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Investigation of cardioprotective effects of *Leea rubra* Blume leaves on isoproterenol-induced left ventricular hypertrophy in mice

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ABSTRACT

Introduction: Cardiac hypertrophy is a risk factor for many cardiovascular diseases (CVDs). Species of the genus *Leea* have traditional cardioprotective uses, but their direct effects on the heart remain unreported. This study evaluated the cardioprotective activities of a crude methanolic extract (CME) of *L. rubra* leaves (LRL) in isoproterenol-induced cardiac hypertrophic mice (IIHM) using atorvastatin as a reference treatment.

Methods: IIHM (induced with isoproterenol, 5 mg/kg, 7 days, intraperitoneally) were treated with CME of LRL (40 mg/kg or 80 mg/kg, 28 days, orally) and compared with a standard treatment group (atorvastatin, 20 mg/kg, 28 days, orally) and a negative control group (IIHM, placebo). The cardioprotective and hepatoprotective effects of the extract were evaluated by analysing the changes in blood biochemical parameters, including lipid profile (total cholesterol [TC], triglyceride [TG], high-density lipoprotein [HDL], and low-density lipoprotein [LDL]), cardiac troponin I (CTnI), serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT). Analysis was conducted using various commercial test kits. The left ventricular weight and body weight (LVW/BW) ratio was also recorded.

Results: LRL significantly improved the lipid profile by lowering TC, TG, and LDL while increasing HDL levels ($P < 0.0001$). Significant cardioprotective (reduced serum CTnI level and LVW/BW ratio ($P < 0.0001$)) and hepatoprotective (reduced serum SGPT and SGOT levels ($P < 0.0001$)) activities were observed in IIHM compared with the negative control group.

Conclusion: The present study found antihyperlipidemic, cardioprotective, and hepatoprotective activity after LRL treatment in IIHM. Further investigation is required to identify the compounds and molecular mechanisms responsible for these effects.

Implication for health policy/practice/research/medical education:
This study identifies *Leea rubra* as a promising candidate for cardioprotective and hepatoprotective agents. Additional research is necessary to isolate active compounds and elucidate their mechanisms, which could lead to commercialization as effective drugs following thorough clinical trials.

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Introduction

In the 1990s, the World Health Organization (WHO) commenced the Global Burden of Disease study, which highlighted childhood illnesses, mental health problems, and road traffic accidents as the primary contributors to global mortality (1). By 2019, the burden had primarily transitioned to non-communicable diseases (NCDs), responsible for almost 42 million fatalities worldwide. Among these, cardiovascular diseases (CVDs) emerged as leading contributors to mortality, irrespective of a country's socioeconomic status (2). According to the WHO, 17.7 million deaths worldwide were caused by CVDs in 2019, accounting for 31% of all deaths, a number that is predicted to rise (3). Eighty percent of these deaths occur in countries with low or middle incomes, such as Bangladesh, where the greatest number of affected people reside (4). CVDs are consequently recognized as a significant global public health issue. In recent decades, Bangladesh has experienced rapid urbanization driven by sustained economic growth, and it has recently attained the status of a developing nation (5). One result of this growth and increased urbanisation is a shift towards more sedentary lifestyles, prompting concerns of further rises in the chronic disease burden. Among CVDs, myocardial infarction (MI) carries a high mortality risk and is a leading global cause of death (6). MI is a common condition linked with ischemic cardiac disease. Despite advancements in the management of coronary artery disease, MI remains the leading cause of mortality in the modern world (7). It also represents a critical acute consequence of myocardial necrosis, resulting from an imbalance between the oxygen demand of the myocardium and the supply of coronary blood. This mismatch induces cardiac ischemia and subsequent degeneration of cardiomyocytes (8). MI is influenced by a range of risk factors such as smoking, excessive alcohol consumption, high-fat and high-carbohydrate diets, deficient in fruits and vegetables, physical inactivity, obesity, hypertension, and hyperlipidemia (9).

A well-established non-invasive model for studying human MI-related complications is the isoproterenol-induced myocardial injury model, which is frequently employed to evaluate the cardioprotective or preventive effects of natural products. Isoproterenol, a synthetic catecholamine and β-adrenergic receptor agonist, at a dose of 85 mg/kg in mice, induces myocardial hypertrophy predominantly through excessive stimulation of β1-adrenergic receptors. This results in increased cardiac workload, oxidative stress, myocardial necrosis, myofibrillar degeneration, fibrosis, calcium overload, and impaired energy metabolism (10). This model closely matches several pathophysiological and biochemical changes observed in human MI, including oxidative stress and inflammatory responses, making it a reliable and reproducible tool for preclinical research (11). However, a key limitation of this model is that it represents an acute pharmacologic injury, rather than the chronic, multifactorial pathogenesis typically seen in human hypertrophy and heart failure (12). Thus, while useful for screening cardioprotective effects, findings in the model may not fully translate to clinical settings.

In this study, we used atorvastatin as a standard treatment. Beyond its lipid-lowering abilities, atorvastatin exerts pleiotropic effects that are relevant to the pathophysiology of left ventricular hypertrophy (LVH). LVH is a condition where the left ventricle becomes heavier due to an increase in wall thickness, an enlargement of the ventricular cavity, or a combination of both (13). Specifically, atorvastatin has been shown to inhibit the activation of the nuclear factor kappa B (NF-κB) pathway, which plays a key role in the inflammatory processes that contribute to cardiac hypertrophy (14). Moreover, atorvastatin has been reported to attenuate myocardial fibrosis and reduce LVH, potentially through the upregulation of angiotensin-converting enzyme 2 (ACE2) expression, which counteracts the hypertrophic effects of the renin–angiotensin system (15).

Due to the various side effects resulting from the prolonged use of synthetic medicines for cardiac complications, there is an urgent need for safer alternatives. Herbal remedies, widely used in traditional medicine, have shown effectiveness due to their cardioprotective, antioxidant, and anti-inflammatory properties (16). With this in mind, we aimed to explore the cardioprotective potential of products of herbal origin with negligible side effects (17). The Vitaceae family encompasses the genus *Leea*, which is distributed across several locations globally, including Australia, Africa, New Guinea, Malaysia, Thailand, Bangladesh, China, and India (18). *Leea* comprises over 70 distinct species. Among these, Bangladesh hosts seven: *L. asiatica*, *L. guineensis*, *L. indica*, *L. macrophylla*, *L. aequata*, *L. rubra,* and *L. alata*. *Leea* species exhibit many pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, hepatoprotective, neuroprotective, and anticancer effects (19). If some species from a genus or family are known to treat a specific disorder based on chemotaxonomic or molecular phylogenetic data, other related species may possess similar biological activity. This is due to genetic similarities within phylogenetic groups, potentially producing equivalent chemical-producing capabilities and offering similar therapeutic advantages (20). *L. rubra* was selected for this study based on this rationale. The objective of the present study was to investigate the cardioprotective effect of a crude methanolic extract (CME) of *L. rubra* leaves (LRL*)* in a mouse model of LVH, isoproterenol-induced cardiac hypertrophic mice (IIHM). This model is representative of non-standard but concerning risks for CVDs, including coronary artery disease, stroke, and cardiac failure (21).

Materials and Methods

Materials

Materials used in this study included isoproterenol (Isolin; Samarth Life Sciences Pvt. Ltd., India), heparin (Panpharma Ltd., Bangladesh), atorvastatin (Square Pharmaceuticals Ltd., Bangladesh), and distilled water. Biochemical parameters, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and cardiac troponin I (CTnI) were determined using commercially available testing kits (Human, Germany). All chemicals utilised met analytical grade standards.

Plant collection

Plant leaves were collected in December 2021 from Bandarban, a district in the Chittagong Division of South-Eastern Bangladesh. Plant specimens were authenticated by a qualified taxonomist from the Bangladesh National Herbarium in Dhaka, and a certificate of authenticity was provided (voucher number: DACB66292).

Extraction of the plant material

After carefully cleaning the collected leaves under running water, they were dried in the shade while being exposed to intermittent daylight over the course of several days. They were then desiccated for 24 hours at a low temperature in an oven to enhance processing. The dried materials were ground into a coarse powder (Capacitor Start Motor, Wuhu Motor Manufacturer, China) and stored at room temperature in the Department of Pharmacy at the University of Rajshahi, Bangladesh, before further use. The powdered leaves (650 g) were then steeped in 1.5 litres of 100% methanol three times. Following Alam's protocol, the extract underwent rotary evaporation at 35 °C and filtration through cotton and Whatman No. 1 filter sheets, producing 80 g of concentrated extract (22). This improved extraction technique paves the way for exploring the medicinal potential of LRL in herbal treatments.

Animals

A total of 35 Swiss Albino male mice, aged 2 months, weighing 28–30 g, were purchased from the Department of Biochemistry, Jahangirnagar University. Prior to the beginning of experiments, all mice were habituated to their new environmental conditions for two weeks. The mice were housed with adequate ventilation and kept at a temperature of 25 °C with access to standard pellets from the International Centre for Diarrhoeal Disease Research, Bangladesh and fresh water throughout the experiment. All mice were housed in cages and kept in a 12-hour natural light/dark cycle. The institutional animal ethics committee's guiding principles for animal ethics were used to guide the experiments.

Acute oral toxicity study

A study of the acute oral toxicity of LRL extract was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) 425 guidelines (23). After a one-week adaptation period, five healthy male mice weighing 28–30 g were utilised for this experiment. Two mice were fasted for 4 hours before being administered a 1000 mg/kg oral dose of LRL extract via a gavage approach. The mice were periodically monitored for 24 hours to observe any immediate signs of poisoning. The other three mice received an identical dose, and their activity was observed over 14 days in their home cages. This procedure was repeated with five further mice with a dose of 2000 mg/kg.

Grouping and dosing of animals

Another 25 fresh mice were randomly assigned to one of five experimental groups (n = 5): a normal group (G-I), a negative control group (G-II), a standard group (G-III), and two treatment groups (G-IV and G-V). All the investigators involved in data collection, and the outcome assessments were blinded to the groups throughout the duration of the study. All groups, except group G-I, received an intraperitoneal injection of isoproterenol to induce the LVH model. In terms of treatment, G-I and G-II received distilled water (DW), G-III was treated with atorvastatin (0.3 mg/kg), and groups G-IV and G-V were treated with 40 and 80 mg/kg of LRL extract, respectively (24).

Preparation of doses of drugs

Atorvastatin was in an amorphous form and freely soluble in DW. Doses of atorvastatin were prepared using DW so that each 100 µLcontained the equivalent dose of 0.3 mg/kg body weight; this is the effective dose for atorvastatin in humans. The LRL extract was also dissolved in DW and diluted to prepare two concentrations, providing doses of 40 and 80 mg/kg per 100 µL. 100 µL of each solution was administered by oral gavage to the mice.

Experimental treatment of mice

The mice in all groups except G-I were intraperitoneally injected with 100 µL of isoproterenol using a 1 mL disposable syringe for 7 consecutive days to induce hypertrophy. This administration was based on an earlier report, in which 5 mg/kg/day isoproterenol injection induced cardiac hypertrophy (25). LVH was confirmed at the end of the study by measuring the left ventricular weight to body weight (LVW/BW) ratio, as individual confirmation during the study would have required sacrificing the animals. This approach was based on our previous study and established literature (25). After 7 days, LRL extract was administered orally to G-IV and V mice (40 and 80 mg/kg, respectively) and atorvastatin (0.3 mg/kg oral) to the G-III mice. Mice were treated for 28

consecutive days before being sacrificed. To confirm the prevention of hypertrophy and the most effective dose, TC, TG, HDL cholesterol, LDL cholesterol, CTnI, SGPT, and SGOT levels were measured and compared between normal mice and IIHM. All assays were carried out using diagnostic kits.

Sacrifice of mice and collection of blood, serum, and heart

Following four weeks of treatment, the mice were anaesthetised using chloroform (Sigma-Aldrich, Germany). Subsequently, following the incision of the abdominal skin, the thoracic artery was exposed, and 1–1.5 mL of blood was collected using heparinised syringes. The heart from each mouse was excised, and the left ventricular part was removed for subsequent analysis. Blood samples were centrifuged at 4000 rpm for 15 minutes at 25°C to separate the serum, which was then used for various biochemical analyses using commercially available ELISA kits (Human, Germany), following the validated protocols provided by the manufacturers.

Measurement of lipid profile

Serum lipid profile, including TC, TG, LDL, and HDL, were assessed using diagnostic kits (Human, Germany) (26).

Cardiac troponin I test

CTnI ELISA test kits were utilised to measure the CTnI level in serum. The test is dependent on the theory of a solid-phase enzyme-linked immunosorbent assay (27).

SGPT and SGOT tests

SGPT and SGOT levels were determined using SGPT and SGOT testing kits (Human, Germany). The kit uses a kinetic method for determining SGPT and SGOT activity without pyridoxal phosphate activation (26).

Determination of left ventricular weight to body weight ratio

Since left ventricular mass increases in LVH, the LVW/BW ratio was used as a key parameter to confirm the LVH. LVW/BW ratios were determined by measuring the weight of the extracted left ventricles. After euthanasia, the hearts were rapidly excised, and the left ventricle carefully separated from the right ventricle and atria under a dissecting microscope. The isolated left ventricles were gently wiped to remove excess fluid. After dissection, the left ventricle was weighed and compared to the overall body weight of the respective mouse (28).

Statistical analysis

The results were represented as mean ± standard error of mean (SEM). GraphPad Prism version 8 was used for statistical analysis. Statistical differences between groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered significant when P values were less than 0.05.

Results

Acute toxicity study

Acute toxicity tests showed that oral doses of 1 and 2 g/kg of CME of LRL were safe. Assessed over 24 hours and 14 days at doses of 1 and 2 g/kg, no mice exhibited any symptoms of toxicity or any changes in normal behaviours, indicating that CME was well tolerated throughout the investigation.

Effects of LRL on serum total cholesterol and triglyceride levels in IIHM

The effects of LRL on TC levels are illustrated in Figure 1a. Mice in G-II exhibited significantly higher TC levels (162.2 ± 2.65 mg/dL) ($P<0.0001$) than the normal mice in G-I (131 ± 3.36 mg/dL). After four weeks of treatment, in comparison to G-II, atorvastatin significantly decreased TC levels (125 ± 2.70 mg/dL) in G-III ($P<0.0001$), to similar levels seen in G-I mice. Mice receiving 40 mg/kg CME in G-IV did not show significant changes in TC levels (158.2 ± 2.13 mg/dL). However, G-V mice receiving 80 mg/kg CME exhibited significantly decreased TC levels (118.2 ± 2.52 mg/dL) ($P<0.0001$), even lower than the atorvastatin group.

The TG values of G-III and G-V mice showed significant differences compared to G-II mice. Mice treated with isoproterenol for seven days exhibited significantly higher TG levels (174.2 ± 3.10 mg/dL) ($P<0.0001$) than normal mice (134 ± 2.95 mg/dL). After four weeks of treatment, atorvastatin in G-III mice significantly decreased TG levels (139 ± 2.05 mg/dL) ($P<0.0001$), similar to the normal mice. In G-IV, 40 mg/kg CME did not induce significant changes in TG levels (170.2 ± 2.22 mg/dL). However, in comparison to G-II, G-V mice at 80 mg/kg CME had significantly decreased TG levels (141.8 ± 2.08 mg/dL) ($P<0.0001$). These results are illustrated in Figure 1b.

Effects of LRL on serum HDL and LDL levels in IIHM

The values for HDL levels in G-III, G-IV, and G-V mice were significantly different from those in G-II, as represented in Figure 2a. Mice in G-II exhibited significantly lower HDL levels (26 ± 0.71 mg/dL) ($P<0.01$) than normal mice (30 ± 0.89 mg/dL) in G-I. After four weeks of treatment, atorvastatin in G-III significantly increased HDL levels (30.8 ± 1.24 mg/dL) ($P<0.01$) to a value similar to that of normal mice. CME also significantly increased HDL levels in G-IV (29.8 ± 0.73 mg/dL) ($P<0.05$) and G-V (35 ± 1.18 mg/dL) ($P<0.0001$) mice, in comparison to the untreated IIHM. HDL levels in G-V mice were higher than those in G-III.

The LDL levels in G-III and G-V were significantly different from the hypertrophic control group (G-II), as shown in Figure 2b. Mice in G-II exhibited significantly higher LDL levels (81 ± 2.07 mg/dL) ($P<0.0001$) than normal mice in G-I (61.2 ± 1.69 mg/dL). After four weeks,

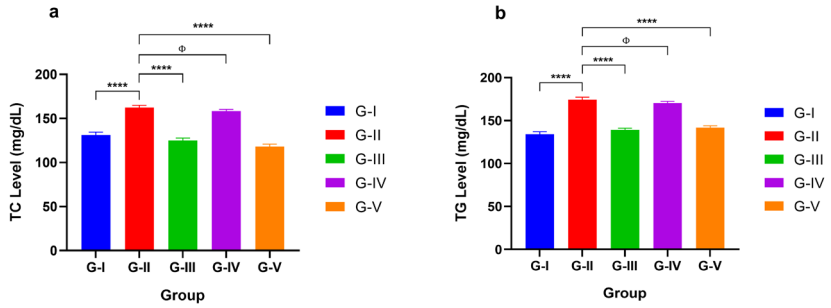

Figure 1. Effects of *Leea rubra* leaves on serum total cholesterol and triglyceride levels in isoproterenol-induced cardiac hypertrophic mice. Values imply mean ± SEM (n=5). TC: total cholesterol, TG: triglyceride, G-I: normal group, G-II: negative control group, G-III: standard group, G-IV and G-V: treatment groups. $^{\Phi}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

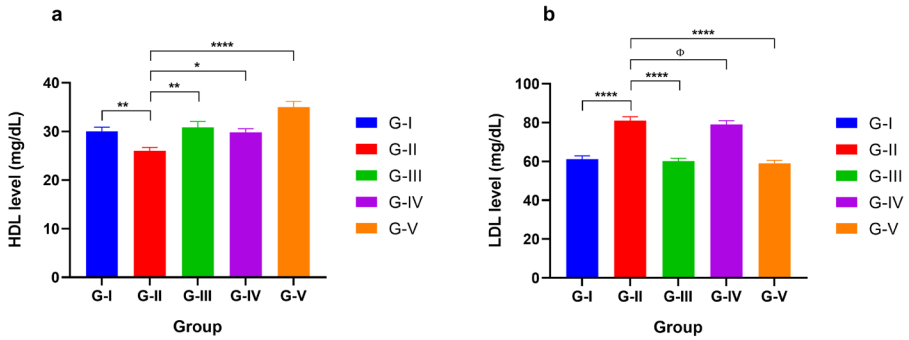

Figure 2. Effects of *Leea rubra* leaves on serum high-density lipoprotein and low-density lipoprotein levels in isoproterenol-induced cardiac hypertrophic mice. Values imply mean ± SEM (n=5). HDL: high-density lipoprotein, LDL: low-density lipoprotein, G-I: normal group, G-II: negative control group, G-III: standard group, G-IV and G-V: treatment groups. $^{\Phi}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

atorvastatin treatment in G-III significantly decreased LDL levels (60.2 ± 1.46 mg/dL) ($P < 0.0001$), bringing them close to normal levels. In G-IV, CME at a dose of 40 mg/kg did not cause a significant change in LDL levels (79 ± 2.00 mg/dL). However, compared to G-II mice, CME at a dose of 80 mg/kg significantly reduced LDL levels (59 ± 1.64 mg/dL) ($P < 0.0001$) in G-V mice, similar to the impact of atorvastatin.

Effects of LRL on cardiac troponin I level and LVW/BW ratio in IIHM

The values for CTnI levels in G-III, G-IV, and G-V mice were significantly different from the hypertrophic control group (G-II), as presented in Figure 3a. Mice in G-II exhibited significantly higher CTnI levels (1.50 ± 0.03 ng/mL) ($P < 0.0001$) compared to the normal mice (1.19 ± 0.02 ng/mL) in G-I. After four weeks of treatment, atorvastatin significantly decreased CTnI levels (1.20 ± 0.02 ng/mL) ($P < 0.0001$) in G-III mice. In comparison to G-II mice, CME in G-IV significantly decreased CTnI levels (1.19 ± 0.02 ng/mL) ($P < 0.0001$), similar to atorvastatin. Notably, in G-V mice, CME significantly decreased CTnI levels (0.55 ± 0.01 ng/mL) ($P < 0.0001$) to less than half of the level observed with atorvastatin treatment, suggesting a superior cardioprotective effect of LRL.

The values for the LVW/BW ratios of G-III, G-IV, and G-V mice were significantly different from the hypertrophic control group (G-II), as presented in Figure 3b. Mice in G-II exhibited significantly higher LVW/BW ratios (0.00374 ± 0.000051) ($P < 0.0001$) than G-I normal mice (0.00218 ± 0.000058). After the four-week treatment period, atorvastatin significantly decreased the LVW/BW ratio (0.00258 ± 0.000037) ($P < 0.0001$) in G-III mice. In comparison to G-II, CME in G-IV and G-V significantly decreased the LVW/BW ratio (0.0034 ± 0.000071 ($P < 0.01$) and 0.00268 ± 0.000058 ($P < 0.0001$), respectively).

Effects of LRL on serum SGPT and SGOT level in IIHM

SGPT values for G-IV and G-V mice showed significant differences from those in G-II, as presented in Figure 4a. Mice in G-II exhibited significantly higher SGPT levels (86.2 ± 2.35 U/L) ($P < 0.0001$) than G-I normal mice (34 ± 1.26 U/L). After four weeks of treatment, atorvastatin in G-III decreased SGPT levels (82 ± 2.98 U/L), while CME in G-IV had no significant effect (80 ± 3.39 U/L) compared to G-II mice. However, CME at 80 mg/kg in G-V mice significantly decreased the SGPT level (58.2 ± 1.39 U/L) ($P < 0.0001$) in comparison to G-II mice.

SGOT values in G-IV and G-V mice showed significant differences from G-II mice, as shown in Figure 4b. Mice

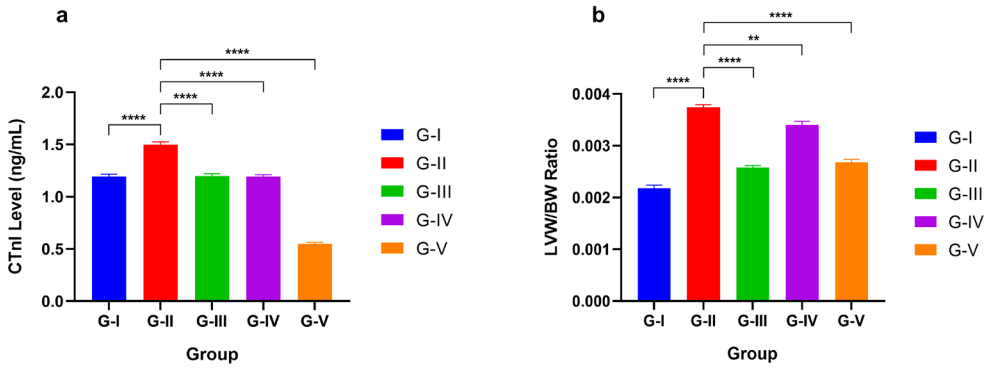

Figure 3. Effects of *Leea rubra* leaves on serum cardiac troponin I (CTnI) levels and left ventricular weight to body weight ratios in isoproterenol-induced cardiac hypertrophic mice. Values imply mean ± SEM (n=5). CTnI: cardiac troponin I, LVW/BW: left ventricular weight to body weight ratio, G-I: normal group, G-II: negative control group, G-III: standard group, G-IV and G-V: treatment groups. $^{\Phi}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

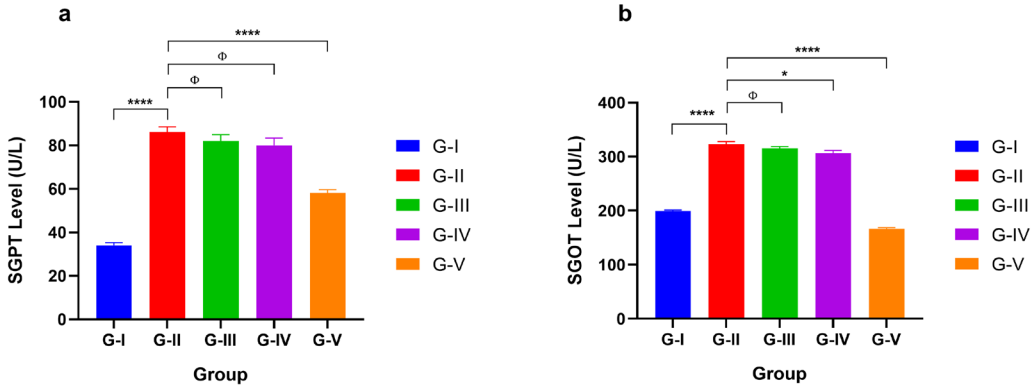

Figure 4. Effects of *Leea rubra* leaves on serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase levels in isoproterenol-induced cardiac hypertrophic mice. Values imply mean ± SEM (n=5). SGPT: serum glutamic pyruvic transaminase, SGOT: serum glutamic oxaloacetic transaminase, G-I: normal group, G-II: negative control group, G-III: standard group, G-IV and G-V: treatment groups. $^{\Phi}P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

in G-II exhibited significantly higher SGOT levels (323.2 ± 4.77 U/L) ($P < 0.0001$) than normal mice (199.2 ± 1.85 U/L) in G-I. After four weeks of treatment, atorvastatin decreased SGOT levels in G-III mice (315.2 ± 3.68 U/L), and CME produced significant changes in SGOT levels for both G-IV (306.4 ± 4.95 U/L) ($P < 0.05$) and G-V (166.2 ± 2.20 U/L) ($P < 0.0001$) mice in comparison to G-II mice.

Discussion

There are many different synthetic medications on the market to treat hypertension and CVDs, but they are typically expensive and have associated side effects. LVH can be adaptive and related to an increase in left ventricular pressure or workload, which can be the result of hypertension, oxidative stress, or high blood cholesterol (29). Our objective was therefore to find novel therapeutics from plants accessible and affordable to treat LVH with fewer adverse effects. Thus, we picked *L. rubra* as a potential treatment, since *Leea* species have been used in traditional medicine worldwide for ailments such as joint pain, sores, leprosy, ulcers, skin diseases, diabetes, wounds, cardiac diseases, cancer, gangrene, fever, and others. These plants contain saponins, tannins, glycosides, cardiac glycosides, polyphenolic compounds, and steroids, all of which are biologically active phytoconstituents responsible for positive health effects (30-32). Saponins are known to have antihyperlipidemic and antioxidant effects that can help reduce blood lipid levels and oxidative stress, key contributors to LVH and other CVDs (33, 34). Glycosides, especially cardiac glycosides, play a direct role in improving cardiac contractility and reducing myocardial oxygen demand (35). Increased concentrations of TC, TG, and LDL and decreased HDL concentrations have been associated with atherosclerotic lesions and a higher chance of developing related clinical symptoms, including LVH, MI, cerebrovascular incidents, and peripheral vascular disease (36). To ascertain the cardioprotective effect of LRL extract, we performed an

in vivo experiment in which two doses of LRL CME were administered to IIHM for four weeks. We observed the effect of the CME on lipid profile, cardiac biomarkers, cardiac-specific enzymes, and LVW. The effects were compared with a common lipid-lowering medication, atorvastatin, which has a secondary beneficial effect in preventing cardiac hypertrophy (37).

IIHM exhibited significantly higher TC, TG, and LDL levels and lower HDL levels than normal mice, while both doses of CME improved their lipid profile. Specifically, LRL CME at 80 mg/kg reduced TC, TG, and LDL levels and increased HDL levels to a greater extent than atorvastatin. These results indicate the potential of LRL CME as a natural anti-hypercholesterolemic agent that could reduce the risk of CVDs (38). CTnI is used as a cardiac biomarker to diagnose myocardial damage due to its tissue specificity and high sensitivity (39). Generally, normal levels of CTnI in the blood are extremely low but increase during myocardial inflammation. In the present study, we found that isoproterenol induction significantly raised CTnI levels in IIHM compared to healthy mice. After four weeks of treatment, both dosages of LRL CME significantly reduced CTnI levels compared to untreated IIHM. Atorvastatin also significantly decreased the CTnI level; however, the levels in the high-dose LRL group were less than half that of those in the atorvastatin group. This remarkable cardioprotective effect of CME may be attributed to its potent antioxidant properties (40), which help mitigate oxidative stress-induced myocardial injury, thereby reducing CTnI release more effectively than atorvastatin.

The LVW/BW ratio is also an indicator of LVH, as myocytes in the left ventricle are enlarged in LVH (41). Both doses of CME significantly reduced the LVW/BW ratio in comparison with untreated IIHM. Reduction of the CTnI level and the LVW/BW ratio are very important metrics, as they indicate a recovery from isoproterenol-induced LVH. According to these findings, we can conclude that LRL CME is a potential candidate for preventing and managing LVH. Isoproterenol-induced cardiac hypertrophy is frequently indicated by significantly elevated serum levels of SGPT and SGOT (42). In our experiment, it was observed that serum SGPT and SGOT levels were considerably higher in IIHM than in normal mice. CME of LRL (at 80 mg/kg) significantly decreased the SGPT and SGOT levels compared to untreated IIHM, achieving levels similar to healthy mice. This indicates a protective effect on liver function, which is often compromised in isoproterenol-induced hypertrophy. In contrast, the effect of atorvastatin on SGPT and SGOT levels was not significant, with values similar to those of untreated IIHM. This is consistent with previous findings, as atorvastatin has a known negative impact on the liver (43).

The findings of the present study demonstrate that CME of LRL has a significant impact on the lipid profile, reducing LVH and hepatic injury. Although statistically significant results were observed, the small sample size (n = 5 per group) warrants cautious interpretation. Future investigations with larger cohorts, incorporating power calculations, are needed to validate these findings and evaluate the effect sizes. While the precise mechanisms remain unclear, the presence of polyphenolic compounds and saponins leads us to hypothesize that the benefits of LRL may involve the modulation of oxidative stress (e.g., ROS reduction) and the inhibition of pro-hypertrophic signalling cascades (e.g., MAPK/ERK1/2 pathways), critical in cardiac remodelling and inflammation (44-46). Further investigations to quantify related proteins (such as MAPKs, ERK1/2, and cAMP) and profile gene expression are needed to confirm these mechanisms. It is important to note that, while isoproterenol-induced LVH in mice is a well-established preclinical model, it may not fully replicate the complexity of human cardiac hypertrophy or CVD progression. Therefore, the translational relevance of our findings should be interpreted with caution. Further investigations, including isolation of cardioprotective compounds, molecular assays, and ultimately clinical trials, are essential to validate the efficacy and safety of LRL in human subjects.

Conclusion

This study highlights the potential cardioprotective activity of LRL extract in IIHM. Cardioprotective activity was evident in the significant improvement in lipid profile, with increased HDL and decreased TC, TG, and LDL levels. The most interesting finding was that CME of LRL normalized troponin I levels, alongside a reduction in the LVW/BW ratio, metrics that were markedly increased by isoproterenol induction. Additionally, CME of LRL had a positive impact on liver toxicity, reducing SGPT and SGOT levels. We conclude that LRL has cardioprotective effects by inhibiting LVH and hepatoprotective effects. However, this study is limited by a lack of advanced phytochemical profiling and mechanistic explorations, which should be addressed in future research. Investigations to identify, isolate, and characterize the bioactive constituents of LRL and to explore underlying molecular mechanisms involved in the cardioprotective effects are essential. Overall, our findings suggest that *L. rubra* has potential as the basis for the development of affordable plant-based medications for cardiovascular and hepatic disorders.

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Conflict of Interests

The authors claim to have no conflicting interests.

Ethical considerations

The Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC), Institute of Biological Sciences, Rajshahi University approved the protocol [ref.72(23)/320/IAMEBBC/IBSc] for studying antihyperlipidemic and cardioprotective effects, with mice used as an animal model.

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