008 mg/dl). Following the four-weeks' TILE treatment, it was found that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE decreased the serum creatinine levels to (0.450 ± 0.012 mg/dl), (0.417 ± 0.003 mg/dl), and (0.423 ± 0.015 mg/dl), respectively, in comparison to IIHMs. Additionally, atorvastatin significantly decreased the serum creatinine level (0.423 ± 0.009 mg/dl) (Figure 4B).

3.4.4. Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT) Test

Mice treated with isoproterenol for seven days exhibited significantly higher SGPT and SGOT levels (61.02 ± 2.07 U/L) and (64.80 ± 1.75 U/L) compared to normal mice (20.08 ± 0.64 U/L) and (41.08 ± 0.75 U/L), respectively. Following the four-week TILE treatment, it was found that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE decreased the SGPT levels to (28.49 ± 1.65 U/L), (26.14 ± 0.93 U/L), (37.82 ± 0.88 U/L), and the SGOT level to (49.33 ± 1.10 U/L), (46.92 ± 1.61 U/L), and (52.87 ± 0.96 U/L) respectively in comparison to IIHMs. Additionally, atorvastatin significantly decreased the SGPT and SGOT levels (22.25 ± 1.46 U/L) and (44.20 ± 1.67 U/L) (Figure 5).

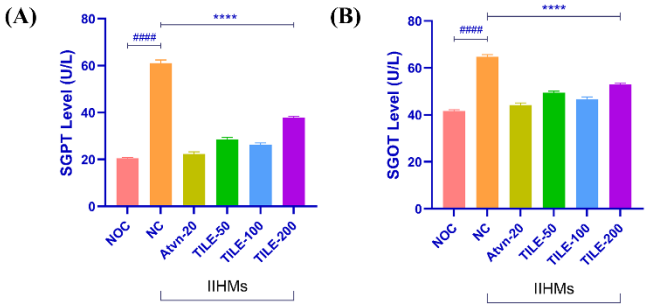

Figure 5. Effects of TILE on (A) SGPT level (B) SGOT level in IIHMs for four weeks in comparison with IIHMs. Data are represented as mean ± SEM, n = 7 in each group. #### $p < 0.0001$ (*t*-test) vs. normal and **** $p < 0.0001$ vs. IIHMs (ANOVA followed by Dunnett's test).

3.4.5. Estimation of SOD & CAT Enzyme Level

Mice treated with isoproterenol for seven days exhibited significantly lower SOD levels (0.73 ± 0.03 U/mg) than normal mice (1.64 ± 0.04 U/mg). Following the four-week TILE treatment, it was observed that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE increased the SOD levels to 1.24 ± 0.02 U/mg, 1.34 ± 0.03 U/mg, and 1.13 ± 0.03 U/mg, respectively, in comparison to IIHMs. Additionally, atorvastatin significantly increased the SOD level (1.37 ± 0.02 U/mg) (Figure 6A).

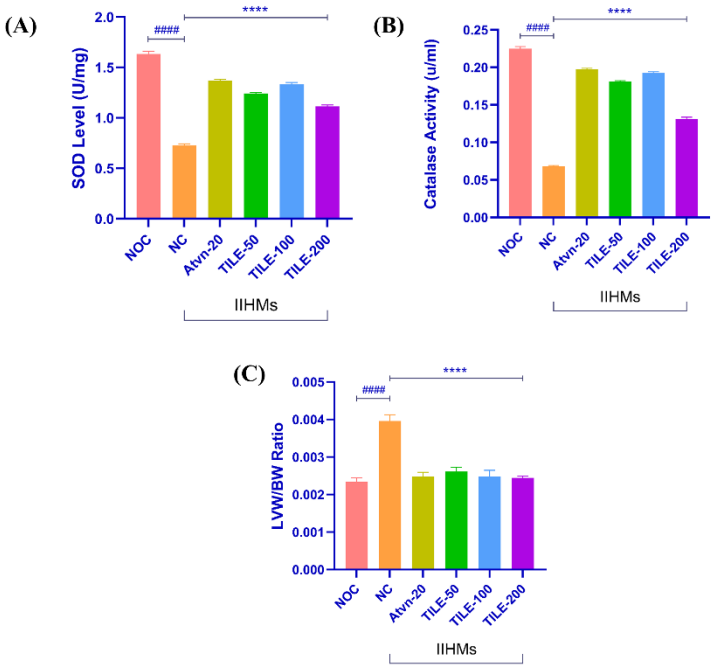

Figure 6. Effects of TILE on (A) SOD level (B) catalase level (C) LVH in IIHMs for four weeks in comparison with IIHMs. Data are represented as mean ± SEM, n = 7 in each group. #### $p < 0.0001$ vs. normal (*t*-test) and **** $p < 0.0001$ vs. IIHMs (ANOVA followed by Dunnett's test).

Mice treated with isoproterenol for seven days exhibited significantly lower catalase enzyme levels (0.068 ± 0.002 U/mL) than normal mice (0.225 ± 0.005 U/mL). Following a four-week TILE treatment, it was found that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE increased the catalase enzyme levels to (0.181 ± 0.002 U/mL), (0.192 ± 0.003 U/mL), and (0.131 ± 0.004 U/mL), respectively, in comparison to IIHMs. Additionally, atorvastatin significantly increased the catalase enzyme level (0.197 ± 0.002 U/mL) (Figure 6B).

3.4.6. LVW/BW Ratio

The values of the other five groups showed a significant change from the hypertrophic control group. Mice treated with isoproterenol for seven days exhibited a significantly increased left ventricle weight/body weight ratio (0.0036 ± 0.0002) than normal mice (0.0023 ± 0.0001). Following the four-week TILE treatment, it was found that 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW of TILE decreased the left ventricle weight/body weight (LVW/BW) ratio to (0.0026 ± 0.0001), (0.0024 ± 0.0002), and (0.0024 ± 0.0001), respectively, in comparison to IIHMs. Additionally, atorvastatin significantly decreased left ventricle weight/body weight (LVW/BW) ratio (0.0024 ± 0.0001) (Figure 6C).

3.4.7. Histopathology of Left Ventricle of the Mouse Heart

The image (I) serves as a normal control and displays the baseline configuration of cardiomyocytes in the absence of any therapy. Image (II) most likely depicts cardiomyocytes that have been damaged by the induction of isoproterenol, highlighting the left ventricular hypertrophy. Image (III) illustrates the impact of atorvastatin administration on cardiomyocytes injured by isoproterenol, which was used as standard that has shown the restorative property. The cardiomyocytes exposed to 50 mg/kg BW dose of TILE have been seen in the image (IV), and the initial dosage response is indicated. Additional improvement in the structure of cardiomyocytes, indicating a response that is dose-dependent, meaning that a higher dose, 100 mg/kg BW, produces better results, as shown in image (V). But 200 mg/kg BW TILE didn't improve the cardiomyocyte condition more than 100 mg/kg BW, image (VI). So, it cannot be said that TILE is working in a dose-dependent manner (Figure 7).

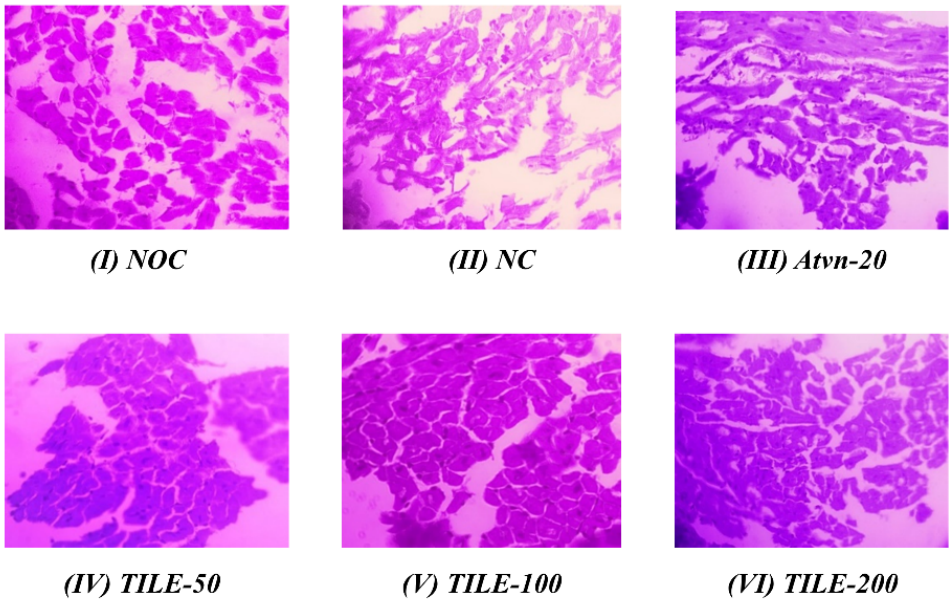

Figure 7. Effects of isoproterenol and TILE on cardiomyocytes of the left ventricle of the mouse heart.

4. Discussion

Our results demonstrate that *T. indica* leaves, rich in polyphenolic compounds, possess substantial antioxidant and cardioprotective properties. The phytochemical analysis of the ethanolic extract confirmed the presence of bioactive compounds such as alkaloids, cardiac glycosides, steroids, tannins, phenolic compounds, flavonoids, and terpenoids. These compounds are known for their potential therapeutic benefits, which are crucial in mitigating oxidative stress, a key factor in the progression of LVH [34].

In vitro assays revealed that TILE exhibits potent antioxidant activity. This effect is likely related to its high phenolic and flavonoid content. In both DPPH and hydroxyl (OH) radical scavenging assays, TILE showed greater

scavenging activity than the reference antioxidants BHT and catechin, respectively. These results suggest that the antioxidant capacity of *T. indica* may contribute to its protective effects against oxidative stress, a key factor involved in the development of LVH [35].

In vivo experiments further corroborated the cardioprotective effects of TILE. Although no formal acute toxicity study was conducted, no mortality or obvious signs of behavioral toxicity were observed during the four-week treatment period. Treatment with different doses of TILE significantly improved lipid profiles in isoproterenol-induced hypertrophic mice (IIHMs). Specifically, TILE reduced TG, TC, and LDL levels while increasing HDL levels, effects that were comparable to those observed with atorvastatin, a standard lipid-lowering drug. These results underscore the potential of TILE as a natural hypocholesterolemic agent that could reduce the risk of atherosclerosis and subsequent CVDs [36].

Furthermore, TILE administration led to a significant reduction in cTnI levels, a sensitive biomarker for myocardial injury. The reduction in cTnI levels suggests that TILE not only mitigates the biochemical markers of cardiac damage but also offers protection against myocardial inflammation [37]. Additionally, TILE effectively reduced serum levels of SGPT and SGOT, indicating a protective effect on liver function, which is often compromised in conditions of cardiac hypertrophy. The normalization of serum creatinine levels further suggests that TILE mitigates the renal toxicity associated with isoproterenol-induced hypertrophy.

The study also highlighted the antioxidative effects of TILE in vivo, as evidenced by the restoration of SOD and CAT enzyme levels in IIHMs. The balance between oxidative stress and antioxidant defenses is crucial in preventing the progression of LVH and other oxidative stress-related diseases [38]. Notably, TILE at a dose of 100 mg/kg showed the most pronounced effects, comparable to those of atorvastatin, in restoring antioxidant enzyme levels. Although restoration of SOD and catalase activities suggests that antioxidant effects may contribute to the cardioprotective action of TILE, the present study did not directly evaluate oxidative stress biomarkers such as MDA, ROS, lipid peroxidation products, or signaling pathways involved in cardiac hypertrophy and oxidative stress. Therefore, the antioxidant-mediated mechanism proposed in this study should be considered preliminary. Further investigations involving molecular and signaling pathway analyses, including Nrf2, NF-κB, MAPK, and RhoA/ROCK pathways, are required to confirm the underlying mechanisms.

The histopathological analysis further confirmed the protective effect of TILE on cardiac tissue. The reduction in the LVW/BW ratio and cardiac myocyte size in TILE-treated mice indicates a reversal of isoproterenol-induced LVH. These findings are significant, as they suggest that TILE could be an effective natural treatment for preventing and managing LVH. The assessment of hypertrophy was limited to LVW/BW ratio and histopathological observations. Molecular markers such as ANP, BNP, β-MHC, collagen deposition, and quantitative morphometric analyses of cardiomyocyte cross-sectional area were not evaluated and should be included in future studies.

However, though our research offers strong proof of TILE's cardioprotective benefits, it's crucial to acknowledge its limitations. The precise molecular mechanisms underlying the anti-hypertrophic effects of TILE remain unclear. Previous studies have indicated the involvement of several signaling pathways in cardiac hypertrophy, including the Raf-MEK1/2-ERK1/2 and DAG-PKC-Rho kinase pathways [39,40]. Future research should focus on elucidating these mechanisms through protein concentration analysis and gene expression studies.

5. Conclusions

In this study, it has been found that the TILE has significant cardioprotective effects against LVH in mice. TILE effectively improved lipid profiles, reduced cardiac injury markers, and enhanced antioxidant defenses, indicating its potential as a natural treatment for cardiovascular diseases. While promising, further research is required to elucidate the precise molecular mechanisms underlying these effects, particularly through the isolation and characterization of the active phytoconstituents responsible for the observed activity, to better establish their therapeutic applicability in LVH and other cardiovascular diseases. Overall, these findings strongly indicate that TILE exhibits substantial cardioprotective potential and may serve as a valuable source for identifying novel bioactive compounds for the management of cardiac hypertrophy and related cardiovascular disorders.

Author Contributions

F.S.: Investigation, Writing—original draft; F.H.: Data curation, Visualization, Writing—review and editing; J.S.: Software, Validation, Writing—review and editing; I.H.: Formal analysis; K.K.: Data curation, Validation; M.I.: Methodology, Resources; R.K.: Methodology, Validation; A.B.M.A.: Writing—review and editing; A.H.M.K.A.: Writing—review and editing; M.R.: Supervision, Conceptualization, Project administration, Correspondence. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Science and Technology (NST) Fellowship, Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (IAMEBBC) at the Institute of Biological Science, University of Rajshahi, Bangladesh (Ethical clearance number: 249 and date of approval: 16 November 2022).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data will be made available on request.

Acknowledgments

The authors would like to express sincere gratitude to the Department of Pharmacy, University of Rajshahi, for providing a well-organized lab and other support. The authors also thank the Ministry of Education, Bangladesh, for providing funds.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

List of Abbreviations

CVD, cardiovascular disease; LVH, Left ventricular hypertrophy; ROCK, Rho kinase; *T. indica, Tamarindus indica;* TCA, tri-chloroacetic acid; FCR, ferric chloride; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TILE, *Tamarindus indica* leaf extract; DPPH, 1, 1-Diphenyl-2-picrylhydrazyl; IIHMs, isoproterenol induced hypertrophic mice; SGPT, Serum glutamic pyruvate transaminase; SGOT, serum glutamic oxaloacetate transaminase; IFCC, International Federation of Clinical Chemistry; SOD, superoxide dismutase; CAT, catalase; BW, body weight.

References

1. Messerli, F.H.; Ketelhut, R. Left ventricular hypertrophy: An independent risk factor. *J. Cardiovasc. Pharmacol.* **1991**, *17* (Suppl. S4), S59–S67.
2. Verdecchia, P.; Porcellati, C.; Reboldi, G.; et al. Left ventricular hypertrophy as an independent predictor of acute cerebrovascular events in essential hypertension. *Circulation* **2001**, *104*, 2039–2044. https://doi.org/10.1161/hc4201.097944.
3. Shimizu, I.; Minamino, T. Physiological and pathological cardiac hypertrophy. *J. Mol. Cell Cardiol.* **2016**, *97*, 245–262. https://doi.org/10.1016/j.yjmcc.2016.06.001.
4. Bruckschlegel, G.; Holmer, S.R.; Jandeleit, K.; et al. Blockade of the renin-angiotensin system in cardiac pressure-overload hypertrophy in rats. *Hypertension* **1995**, *25*, 250–259. https://doi.org/10.1161/01.hyp.25.2.250.
5. Alexander, R.W.; Brock, T.A.; Gimbrone, M.A., Jr.; Rittenhouse, S.E. Angiotensin increases inositol trisphosphate and calcium in vascular smooth muscle. *Hypertension* **1985**, *7*, 447–451.
6. Griendling, K.K.; Rittenhouse, S.E.; Brock, T.A.; et al. Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. *J. Biol. Chem.* **1986**, *261*, 5901–5906.
7. Vanderheyden, V.; Devogelaere, B.; Missiaen, L.; et al. Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release by reversible phosphorylation and dephosphorylation. *Biochim. Biophys. Acta* **2009**, *1793*, 959–970. https://doi.org/10.1016/j.bbamcr.2008.12.003.

8. Kolczynska, K.; Loza-Valdes, A.; Hawro, I.; et al. Diacylglycerol-evoked activation of PKC and PKD isoforms in regulation of glucose and lipid metabolism: A review. *Lipids Health Dis.* **2020**, *19*, 113. https://doi.org/10.1186/s12944-020-01286-8.
9. Shimokawa, H.; Takeshita, A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 1767–1775. https://doi.org/10.1161/01.ATV.0000176193.83629.c8.
10. Striessnig, J.; Pinggera, A.; Kaur, G.; et al. L-type $Ca^{(2+)}$ channels in heart and brain. *Wiley Interdiscip. Rev. Membr. Transp. Signal* **2014**, *3*, 15–38. https://doi.org/10.1002/wmts.102.
11. Molkentin, J.D.; Dorn, G.W., 2nd. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu. Rev. Physiol.* **2001**, *63*, 391–426. https://doi.org/10.1146/annurev.physiol.63.1.391.
12. Proud, C.G. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. *Cardiovasc. Res.* **2004**, *63*, 403–413. https://doi.org/10.1016/j.cardiores.2004.02.003.
13. Rashid, M.; Tawara, S.; Fukumoto, Y.; et al. Importance of Rac1 signaling pathway inhibition in the pleiotropic effects of HMG-CoA reductase inhibitors. *Circ. J.* **2009**, *73*, 361–370. https://doi.org/10.1253/circj.cj-08-0817.
14. Lezoualc'h, F.; Métrich, M.; Hmitou, I.; et al. Small GTP-binding proteins and their regulators in cardiac hypertrophy. *J. Mol. Cell Cardiol.* **2008**, *44*, 623–632. https://doi.org/10.1016/j.yjmcc.2008.01.011.
15. Liu, W.; Zi, M.; Naumann, R.; et al. Pak1 as a novel therapeutic target for antihypertrophic treatment in the heart. *Circulation* **2011**, *124*, 2702–2715. https://doi.org/10.1161/circulationaha.111.048785.
16. Kuru, P. *Tamarindus indica* and its health related effects. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 676–681. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0173.
17. Devi, B.; Boruah, T. *Tamarind (Tamarindus indica)*; Springer: Singapore, 2020; pp. 317–332.
18. Yogeswari, M.; Baharin, B.; Ganesan, P. Effect of Room Temperature Storage on the Physicochemical and Antioxidant Properties of Oven Dried Young Tamarind Leaves (*Tamarindus Indica*) Chutney Powder. *Indian J. Sci. Technol.* **2016**, *9*, 1–8 https://doi.org/10.17485/ijst/2016/v9i48/91997.
19. Imam, S.; Azhar, I.; Hasan, M.M.; et al. Two triterpenes lupanone and lupeol isolated and identified from *Tamarindus indica* linn. *Pak. J. Pharm. Sci.* **2007**, *20*, 125–127.
20. Pino, J.; Escalona Arranz, J.; Licea, I.; et al. Leaf Oil of *Tamarindus indica* L. *J. Essent. Oil Res.* **2002**, *14*, 187–188. https://doi.org/10.1080/10412905.2002.9699819.
21. Hossain, M.S.; Jami, M.A.B.S.; Ashraful, A.; et al. Unveiling the anti-inflammatory activity of chloroform fraction of *curcuma wallichii* and its phytoconstituents by *in vivo* and *in silico* studies. *Sci. Rep.* **2026**, *16*, 1762.
22. Islam, M.T.; Saha, S.; Ashraful, A.; et al. Phytochemical Profile, Antioxidant, Antidiabetic, and Antidiarrheal Potentials of *Hylocereus undatus* Leaf Extract: GC–MS and In Silico Insights. *J. Food Biochem.* **2026**, *2026*, 3039549.
23. Reza, A.A.; Haque, M.A.; Sarker, J.; et al. Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of *Achyranthes ferruginea* Roxb. in cancer cell line. *Food Sci. Nutr.* **2021**, *9*, 3777–3805.
24. Hossain, M.S.; Ashraful, A.; Rahman, A.A.; et al. Anti-ROS and Anticancer Potential of Rhizomes and a Polyunsaturated Fatty Acid From Chloroform Fraction of *Curcuma wallichii* as a Bioactive Compound. *J. Food Biochem.* **2025**, *2025*, 9517484.
25. Sarker, J.; Reza, A.A.; Ashraful, A.; et al. In Vitro Antioxidant and p53-Targeted Anticancer Activity of *Aerva sanguinolenta* (L.) Blume Across Different Human Cancer Cell Lines and Ehrlich Ascites Carcinoma Model. *J. Chem. Health Risks* **2026**, *16*, 1292–1316
26. Hossain, F.; Zamaly, S.; Ashraful, A.; et al. Exploring the therapeutic potential of *Leea rubra* (Vitaceae): Antioxidant efficacy, brine shrimp lethality, and in vivo anticancer activity against EAC cells in Swiss albino mice. *J. Biol. Act. Prod. Nat.* **2025**, *15*, 181–195.
27. Zhang, Y.; Xu, J.; Long, Z.; et al. Hydrogen (H_2) inhibits isoproterenol-induced cardiac hypertrophy via antioxidative pathways. *Front. Pharmacol.* **2016**, *7*, 217818.
28. Haroon, H.B.; Ahmed, N.; Sampath, M.K.; et al. *Tamarindus indica*. Linn leaves ameliorates experimental induced heart failure in Wistar rats. *J. Basic. Clin. Physiol. Pharmacol.* **2022**, *33*, 363–371.
29. Tushar, M.A.N.; Rashid, M.M.; Hossain, F.; et al. Investigation of cardioprotective effects of *Leea rubra* Blume leaves on isoproterenol-induced left ventricular hypertrophy in mice. *J. Herbmed Pharmacol.* **2026**, *15*, 27–35.
30. 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*, 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.
31. Hadwan, M.H. Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC Biochem.* **2018**, *19*, 7. https://doi.org/10.1186/s12858-018-0097-5.
32. Khatun, R.; Sarker, J.; Sharmin, F.; et al. Attenuation of Left Ventricular Hypertrophy by *Psidium guajava* Leaf Extract Via Inhibition of Oxidative Stress and Cardiac Troponin I Levels in Adrenaline-Induced Hypertrophic Rats. *J. Med. Nat. Prod.* **2026**, *3*, 100011.
33. Slaoui, M.; Fiette, L. Histopathology procedures: From tissue sampling to histopathological evaluation. *Methods Mol. Biol.* **2011**, *691*, 69–82. https://doi.org/10.1007/978-1-60761-849-2_4.

34. Sookying, S.; Duangjai, A.; Saokaew, S.; et al. Botanical aspects, phytochemicals, and toxicity of Tamarindus indica leaf and a systematic review of antioxidant capacities of *T. indica* leaf extracts. *Front Nutr.* **2022**, *9*, 977015.
35. Ołdakowski, Ł.; Taylor, J.R. Oxidative damage and antioxidant defense are assay and tissue-dependent both in captive and wild-caught bank voles (*Myodes glareolus*) before and after reproduction. *Ecol. Evol.* **2018**, *8*, 7543–7552.
36. McGill, H.C., Jr.; McMahan, C.A.; Zieske, A.W.; et al. Association of Coronary Heart Disease Risk Factors with microscopic qualities of coronary atherosclerosis in youth. *Circulation* **2000**, *102*, 374–379. https://doi.org/10.1161/01.cir.102.4.374.
37. Sharma, S.; Jackson, P.G.; Makan, J. Cardiac troponins. *J. Clin. Pathol.* **2004**, *57*, 1025–1026. https://doi.org/10.1136/jcp.2003.015420.
38. Halliwell, B. Antioxidants: The basics--what they are and how to evaluate them. *Adv. Pharmacol.* **1997**, *38*, 3–20. https://doi.org/10.1016/s1054-3589(08)60976-x.
39. Gilbert, C.J.; Longenecker, J.Z.; Accornero, F. ERK1/2: An Integrator of Signals That Alters Cardiac Homeostasis and Growth. *Biology* **2021**, *10*, 346. https://doi.org/10.3390/biology10040346.
40. Moreau, S. *Characterizing Rho Kinase Activity Using a Novel PET Tracer in Hypertrophied Cardiomyocytes*; University of Ottawa: Ottawav, Canada, 2012.