RESEARCH PAPER

Protective Role of Rosmarinic Acid in Isoproterenol-Induced Myocardial Infarction Associated with Triton X-100-Induced Atherosclerosis in Comorbid Rat Models

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ABSTRACT
Atherosclerosis (ATS) and myocardial infarction (MI) are closely interconnected cardiovascular disorders driven by dyslipidemia, oxidative stress, inflammation, and endothelial dysfunction. As these conditions frequently coexist in clinical practice, therapies targeting multiple pathological pathways are required. This study investigated the cardioprotective potential of Rosmarinic acid (RA) in an experimental comorbid model of ATS and MI. A comorbid condition of ATS and MI was induced in Wistar rats by sequential administration of Triton X-100 followed by isoproterenol. RA was administered orally at doses of 25 and 50 mg/kg, while rosuvastatin (1 mg/kg, p.o.) served as the standard. Serum lipid profile, atherogenic indices, cardiac biomarkers, inflammatory and hepatic markers, cardiac antioxidant enzymes, electrocardiographic alterations, and histopathological changes were systematically evaluated. The comorbid model produced marked cardiovascular dysfunction characterized by a significant reduction in heart rate (254.00 ± 1.23 to 200.33 ± 0.57 bpm), severe dyslipidemia with increased total cholesterol (163.5 ± 0.18 mg/dL), triglycerides (161.3 ± 0.34 mg/dL), LDL-C (111.8 ± 0.25 mg/dL), and reduced HDL-C (19.5 ± 0.47 mg/dL). Cardiac injury biomarkers were markedly elevated, including CK-MB (13.2 ± 0.6 to 35.0 ± 0.5 IU/L) and LDH (19.5 ± 0.2 to 174.2 ± 0.7 U/L), together with increased CRP (2.83 ± 0.29 to 25.83 ± 0.34 mg/L), SGOT (49.7 ± 0.5 to 93.0 ± 0.4 U/L), SGPT (48.2 ± 0.4 to 100.3 ± 0.3 U/L), and BUN (8.7 ± 0.4 to 10.8 ± 0.3 mg/dL). Myocardial oxidative stress was evidenced by depletion of GSH (30.00 ± 0.52 to 11.67 ± 0.58 µg/g), CAT (34.50 ± 0.44 to 13.67 ± 0.61 U/g), and SOD (22.50 ± 0.44 to 7.83 ± 0.37 U/g), accompanied by ECG abnormalities with ST-segment depression and severe histopathological injury (total myocardial injury score 8.90 ± 0.10). RA treatment significantly improved these pathological changes in a dose-dependent manner. At 50 mg/kg, RA restored heart rate to 238.83 ± 0.53 bpm, reduced total cholesterol to 123.5 ± 0.86 mg/dL, triglycerides to 125.3 ± 0.62 mg/dL, LDL-C to 68.0 ± 1.64 mg/dL, while increasing HDL-C to 30.5 ± 0.55 mg/dL. It also decreased CK-MB to 20.3 ± 0.4 IU/L, LDH to 131.7 ± 1.1 U/L, CRP to 15.83 ± 0.17 mg/L, restored GSH (19.83 ± 0.43 µg/g), CAT (23.00 ± 0.36 U/g), and SOD (16.50 ± 0.36 U/g), improved ECG morphology, attenuated ST-segment depression, and markedly reduced the total myocardial injury score to 3.00 ± 0.20, indicating substantial preservation of myocardial architecture. RA exerted significant cardioprotective effects against experimental ATS-associated MI through multitarget mechanisms involving lipid regulation, antioxidant enhancement, anti-inflammatory activity, and preservation of myocardial structural and functional integrity. These findings suggest that RA is a promising natural therapeutic candidate for the management of complex cardiovascular comorbidities and warrant further mechanistic and clinical investigations.

Keywords: *Rosmarinic acid, Atherosclerosis; Myocardial infarction, Comorbidity; Oxidative stress, Dyslipidemia, Cardioprotection, isoproterenol, Triton X-100.*
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1. INTRODUCTION

Globally, cardiovascular diseases (CVDs) represent the principal cause of morbidity and mortality, with annual deaths exceeding 20 million and imposing a substantial socioeconomic burden on healthcare systems [1,2]. Among the various cardiovascular disorders, coronary artery disease is the predominant cause of myocardial infarction (MI) and contributes significantly to premature mortality

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and disabilities [3]. The growing incidence of CVDs is closely allied with ageing, urbanization, unhealthy dietary habits, physical inactivity, obesity, diabetes mellitus, hypertension, and hyperlipidemia, all of which collectively accelerate vascular injury and disease progression [4,5].

Atherosclerosis (ATS) is the primary pathological process underlying most ischemic cardiovascular disorders. It is a chronic inflammatory disease characterized by lipid buildup, endothelial function impairment, exposure to oxidative stress, long-standing inflammatory cell infiltration, and progressive plaque formation within the arterial walls [6]. Plaque progression narrows the vascular lumen, whereas plaque rupture triggers thrombus formation and acute coronary occlusion, culminating in myocardial ischemia and infarction [7]. In addition to lipid accumulation, persistent oxidative stress and chronic inflammation amplify vascular injury, making these interconnected pathways attractive therapeutic targets [8,9].

MI is the terminal consequence of prolonged myocardial ischemia and remains a major cause of cardiovascular mortality worldwide [10,11]. Despite considerable advances in pharmacological management and secondary prevention strategies, MI continues to impose a considerable clinical burden owing to persistent oxidative stress, continuous inflammation, endothelial function alterations, mitochondrial injury, and dysregulated lipid metabolism, which continue to promote adverse cardiac remodelling and recurrent ischemic events [12–14]. As these pathological mechanisms are uncontrollably involved throughout disease progression, most of the current therapies directed at a single or two molecular targets often provide only partial protection.

The current management of ATS-associated MI largely depends on combinations of lipid-lowering agents, antiplatelets, anticoagulants, β-blockers, ACE inhibitors, and other cardioprotective medications. Although these therapies effectively reduce mortality and recurrent cardiovascular events, long-term multidrug treatment is frequently associated with adverse effects, drug–drug interactions, increased healthcare costs, and poor patient adherence, particularly in individuals with multiple comorbidities [15–17]. Moreover, currently available therapies primarily control disease progression rather than comprehensively addressing the multiple pathological mechanisms responsible for vascular and myocardial injuries [18,19]. These limitations have stimulated interest in identifying **multitarget therapeutic agents** capable of concurrently modulating oxidative imbalance, activation of inflammatory pathways, dyslipidemia, endothelial dysfunction, and myocardial damage.

Natural products have arisen as valuable sources of multitarget therapeutic agents because they contain bioactive molecules that can simultaneously modulate multiple signalling pathways [20]. Numerous phytoconstituents have demonstrated antioxidant, anti-inflammatory, antihyperlipidemic, anti-atherosclerotic, and vasoprotective activities in investigational models of CVDs [21,22]. Unlike many synthetic drugs that predominantly act on individual molecular targets, plant-derived bioactive compounds often exhibit **pleiotropic pharmacological actions**, enabling the concurrent regulation of endogenous lipid metabolism, oxidative stress, ongoing inflammatory signalling, endothelial function, and cellular survival pathways[23,24]. Such multitarget mechanisms may promise greater protection against the complex pathophysiology of ATS-associated myocardial injury.

Rosmarinic acid (RA) is a bioactive polyphenolic ester widely distributed in medicinal herbs and has received considerable attention for its broad spectrum of cardiovascular pharmacological effects. Many previous investigational studies have demonstrated that RA exerts suppression of oxidative stress**, anti-inflammatory, hypolipidemic, anti-atherogenic, and endothelial protective** effects by modulating multiple molecular pathways [25-27]. These complementary mechanisms distinguish RA from conventional single-target pharmacological interventions and highlight its potential as a promising cardioprotective phytoconstituent.

Existing studies suggest that RA exerts significant cardiovascular protective effects; its therapeutic efficacy in the clinically relevant comorbid condition of hyperlipidemia-associated ATS complicated by MI remains inadequately characterized. Many previous experimental investigational studies have focused on isolated models of dyslipidemia or myocardial injury, whereas the integrated pathological interactions between these conditions have received limited attention. Therefore, the current investigational study was designed to evaluate the cardioprotective potential of RA in experimental ATS–comorbid MI models by integrating biochemical, lipid profile, antioxidant, electrocardiographic, and histopathological assessments of the heart. This method may provide additional understanding into the multitarget therapeutic potential of RA in complex CVDs.

2. MATERIAL AND METHODS

2.1. General

Rosmarinic Acid was bought from JK Botanicals. All chemicals were acquired from different vendors: Isoproterenol Hydrochloride (TCI), Cholesterol Kit (Transasia Bio-Medicals Ltd.), High-Q SGPT (Pariksha Biotech Pvt Ltd.), and High-Q CK-MB (Pariksha Biotech Pvt Ltd.). Digital Physiograph (Meditech Technologies India Pvt. Ltd.) and microscope (Labomed).

2.2. Experimental Animals

Fully-grown Male Wistar rats (150–200 g) were acquired from the central animal facility. Animals were kept in polypropylene cages under precise environmental conditions (temperature 22 ± 2 °C, 12-hour light/dark cycle, 50–60% humidity) with free access to standard pellet diet and water ad libitum. Before experimentation, the animals underwent a 7-day acclimatization period in a laboratory environment [28]. The present study protocol was

permitted by the IAEC of the University of Hyderabad (Approval No. UH/IAEC/SK/2023-1/23), and all procedures obeyed with CPCSEA guidelines (Govt. of India, 2018) and ARRIVE 2.0 reporting guidelines. Efforts were made to lessen animal suffering by providing acclimatization, gentle handling, and daily monitoring for signs of distress.

2.3. Experimental design

The current study evaluated the cardioprotective effects of RA in a rat model of ATS and MI induced by Triton X-100 and ISO, respectively. A 30 healthy adult Male Wistar rats (150–200 g) were randomly separated into five groups (n = 6 per group), as shown in Table 1. Group I was labelled as the normal control, while Group II represented the disease control, receiving Triton X-100 and ISO to induce comorbid conditions. Group III received standard treatment (rosuvastatin), and Groups IV and V received low and high doses of RA, respectively [29].

The selected doses of RA were based on earlier studies indicating significant antioxidant and cardioprotective activities of RA within this range in rodent models. [30,31]

2.4. Experimental Induction of Comorbid

Healthy male Wistar rats (200–350 g) were acclimatized and computer-randomized into five experimental groups (n = 6). Following an 18 h fast, ATS was induced by intraperitoneal administration of Triton X-100 (200 mg/kg on day 1, followed by 50 mg/kg on alternate days). The respective treatments (rosuvastatin or RA) were administered orally according to the experimental design. MI was induced by subcutaneous administration of ISO at 5.25 mg/kg on days 20 and 21, followed by 8.5 mg/kg once daily from days 22–28.

Table 1: Experimental Treatment Schedule for Evaluating the Protective Effect of RA in Comorbid Rat Models

Group	Treatment
Group I (Normal Control)	Normal saline (1 mL/kg, p.o.) from Day 1 to Day 28.
Group II (Comorbid)	Triton X-100 (200 mg/kg, i.p., Day 1; 50 mg/kg, i.p., on alternate days) + ISO (5.25 mg/kg, i.p., Days 20–21; 8.5 mg/kg, s.c., Days 22–28).
Group III (Standard)	Rosuvastatin (1 mg/kg, p.o), once daily (Days 1–28) + Comorbid
Group IV (Low Dose)	RA (25 mg/kg, p.o.) once daily (Days 1–28) + Comorbid
Group V (High Dose)	RA (50 mg/kg, p.o.) once daily (Days 1–28) + Comorbid

Note: I.P: Intraperitoneal; S.C: subcutaneous; ISO: Isoproterenol; RA: Rosmarinic Acid**;** P.O: Per os

2.7. Electrocardiographic Recording and Analysis

At the termination of the treatment period, ECG copies were obtained using a digital physiograph (Polygraph Digital-3 Channel; RMS, India). Rats were anesthetized with ketamine (60 mg/kg i.p) and xylazine (9 mg/kg). After confirming full anesthesia, subcutaneous needle electrodes were placed in the left upper and lower chest and right upper and lower limbs according to the standard limb-lead configuration. ECG signals were recorded to assess key cardiac electrical parameters, including the RR interval (s), PR interval (s), QRS duration (s), QT interval (s), P-wave amplitude (mV), R-wave amplitude (mV), and heart rate (beats/min), which have been widely validated in rodent cardiotoxicity studies [32].

2.5. Estimation of Biochemical Parameters

After ECG recording, blood samples were obtained from each animal using retro-orbital blood collection. The procedure was performed using sterile capillary tubes, and gentle pressure was applied to achieve hemostasis and minimize discomfort, following CPCSEA (2018) and ARRIVE 2.0 guidelines [33,34]. The collected blood was allowed to clot at normal temperature for 20 min and centrifuged at 2500 rpm for 15 min to get serum, which was preserved at −20 °C till next biochemical analysis [35].

Serum was used to estimate cardiac biomarkers (CK-MB and LDH), lipid profiles (TC, TG, HDL-C, LDL-C, and VLDL-C), liver and renal function markers (AST, ALP, and BUN), and atherogenic indices [36]. Following euthanasia, the hearts of all rats were carefully excised and washed with ice-cold saline solution. One half was homogenized to estimate cardiac oxidative stress markers (SOD, CAT, and GSH), whereas the remaining half was fixed in 10% neutral- buffered formalin for histopathological examination [37].

2.6. Estimation of Cardiac Antioxidant Parameters

The excised cardiac tissues were washed with ice-cold saline to remove residual blood and immediately homogenized in phosphate buffer (pH 7.4) using a homogenizer. The obtained homogenate solution was centrifuged at 3000 rpm for 10 min at 4 °C, and the resulting supernatant was used for the biochemical estimation of endogenous antioxidant enzymes. The myocardial levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were estimated spectrophotometrically using commercial diagnostic kits, as per the manufacturer's directions and standard validated analytical protocols under appropriate quality-controlled laboratory conditions [38]. All assays were performed in triplicate. Antioxidant enzyme activities were normalized to the protein content of the myocardial homogenates and indicated as units per milligram protein. Total Protein

quantification was determined by the Lowry method against a bovine serum albumin (BSA) standard curve [39].

2.8. Assessment of Cardiac Tissue Histological Parameters

The apical portion of the left ventricle was fixed in a solution of 10% neutral buffered formalin, and then paraffin-embedded, sectioned at 4–5 µm thickness, and marked with application of hematoxylin and eosin (H&E) for histopathological examination. Myocardial injury was assessed using a blinded, semi-quantitative scoring system based on interstitial oedema, myocyte necrosis, and inflammatory cell infiltration (0 = none, 1 = mild, 2 = moderate, and 3 = severe), in accordance with established experimental cardiology criteria [40]. Four non-overlapping microscopic arenas per section were evaluated at 400× magnification, and the cumulative injury score (0–9) was calculated for mean ± SEM for each experimental group.

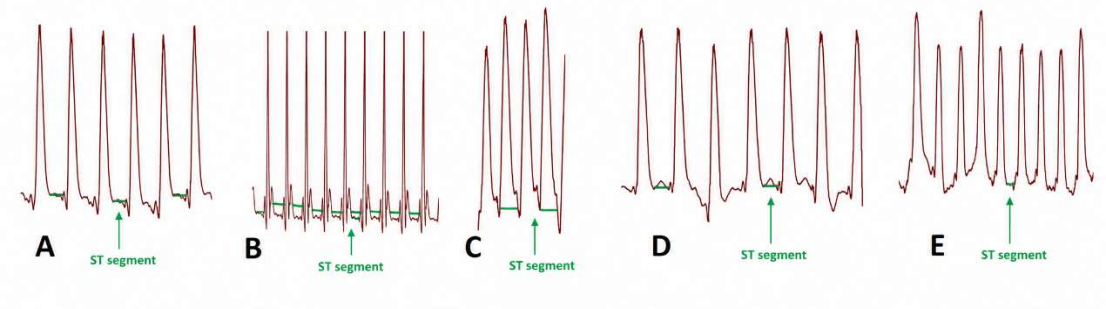
2.9. Statistical Analysis

Data are denoted as **mean ± SEM** (n = 6). Statistical calculations were performed using **GraphPad Prism version 8.0** (GraphPad Software Inc., San Diego, CA, USA). Group comparisons were conducted using one-way ANOVA, followed by Dunnett's post hoc assessment for multiple comparisons, against the comorbid control group. In the figures, **ns** indicates non-significant; †, ‡, and **§** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, compared with the NC group, whereas **a, b**, and **c** denote $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, compared with the Comorbid Control group.

3. RESULTS AND DISCUSSION

3.1 Effect of RA on Electrocardiographic parameters in rats with comorbidities

The representative ECG tracings shown in Graph 1 demonstrate the effect of RA on cardiac electrical activity in rats with comorbidities. Group I (Normal Control) exhibited a regular ECG pattern with a normal sinus rhythm and well-defined P waves, QRS complexes, normal ST segment, and T waves, indicating normal cardiac function. In contrast, Group II (Comorbid Control) showed marked electrocardiographic abnormalities characterized by an irregular rhythm, altered QRS morphology, prominent ST-segment depression, and reduced T-wave amplitude, findings consistent with myocardial ischemic injury induced by Triton X-100-mediated hyperlipidemia and ISO administration. The pronounced ST-segment depression and prominent change in T amplitude serve as characteristic indicators of MI and confirm successful induction of myocardial injury[41]. Group III (standard) displayed a near-normal ECG pattern with restoration of regular rhythm and normalization of the QRS complex, ST segment, and T-wave morphology, indicating significant cardioprotection. Group IV (RA, low-dose) showed partial improvement in ECG morphology, with attenuation of ST-segment depression, improved T-wave morphology, and restoration of a more regular rhythm compared with Group I with comorbidities. Group V (RA, high-dose) demonstrated a more pronounced restoration of normal ECG architecture, exhibiting a regular rhythm with near-normal QRS complexes, minimal ST-segment depression, and improved T-wave morphology, indicating dose-dependent protection against myocardial injury. These findings suggest that RA effectively attenuates myocardial electrical disturbances associated with comorbid conditions, with a higher dose providing greater cardioprotective efficacy.



Graph 1. Effect of RA on ECG Tracings of Comorbid Rats A) Group I B) Group II C) Group III D) Group IV E) Group V

The ECG parameters presented in Table 1Tables 2 andTable 3 revealed marked alterations in the comorbid control group (Group II) compared with the normal group (Group I), confirming significant impairment of cardiac electrical activity following Triton X-100-induced hyperlipidemia and ISO-induced myocardial injury. HR decreased significantly from 254.00 ± 1.23 to 200.33 ± 0.57 bpm ($p < 0.001$), while P-wave, R-wave, and T-wave amplitudes increased from 0.04 ± 0.04, 0.24 ± 0.02, and 0.04 ± 0.04 to 0.19 ± 0.02, 1.03 ± 0.01, and 0.32 ± 0.004, respectively ($p < 0.001$). In contrast, QT, QRS, and QTc intervals were significantly reduced from 0.35 ± 0.004, 0.13 ± 0.002, and 0.07 ± 0.003 to 0.13 ± 0.004, 0.05 ± 0.009, and 0.03 ± 0.002, respectively ($p < 0.001$), whereas the RR interval increased significantly (0.23 ± 0.033 to 0.27 ± 0.019, $p < 0.05$). These changes, together with ST-

segment depression, indicate impaired cardiac conduction and myocardial electrical instability.

Rosuvastatin (Group III) significantly restored the altered ECG parameters, increasing heart rate to 243.33 ± 0.96 bpm ($p < 0.01$), normalising P-wave (0.04 ± 0.011), R-wave (0.26 ± 0.038), QRS (0.09 ± 0.017), QT (0.25 ± 0.072), and QTc (0.06 ± 0.008) intervals, with only nonsignificant changes in RR and PR intervals.

RA treatment also improved the ECG abnormalities in a dose-dependent pattern. The low dose (Group IV) increased the heart rate to 222.00 ± 0.55 bpm and significantly reduced P-wave (0.15 ± 0.024), R-wave (0.90 ± 0.028), and T-wave (0.24 ± 0.057) amplitudes while improving QT (0.16 ± 0.023), QRS (0.07 ± 0.012), and QTc (0.04 ± 0.016) intervals ($p < 0.05$–0.01). The high dose (Group V) produced greater cardioprotection, increasing the heart rate to 238.83 ± 0.53 bpm and restoring P-wave (0.12 ± 0.028), T-wave (0.17 ± 0.080), QRS (0.09 ± 0.025), and QTc (0.12 ± 0.196) toward normal values, with concurrent improvement in the remaining ECG parameters. Overall, RA effectively attenuated ECG abnormalities and ST-segment depression with increasing doses, although rosuvastatin produced the greatest restoration of normal cardiac electrical activity.

Table 2: Effect of RA on ECG Parameters (QT, RR, PR, QRS, and QTc Intervals) in rats with comorbidities.

Groups	QT Intervals Sec.	RR Intervals Sec.	PR Intervals Sec.	QRS Intervals Sec.	QTc Intervals Sec.
Group-I	0.35±0.004	0.23±0.033	0.1±0.066	0.13±0.002	0.07±0.003
Group-II	0.13±0.004§	0.27±0.019†	0.14±0.083^{ns}	0.05±0.009§	0.03±0.002§
Group-III	0.25±0.072^{a}	0.23±0.018^{ns}	0.11±0.04^{ns}	0.09±0.017^{c}	0.06±0.008^{c}
Group-IV	0.16±0.023^{b}	0.26±0.015^{ns}	0.09±0.079^{ns}	0.07±0.012^{a}	0.04±0.016^{a}
Group-V	0.20±0.025	0.25±0.012	0.13±0.005	0.09±0.025^{b}	0.12±0.196^{b}

Table 3: Impact of RA on ECG P, R, T Amplitude and Heart rate in comorbid rats.

Groups	Heart rate (HR) Beats/Min	P Amplitude mV	R Amplitude mV	T Amplitude mV
Group-I	254.00±1.234	0.04 ±0.040	0.24±0.021	0.04±0.036
Group-II	200.33±0.571§	0.19±0.019§	1.03±0.014§	0.32±0.004§
Group-III	243.33±0.955^{b}	0.04±0.011^{c}	0.26±0.038^{a}	0.06±0.013
Group-IV	222.00±0.549^{a}	0.15±0.024^{a}	0.90±0.028^{b}	0.24±0.057^{b}
Group-V	238.83±0.533^{a}	0.12±0.028^{b}	0.61±0.124	0.17±0.080^{c}

3.2 Effect of RA on Serum Lipid Parameters in Comorbid Rats

The influence of RA on the serum lipid profile and cardiac risk indices in Triton-induced ATS combined with ISO-induced MI is presented in Table 4**Error! Reference source not found.** and Figure 1. The comorbid control group (Group II) exhibited a significant ($p < 0.001$) increase in serum TC (163.5 ± 0.18 mg/dL), TG (161.3 ± 0.34 mg/dL), VLDL (32.3 ± 0.15 mg/dL), LDL (111.8 ± 0.25 mg/dL), AIP (0.9 ± 0.04), CRR (8.7 ± 0.2), and AC (7.7 ± 0.3), together with a marked reduction in HDL cholesterol (19.5 ± 0.47 mg/dL) compared with the Group I. As shown in Table 4, these findings confirm the successful induction of dyslipidemia and enhanced cardiovascular risk linked with the comorbid ATS-MI model.

Treatment with the standard drug (Group III) significantly ($p < 0.001$) restored the altered lipid profile, producing substantial reductions in TC, TG, VLDL, LDL, AIP, CRR, and AC, while markedly increasing HDL levels toward normal values. As illustrated in Table 4, the standard-treated group exhibited near-normal lipid parameters, demonstrating its effectiveness in attenuating hyperlipidemia and reducing cardiovascular risk.

Administration of RA also significantly improved serum lipid parameters compared with Group I. As shown in Table 4**Error! Reference source not found.** and Figure 1, RA treatment reduced TC, TG, VLDL, and LDL levels while significantly elevating HDL cholesterol. The response was dose-dependent, with the higher-dose RA-treated group (Group V) showing greater normalization of lipid parameters than the lower-dose group (Group IV). Group V exhibited significantly lower TC (123.5 ± 0.86 mg/dL), TG (125.3 ± 0.62 mg/dL), VLDL (22.9 ± 0.28 mg/dL), LDL (68.0 ± 1.64 mg/dL), and higher HDL cholesterol (30.5 ± 0.55 mg/dL), indicating superior antihyperlipidemic activity.

Similarly, the elevated cardiovascular risk indices detected in the comorbid group were significantly mimimized after RA administration. As depicted in Figure 1, both RA doses significantly decreased AIP, CRR, and AC, with the higher dose producing greater reductions than the lower dose. As these indices are recognized predictors of ATS progression and cardiovascular events, their improvement indicates that RA effectively reduces the overall cardiovascular risk

related with combined hyperlipidemia and myocardial injury.

Table 4: Effect of RA on Serum Lipid Parameters in Comorbid Rat Groups

Groups	Total CH mg/dL	TG mg/dL	HDL mg/dL	VLDL mg/dL	LDL mg/dL
Group-I	102.8±0.49	73.8±0.28	46.5±0.19	14.8±0.12	41.5±0.71
Group-II	163.5±0.18§	161.3±0.34§	19.5±0.47§	32.3±0.15§	111.8±0.25§
Group-III	116.3±0.28^{c}	79.5±0.21^{c}	41.3±0.13^{c}	15.9±0.09^{c}	59.3±0.36^{c}
Group-IV	135.5±0.85^{b}	135.8±0.44^{a}	25.8±0.61^{a}	27.2±0.20^{a}	82.5±0.78^{b}
Group-V	123.5±0.86^{c}	125.3±0.62^{b}	30.5±0.55^{a}	22.9±0.28^{c}	68.0±1.64^{c}

Note: TC = Total Cholesterol; TG = Triglycerides; HDL = High-Density Lipoprotein; LDL = Low-Density Lipoprotein; VLDL = Very-Low-Density Lipoprotein.

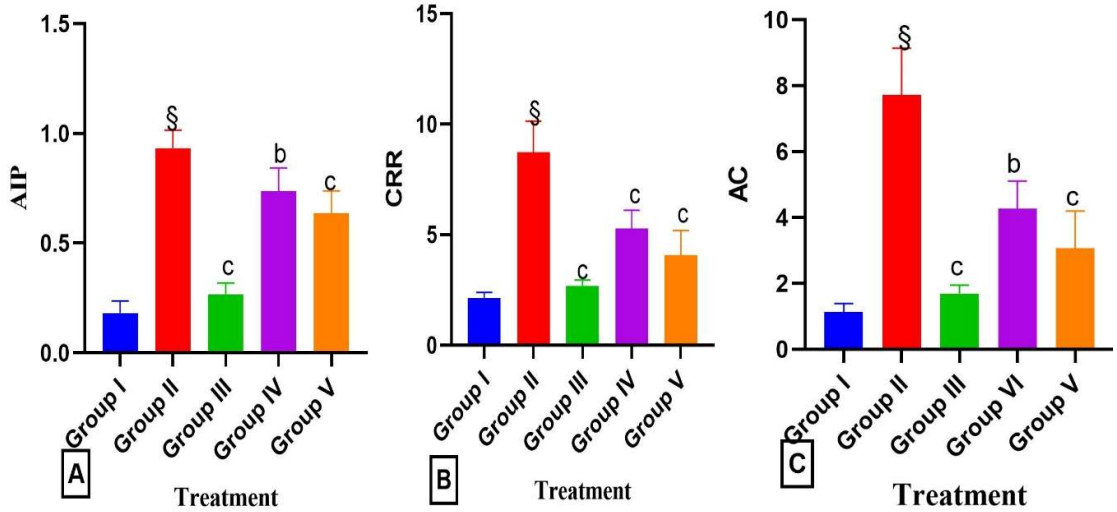


Figure 1:. Effect of RA on Cardiac Risk Indices in Comorbid Rats:(A) CRR, (B) AIP, and (C) AC.

Note: AIP = Atherogenic Index of Plasma; CRR = Cardiac Risk Ratio; AC = Atherogenic Coefficient.

3.3 Effect of RE on Serum Biochemical Markers

The serum biochemical parameters shown in Table 5 revealed significant alterations in the comorbid control group (Group II) compared with those in Group I. Serum CK-MB levels were raised significantly from 13.2 ± 0.6 IU/L in Group I to 35.0 ± 0.5 IU/L in Group II ($p < 0.001$), while LDH levels were raised from 19.5 ± 0.2 U/L to 174.2 ± 0.7 U/L ($p < 0.001$), indicating severe myocardial injury. Similarly, BUN levels were also raised modestly but significantly from 8.7 ± 0.4 mg/dL to 10.8 ± 0.3 mg/dL ($p < 0.05$), suggesting mild renal impairment. The hepatic enzymes SGOT and SGPT were markedly elevated from 49.7 ± 0.5 to 93.0 ± 0.4 mg/dL and from 48.2 ± 0.4 to 100.3 ± 0.3 mg/dL, respectively ($p < 0.001$), indicating hepatocellular injury, which may be related to lipid accumulation and oxidative stress in the liver. Furthermore, the CRP levels increased significantly from 2.83 ± 0.29 mg/L to 25.83 ± 0.34 mg/L ($p < 0.001$), confirming the existence of systemic inflammation.

Treatment with the standard rosuvastatin (Group III) significantly restored all biochemical parameters to normal values. CK-MB and LDH levels decreased to 14.2 ± 0.5 IU/L and 69.8 ± 0.8 U/L, respectively ($p < 0.001$), and BUN decreased to 8.2 ± 0.3 mg/dL ($p < 0.05$). SGOT and SGPT levels were significantly decreased to 52.7 ± 0.5 mg/dL and 55.2 ± 0.2 mg/dL, respectively ($p < 0.001$), and CRP declined to 10.00 ± 0.27 mg/L ($p < 0.001$) compared with the comorbid control group.

RA treatment also produced dose-dependent improvements. The lower dose (Group IV) significantly reduced CK-MB (27.2 ± 0.5 IU/L, $p < 0.05$), LDH (146.0 ± 1.0 U/L, $p < 0.05$), SGOT (80.2 ± 0.5 mg/dL, $p < 0.05$), SGPT (88.5 ± 0.3 mg/dL, $p < 0.01$), and CRP (20.67 ± 0.32 mg/L, $p < 0.01$) levels, whereas the reduction in BUN (9.0 ± 0.1 mg/dL) was non-significant. In contrast, the higher dose of RA (Group V) produced greater protection, significantly decreasing CK-MB to 20.3 ± 0.4 IU/L ($p < 0.01$), LDH to 131.7 ± 1.1 U/L ($p < 0.01$), BUN to 8.5 ± 0.3 mg/dL ($p < 0.05$), SGOT to 71.5 ± 0.6 mg/dL ($p < 0.001$), SGPT to 74.7 ± 0.5 mg/dL ($p < 0.001$), and CRP to 15.83 ± 0.17 mg/L ($p < 0.001$) compared to the comorbid group.

Table 5: Effect of RA on serum cardiac biomarkers (CK-MB, LDH), renal marker (BUN), hepatic enzymes (SGOT, SGPT), and inflammatory marker (CRP) in rats with comorbidities.

Groups	CK MB IU/L	LDH U/L	BUN mg/dL	SGOT mg/dL	SGPT mg/dL	CRP mg/L
Group-I	13.2±0.6	19.5±0.2	8.7±0.4	49.7±0.5	48.2±0.4	2.83±0.29
Group-II	35.0±0.5§	174.2±0.7§	10.8±0.3†	93.0±0.4§	100.3±0.3§	25.83±0.34§
Group-III	14.2±0.5^{c}	69.8±0.8^{c}	8.2±0.3^{a}	52.7±0.5^{c}	55.2±0.2^{c}	10.00±0.27^{c}
Group-IV	27.2±0.5^{a}	146.0±1.0^{a}	9.0±0.1^{ns}	80.2±0.5^{a}	88.5±0.3^{b}	20.67±0.32^{b}
Group-V	20.3±0.4^{b}	131.7±1.1^{b}	8.5±0.3^{a}	71.5±0.6^{c}	74.7±0.5^{c}	15.83±0.17^{c}

Note: CK-MB, Creatine Kinase-MB; LDH, Lactate Dehydrogenase; BUN, Blood Urea Nitrogen; SGOT, Serum Glutamate Oxaloacetate Transaminase (AST); SGPT, Serum Glutamate Pyruvate Transaminase (ALT); CRP, C-Reactive Protein.

3.4 Effect of RA on Cardiac Antioxidant Markers in Comorbid Rats:

The cardiac antioxidant parameters presented in Table**Table 6** revealed a significant decline in the comorbid control group (Group II) compared with Group I, indicating increased oxidative stress in the myocardial tissue. Cardiac GSH levels decreased significantly from 30.00 ± 0.52 µg/g in Group I to 11.67 ± 0.58 µg/g in Group II ($p < 0.001$). Similarly, CAT activity declined from 34.50 ± 0.44 U/g to 13.67 ± 0.61 U/g ($p < 0.001$), and SOD activity decreased from 22.50 ± 0.44 U/g to 7.83 ± 0.37 U/g ($p < 0.001$). These findings indicate severe depletion of endogenous antioxidant defences due to excessive ROS generation associated with hyperlipidemia and ISO-induced myocardial injury.

Treatment with the standard drug rosuvastatin (Group III) significantly restored cardiac antioxidant status. GSH levels increased to 27.50 ± 0.45 µg/g ($p < 0.001$), CAT activity increased to 30.00 ± 0.23 U/g ($p < 0.001$), and SOD activity increased to 19.67 ± 0.22 U/g ($p < 0.001$) compared with the comorbid control group, approaching normal values.

RA treatment also improved the antioxidant defence system in a dose-dependent way. Compared with the comorbid group, the lower dose (Group IV) significantly increased GSH to 15.00 ± 0.49 µg/g ($p < 0.05$), CAT to 17.67 ± 0.42 U/g ($p < 0.05$), and SOD to 12.50 ± 0.20 U/g ($p < 0.01$). The higher dose (Group V) produced a more pronounced restoration, with GSH increasing to 19.83 ± 0.43 µg/g ($p < 0.01$), CAT to 23.00 ± 0.36 U/g ($p < 0.01$), and SOD to 16.50 ± 0.36 U/g ($p < 0.01$) compared with the comorbid group. Although the antioxidant enzyme levels remained lower than those in the normal and standard-treated groups, the significant dose-dependent improvement demonstrated that RA effectively enhanced endogenous antioxidant defenses, thereby mitigating oxidative stress-induced myocardial damage in rats with comorbidities.

Table 6: Effect of RA on cardiac antioxidant parameters

Groups	GSH µmol/mg tissue	CAT U/mg tissue	SOD U/ mg tissue
Group-I	30.00±0.52	34.50±0.44	22.50±0.44
Group-II	11.67±0.58§	13.67±0.61§	7.83±0.37§
Group-III	27.50±0.45^{c}	30.00±0.23^{c}	19.67±0.22^{c}
Group-IV	15.00±0.49^{a}	17.67±0.42^{a}	12.50±0.2^{b}
Group-V	19.83±0.43^{b}	23.00±0.36^{b}	16.50±0.36^{b}

Note: Glutathione (GSH); Catalase (CAT); Superoxide Dismutase (SOD).

3.5 Effect of RA on Histopathological Changes in the Myocardial Tissue of Comorbid Rats

Histopathological examination of cardiac tissue showed distinct morphological alterations among the experimental groups. Group I (Normal Control; Figure 2A) exhibited normal myocardial histoarchitecture with intact cardiac muscle fibers, clear tiny centrally located nuclei, and well-defined longitudinal and transverse (cross) striations, without any evidence of pathological lesions. In contrast, Group II (Comorbid Control; Figure 2B) demonstrated severe myocardial injury characterised by extensive loss of cross-striations (yellow circle), myocardial necrosis (green arrow), splitting and waviness of cardiac muscle fibres (red arrows), interstitial oedema, and prominent inflammatory cell infiltration (black arrow), whereas longitudinal striations and a few nuclei remained discernible. The histopathological scores for edema (2.95 ± 0.05), necrosis (3.00 ± 0.00), inflammatory infiltration (2.95 ± 0.05), and total myocardial injury (8.90 ± 0.10) were significantly higher in Group II than in Group I ($P < 0.001$).

Treatment with rosuvastatin (Group III) markedly restored myocardial architecture, with preservation of cardiac muscle fibers, reduced edema and inflammatory infiltration, and significantly lower scores for edema (1.15 ± 0.10), necrosis (1.20 ± 0.15), inflammatory infiltration (1.05 ± 0.10), and total injury (3.40 ± 0.30) ($p < 0.001$ vs. comorbid control). Group IV (RA, 25 mg/kg; Figure 2D)

showed partial refurbishment of the myocardial architecture with reduced degeneration and inflammatory changes, although mild waviness of muscle fibers persisted, which was reflected by moderate reductions in edema (1.85 ± 0.10), necrosis (2.00 ± 0.10), inflammatory infiltration (1.90 ± 0.12), and total injury score (5.75 ± 0.25). Group V (RA, 50 mg/kg, Figure 2E) exhibited near-normal myocardial architecture with prominent centrally located nuclei, restoration of longitudinal and transverse striations, minimal edema and inflammatory infiltration, and significantly reduced histopathological scores for edema (0.95 ± 0.08), necrosis (1.00 ± 0.10), inflammatory infiltration (1.05 ± 0.09), and total injury (3.00 ± 0.20) ($p < 0.001$ vs. comorbid control group). These findings demonstrate a dose-dependent cardioprotective effect of RA against myocardial injury induced by comorbid conditions.

Table 7: Histopathological Scoring of Myocardial Tissue in Experimental Groups

Groups	Edema (0–3)	Necrosis (0–3)	Inflammatory infiltration (0–3)	Total injury score (0–9)
Group-I	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group-II	2.95 ± 0.05[§]	3.00 ± 0.00[§]	2.95 ± 0.05[§]	8.90 ± 0.10[§]
Group-III	1.15 ± 0.10[c]	1.20 ± 0.15[c]	1.05 ± 0.10[c]	3.40 ± 0.30[c]
Group-IV	1.85 ± 0.10[b]	2.00 ± 0.10[b]	1.90 ± 0.12[b]	5.75 ± 0.25[a]
Group-V	0.95 ± 0.08[c]	1.00 ± 0.10[c]	1.05 ± 0.09[c]	3.00 ± 0.20[c]

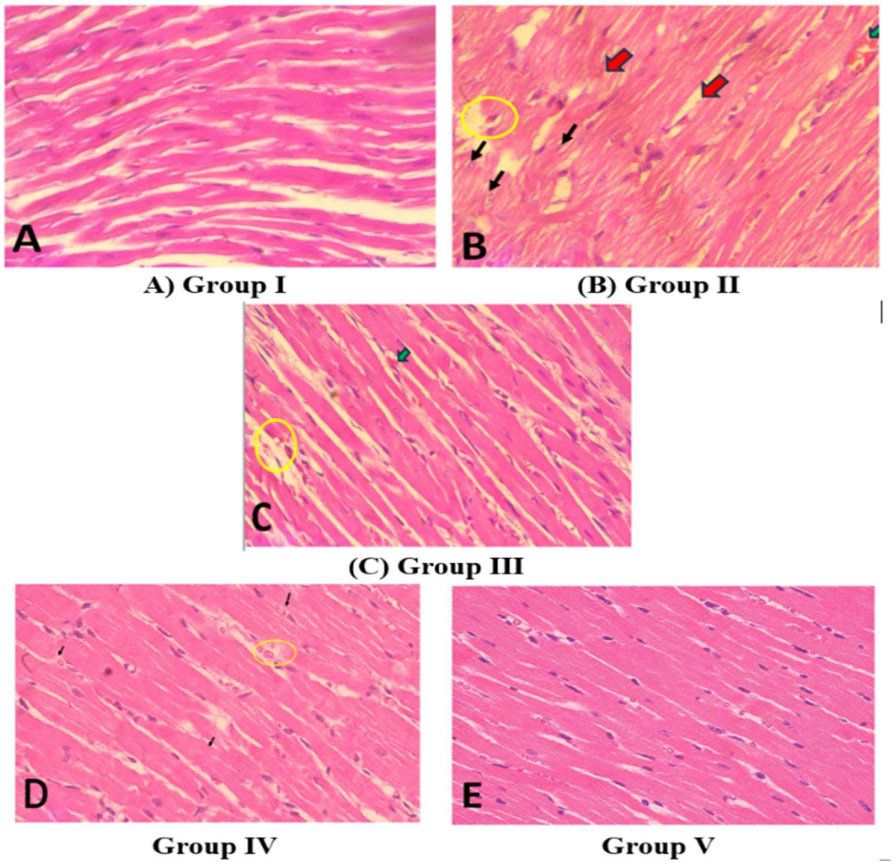


Figure 2: Histopathological Evaluation of Myocardial Tissue in Comorbid Rats Following RA Treatment: A (A) Group I (NC), (B) Group II (DC), (C) Group III (STD), (D) Group IV (RA-25), and (E) Group V (RA-50).

4. SUMMARY

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, with their growing prevalence attributed to multiple metabolic and lifestyle-related risk factors [42,43]. The increasing incidence of CVD among younger individuals further highlights the need for effective preventive and therapeutic strategies [44,45]. Among CVDs, ATS and MI are closely interconnected disorders that play a key role in a substantial proportion of cardiovascular morbidity and mortality. Atherosclerotic

plaque progression and instability predispose to coronary thrombosis, culminating in myocardial ischemia and irreversible cardiomyocyte injury [46]. Enhanced oxidative damage, persistent inflammatory signalling, endothelial functional alterations, mitochondrial damage, and adverse ventricular remodelling are common pathological mechanisms underlying disease progression [47–49].

The coexistence of ATS and MI represents a clinically important comorbid state associated with dyslipidaemia, chronic inflammatory signalling, increased oxidative stress, failure of endothelial function, and accelerated cardiovascular injury, resulting in poorer clinical outcomes [50,51]. Although current pharmacological interventions improve prognosis, they primarily target individual disease pathways and often require combination therapy, which may increase treatment burden and adverse effects [52].

Natural products have attracted extensive interest as multitarget therapeutic agents owing to their antioxidant, anti-inflammatory, lipid-lowering, endothelial-protective, and cardioprotective properties. However, their efficacy against complex cardiovascular comorbidities requires further validation in well-designed experimental models before clinical translation.

The Triton X-100-induced ATS and ISO-induced MI model successfully established a clinically relevant comorbid cardiovascular state, as evidenced by severe dyslipidaemia, increased atherogenic indices, myocardial injury, oxidative stress, electrophysiological disturbances, and characteristic histopathological alterations. The marked elevation of serum TC, TG, LDL-C, CK-MB, LDH, CRP, hepatic enzymes, and BUN, together with depletion of endogenous antioxidant enzymes (GSH, CAT, and SOD), confirmed the successful induction of hyperlipidemia-associated myocardial injury and systemic inflammation.

Rosuvastatin significantly restored these pathological changes, validating the experimental model, whereas RA produced dose-dependent antihyperlipidaemic and cardioprotective effects. The higher dose markedly improved the lipid profile, reduced atherogenic indices, attenuated myocardial injury biomarkers, restored endogenous antioxidant defences, normalised ECG abnormalities, and preserved myocardial histoarchitecture.

The observed antihyperlipidaemic effect of RA is consistent with previous reports, representing the development of reverse cholesterol transport through upregulation of SR-B1, LDL-R, ABCG5/ABCG8, and CYP7A1, together with stimulation of the AMPK–CPT1A pathway, thereby promoting fatty acid β-oxidation and reducing lipid accumulation and atherosclerotic progression.[53]. The significant reduction in CK-MB, LDH, CRP, and hepatic injury markers further supports the cardioprotective potential of RA, which has been attributed to inhibition of NF-κB, JNK, and STAT3 signalling pathways, resulting in suppression of pro-inflammatory cytokine production and inflammatory cell infiltration. In addition, RA has been reported to attenuate NHE-1 overactivation, thereby preventing intracellular sodium and calcium overload and protecting heart tissue against ischemia-induced injury[54, 55].

Restoration of GSH, CAT, and SOD activities indicates that RA effectively counteracted oxidative stress, a major contributor to both ATS and MI. These results are reinforced by previous studies showing stimulation of the Nrf2/ARE signalling pathway, leading to enhanced antioxidant enzyme expression, reduced ROS generation, and inhibition of lipid peroxidation [56]. The improvement in ECG parameters and preservation of myocardial architecture further corroborate the biochemical findings and suggest attenuation of myocardial electrical instability and structural damage. Consistent with earlier investigations, the histological protection afforded by RA may also involve inhibition of RIPK1/RIPK3/MLKL-mediated necroptosis, suppression of MMP-2/MMP-9 activity, and limitation of adverse ventricular remodelling following MI [57,58].

Considering all together, these results with previous reports demonstrate that RA exerts comprehensive cardioprotective effects against hyperlipidaemia-associated myocardial injury through multitarget mechanisms involving lipid regulation, antioxidant defence, anti-inflammatory activity, preservation of myocardial integrity, and inhibition of adverse cardiac remodelling, highlighting its therapeutic potential for the management of complex cardiovascular comorbidities.

CONCLUSION

The current study successfully established a clinically relevant comorbid rat model of ATS and MI using Triton X-100 and ISO, which reproduced key pathological features of human cardiovascular disease, including severe dyslipidaemia, enhanced atherogenic risk, myocardial injury, oxidative stress, inflammation, electrophysiological abnormalities, and histopathological alterations. Rosmarinic acid (RA) produced significant dose-dependent cardioprotective effects, with the higher dose demonstrating greater efficacy in improving the lipid profile, reducing atherogenic indices, attenuating cardiac biomarker release, restoring endogenous antioxidant enzymes, normalising ECG abnormalities, and preserving myocardial histoarchitecture. These findings indicate its pharmacological activity.

The overall cardioprotective effect of RA is likely mediated through multiple corresponding mechanisms, including positive modulation of lipid metabolism, enhancement of antioxidant defence, suppression of inflammatory signalling, preservation of myocardial cell integrity, and attenuation of adverse cardiac remodelling. Such multitarget actions make RA a promising therapeutic candidate for the management of complex cardiovascular comorbidities where oxidative stress, inflammation, dyslipidaemia, and myocardial injury coexist.

Although the current model results provide strong preclinical evidence supporting the cardioprotective potential of RA, supplementary studies are essential to

elucidate its molecular targets, pharmacokinetic profile, long-term safety, and efficacy in clinically relevant disease models and human trials before its therapeutic application can be established.

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