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Evaluation of Cardioprotective Effect of Nigella Sativa Seed and Solanum Melongena Seed with Special Reference to Antioxidant Activity

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ABSTRACT

Background: Cardiovascular diseases (CVDs) remain the leading cause of global mortality, with oxidative stress–mediated myocardial injury being a major underlying mechanism. Isoproterenol (ISO), a synthetic β-adrenergic agonist, induces severe cardiotoxicity through excessive reactive oxygen species (ROS) generation, lipid peroxidation, and subsequent myocardial degeneration. Natural antioxidants from medicinal plants offer promising cardioprotective alternatives.

Objective: To evaluate the cardioprotective and antioxidant effects of ethanolic seed extracts of Nigella sativa (EENS) and Solanum melongena (EESM), individually and in combination, against ISO-induced cardiotoxicity in Wistar rats.

Methods: Forty-two male Wistar rats were divided into eight groups. Animals received EENS (200/400 mg/kg), EESM (200/400 mg/kg), their combination (100 mg/kg each), or Terminalia arjuna (standard) for 21 days. ISO (85 mg/kg, s.c.) was administered on days 20–21 to induce myocardial injury. Electrocardiographic (ECG) parameters, serum biomarkers (CK-MB, AST, ALT, LDH), lipid profile, antioxidant markers (LPO, GSH, SOD, CAT, GPx), and histopathology of heart tissue were assessed.

Results: ISO significantly elevated heart weight, disrupted ECG patterns, increased cardiac enzyme leakage, induced dyslipidemia, and caused severe oxidative stress with marked histological damage. Pretreatment with EENS and EESM significantly ($p < 0.01$–0.001) restored ECG parameters, reduced CK-MB and LDH, corrected lipid abnormalities, suppressed LPO, and enhanced antioxidant enzymes. The combination group showed greater improvement than individual extracts. Histopathological findings confirmed reduced myofibrillar degeneration and improved cardiac architecture in treated groups.

Conclusion: EENS and EESM exhibit potent cardioprotective and antioxidant activity against ISO-induced myocardial injury. Their combination provides synergistic protection comparable to T. arjuna, supporting their therapeutic potential in oxidative stress-mediated cardiovascular disorders.

KEYWORDS: Nigella sativa; Solanum melongena; Cardioprotection; Isoproterenol; Oxidative stress; Antioxidant activity; ECG; Lipid profile; Histopathology.

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of global morbidity and mortality, accounting for nearly one-third of all deaths worldwide. Myocardial infarction, a major manifestation of CVD, results from prolonged ischemia leading to irreversible myocardial cell injury, necrosis, and cardiac dysfunction [1]. Experimental models employing isoproterenol (ISO), a synthetic catecholamine and non-selective β-adrenergic agonist, are widely used to mimic human myocardial damage due to their ability to induce severe oxidative stress, calcium overload, cardiomyocyte necrosis, and electrophysiological abnormalities. ISO undergoes auto-oxidation to generate excessive reactive oxygen species (ROS), which initiate lipid peroxidation, disrupt membrane integrity, and cause mitochondrial dysfunction ultimately precipitating acute cardiotoxicity [2]. Oxidative stress is recognized as a central mechanism in the pathogenesis of myocardial injury, and antioxidant-rich natural products have gained scientific interest as complementary cardioprotective agents. *Nigella sativ*a (black seeds), belonging to the family Ranunculaceae, contains potent bioactive constituents such as thymoquinone, nigellone, flavonoids, phenolic acids, and essential fatty acids, all of which demonstrate strong free radical–scavenging, anti-inflammatory, and membrane-stabilizing properties. Similarly, Solanum melongena (eggplant) seeds are rich in flavonoids (e.g., nasunin), alkaloids, phenolics, anthocyanins, and steroidal compounds, which exhibit antioxidant, antihyperlipidemic, and cardioprotective effects [3]. While both plants are traditionally used for various ailments, their comparative and combined cardioprotective potential against ISO-induced myocardial injury has not been adequately explored. Although several studies have reported the antioxidant and therapeutic properties of N. sativa and S. melongena individually, no study has systematically evaluated their seed extracts—alone and in combination—in a standardized ISO-induced cardiotoxicity model, particularly with comprehensive assessment of ECG changes, serum biomarkers, lipid profile, oxidative stress parameters, and histopathology. Therefore, the present study was designed to investigate the

cardioprotective effects of ethanolic seed extracts of Nigella sativa (EENS) and Solanum melongena (EESM), individually and in combination, against ISO-induced myocardial damage in rats, with special reference to their antioxidant potential [4].

MATERIALS AND METHODS

Plant Material and Extract Preparation

Dried seeds of *Nigella sativa* (NS) and *Solanum melongena* (SM) were procured from Chahaniya Krishi Vikas Kendra, Chandauli (Uttar Pradesh, India) and taxonomically authenticated at the Botanical Survey of India, Western Regional Centre, Pune (Specimen No.: BSI/WRC/120-12/TECH/2024). Seeds were shade-dried, powdered, and subjected to Soxhlet extraction using ethanol. The extracts were concentrated at 40 °C, stored in airtight containers, and kept in a desiccator until use.

Preliminary Phytochemical Screening

The ethanolic seed extracts of NS (EENS) and SM (EESM) were qualitatively screened for alkaloids, flavonoids, tannins, saponins, glycosides, phenols, carbohydrates, steroids, and resins using standard protocols described by Kokate and Harborne [5].

Experimental Animals

Adult male Wistar rats (150–200 g) were housed under controlled environmental conditions (22 ± 3 °C, 50–60% humidity, 12-h light/dark cycle) with free access to standard food and water. All procedures complied with CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee.

Experimental Design

Animals were acclimatized for 7 days and randomly divided into eight groups (n = 5). All treatments were administered orally for 21 days, while myocardial injury was induced by subcutaneous isoproterenol (ISO) injection (85 mg/kg) on days 20 and 21 [6]. The grouping and treatment schedule are summarized in **Table 1**.

Table 1. Experimental Grouping and Treatment Schedule

Group	Treatment	Dose & Route	ISO Induction (Days 20–21)
I	Vehicle control	0.3% CMC-Na (2 mL/kg, p.o.)	Normal saline (1 mL/kg, s.c.)
II	Disease control	0.3% CMC-Na (2 mL/kg, p.o.)	ISO 85 mg/kg, s.c.
III	EENS low dose	EENS 200 mg/kg, p.o.	ISO 85 mg/kg, s.c.
IV	EENS high dose	EENS 400 mg/kg, p.o.	ISO 85 mg/kg, s.c.
V	EESM low dose	EESM 200 mg/kg, p.o.	ISO 85 mg/kg, s.c.
VI	EESM high dose	EESM 400 mg/kg, p.o.	ISO 85 mg/kg, s.c.
VII	Combination	EENS 100 mg/kg + EESM 100 mg/kg, p.o.	ISO 85 mg/kg, s.c.
VIII	Standard drug	*Terminalia arjuna* 250 mg/kg, p.o.	ISO 85 mg/kg, s.c.

(+ p.o. = oral gavage; s.c. = subcutaneous)

Electrocardiographic (ECG) Analysis

On day 22, rats were lightly anesthetized with ether, and Lead II ECG was recorded using a student physiograph (Biodevice). Needle electrodes were placed subcutaneously in the right forelimb, right hindlimb, and left hindlimb. QT interval, QRS duration, and R–R interval were analyzed [7].

BIOCHEMICAL ESTIMATIONS

Blood samples were collected from the retro-orbital plexus under light ether anesthesia, and the serum was separated for biochemical analysis. The following serum lipid parameters were estimated using standard laboratory procedures as described in the original study: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C, calculated using the Sampson equation), and very-low-density lipoprotein cholesterol (VLDL-C). In addition, cardiac injury marker enzymes, including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were quantified using validated commercial diagnostic kits [8]. Biochemical parameters assessed is presented in **Table 2**.

Table 2. Biochemical Parameters Estimated in Serum

Category	Parameters Measured
Lipid Profile	Total cholesterol (TC), Triglycerides (TG), HDL-C, LDL-C*, VLDL-C
Cardiac Marker Enzymes	CK-MB, LDH, AST, ALT

Assessment of Myocardial Oxidative Stress

Myocardial oxidative stress was evaluated using homogenized heart tissue. Standard biochemical methods were used to estimate lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which serve as key indicators of oxidative damage and antioxidant defense status [9].

Histopathological Examination

Heart tissues were fixed in 4% paraformaldehyde for 48 h, dehydrated in graded alcohols, cleared in xylene, embedded in paraffin, and sectioned at 5 µm. Sections were stained with hematoxylin and eosin (H&E) and examined under a trinocular microscope (Carl Zeiss Primostar) by a blinded histopathologist [10].

Statistical Analysis

Data are expressed as mean ± SEM (n = 5). Statistical comparisons were performed using one-way ANOVA followed by appropriate post-hoc tests in GraphPad Prism 5. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening

The qualitative phytochemical analysis (Table 3) showed that both EENS and EESM contained alkaloids, glycosides, carbohydrates, saponins, flavonoids, phenols, steroids, and tannins, while resins were absent. The presence of these Phytoconstituents particularly flavonoids, phenolics, and alkaloids is important because they are known antioxidants capable of scavenging free radicals and stabilizing cell membranes, suggesting that both extracts possess potential cardioprotective activity.

Table 3. Qualitative phytochemical profile of EENS and EESM

Phytochemical Test	Alkaloids	Glycosides	Carbohydrates	Saponins	Flavonoids	Phenols	Steroids	Tannins	Resins
EENS	+	+	+	+	+	+	+	+	–
EESM	+	+	+	+	+	+	+	+	–

(+ Present; – Absent)

Effect on Heart Weight

Isoproterenol administration caused a significant **($p < 0.001$)** elevation in heart weight compared to the control group, indicating ISO-induced myocardial hypertrophy (Table **4**). Pretreatment with EENS and EESM attenuated this increase in a dose-dependent manner. Among the treated groups, EESM 400 mg/kg and the combination group showed significant **($p < 0.01$)** reduction in heart weight, suggesting effective protection against myocardial edema and hypertrophic remodeling. The improvement observed with higher doses and the combination extract indicates a potential synergistic cardioprotective effect.

Table 4. Effect of extracts on heart weight

Group	Heart Weight (g)
Control	1.11 ± 0.03
ISO	1.54 ± 0.05###
EENS 200	1.23 ± 0.06 NS
EENS 400	1.21 ± 0.02 NS
EESM 200	1.39 ± 0.02 NS
EESM 400	1.23 ± 0.03**
Combination	1.28 ± 0.02**
T. arjuna	1.65 ± 0.02**

*(### $p < 0.001$ vs Control; ** $p < 0.01$ vs ISO; NS = Not significant)*

Effect on ECG Parameters

Isoproterenol administration produced marked electrocardiographic abnormalities, evidenced by significant **($p < 0.001$)** prolongation of the QT interval, QRS duration, and R–R interval compared to the control group (Table **5**, Fig. **1**). Pretreatment with EENS 400 mg/kg, EESM 400 mg/kg, and the combination formulation significantly (**$p < 0.01$–0.001**) restored these ECG parameters toward normal physiological values. In contrast, the lower-dose groups (200 mg/kg) produced only modest or nonsignificant improvements. The normalization of QT and QRS intervals in the higher-dose and combination groups indicates stabilization of cardiac electrical activity, restoration of ionic homeostasis, and effective attenuation of ISO-induced ventricular conduction disturbances. These improvements are clearly reflected in the Lead II ECG tracings shown in Fig. **1**, which depict changes in QT interval, QRS duration, and R–R interval across all experimental groups Group I (Control), Group II (ISO), Group III (EENS 200 mg/kg), Group IV (EENS 400 mg/kg), Group V (EESM 200 mg/kg), Group VI (EESM 400 mg/kg), Group VII (Combination), and Group VIII (*T. arjuna*) demonstrating dose-dependent restoration of electrical activity in extract- and standard-treated animals.

Table 5. Effect of extracts on ECG parameters

Group	QT (s)	QRS (s)	R–R (s)
Control	0.32 ± 0.01	0.22 ± 0.03	0.96 ± 0.03
ISO	0.61 ± 0.03###	0.39 ± 0.03###	1.24 ± 0.07###
EENS 200	0.28 ± 0.02 NS	0.21 ± 0.05 NS	0.83 ± 0.06 NS
EENS 400	0.46 ± 0.03***	0.25 ± 0.06**	0.95 ± 0.04**
EESM 200	0.29 ± 0.06 NS	0.16 ± 0.06 NS	0.85 ± 0.07 NS
EESM 400	0.36 ± 0.06***	0.23 ± 0.02**	0.87 ± 0.06***
Combination	0.40 ± 0.03**	0.16 ± 0.02**	0.74 ± 0.06**
T. arjuna	0.46 ± 0.07***	0.21 ± 0.06***	1.43 ± 0.06***

*(### p < 0.001 vs Control; ** p < 0.01, *** p < 0.001 vs ISO; NS = Not significant)*

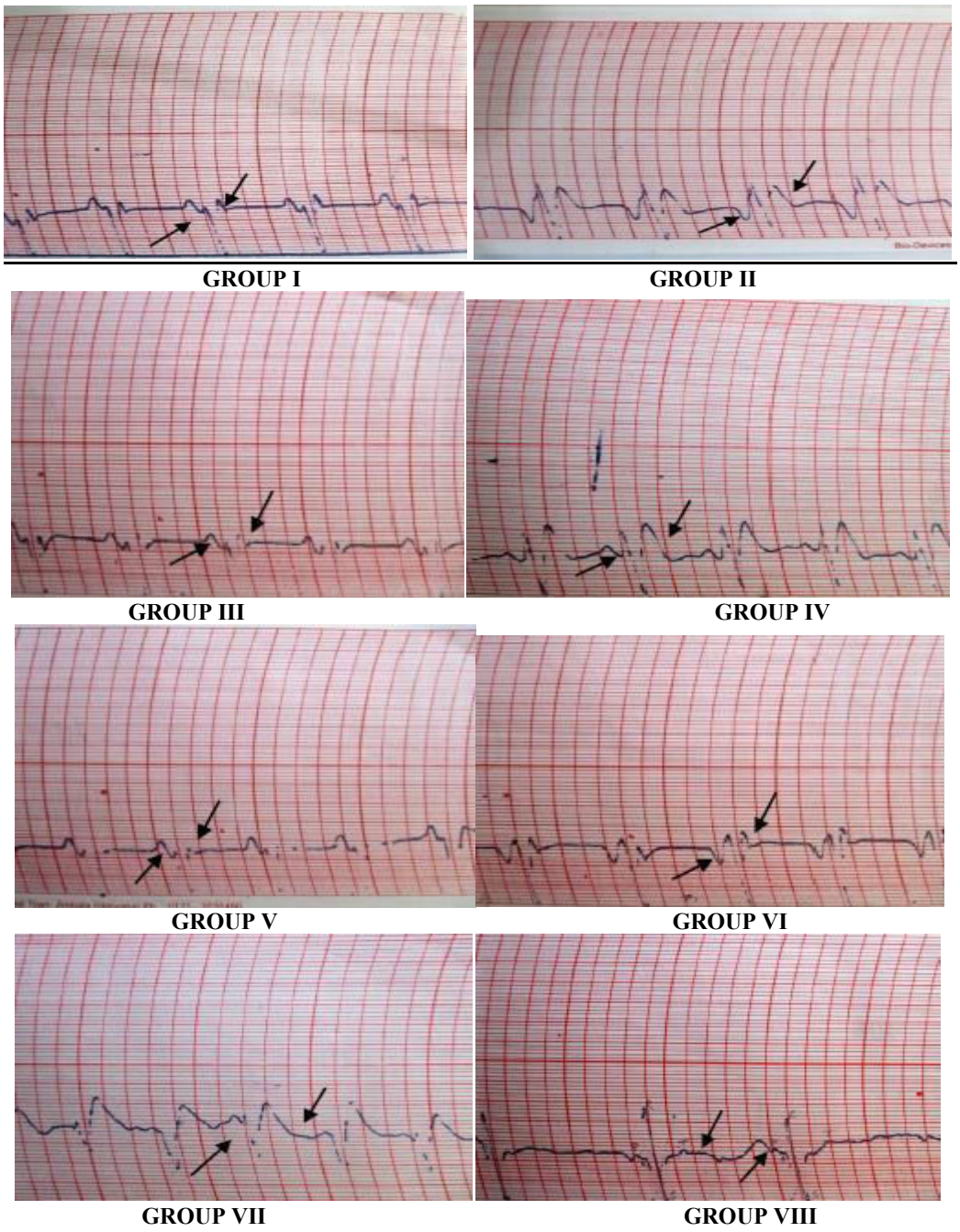

Figure 1. Electrocardiographic (ECG) recordings of experimental groups

EFFECT ON SERUM CARDIAC ENZYMES

ISO administration produced a marked elevation in serum CK-MB, AST, ALT, and LDH levels (**p < 0.001**) compared to the control group, confirming extensive cardiomyocyte damage (Table **6**). Pretreatment with EENS (400 mg/kg), EESM (400 mg/kg), and their combination effectively reduced these elevated enzyme levels (**p < 0.01–0.001**). The decline in biomarker levels indicates restoration of membrane integrity and attenuation of ISO-induced myocardial necrosis. Among the treatments, EESM 400 mg/kg and the combination extract demonstrated responses comparable to the standard drug *Terminalia arjuna*, suggesting strong cardioprotective potential.

Table 6. Effect of extract and T. arjuna on serum CK-MB, AST, ALT, and LDH during ISO-induced cardiotoxicity in rats

Experimental Group	CK-MB	AST	ALT	LDH
Group I - Vehicle control	33.56 ± 1.32	39.56 ± 2.5	31.45 ± 2.43	911.45 ± 12.56
Group II - Disease control (ISO 85 mg/kg)	92.54 ± 5.45###	107.65 ± 4.56###	115.69 ± 2.34###	2234.65 ± 15.32###

Group III - EENS 200 + ISO	43.20 ± 11.9 NS	42.54 ± 3.67 NS	39.11 ± 2.78 NS	567.38 ± 12.65###
Group IV - EENS 400 + ISO	45.34 ± 1.67***	67.57 ± 2.32***	25.76 ± 1.34**	1767.44 ± 9.45**
Group V - EESM 200 + ISO	24.61 ± 3.29 NS	45.01 ± 7.84 NS	42.65 ± 3.11 NS	560.06 ± 4.64***
Group VI - EESM 400 + ISO	46.32 ± 5.45***	50.34 ± 2.45***	34.68 ± 1.17***	1456.23 ± 12.7###
Group VII - Combination (EENS + EESM) + ISO	39.56 ± 1.65***	49.33 ± 3.36***	49.33 ± 1.42*	1316.20 ± 15.65***
Group VIII - *T. arjuna* + ISO	38.63 ± 1.45***	42.34 ± 2.67***	35.16 ± 1.56***	1156.65 ± 12.65***

***NS:** Not significant, **#, ##, ###:** $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. Control, ***, **, ***:** $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. ISO*

Effect on Lipid Profile

ISO administration caused significant (**$p < 0.001$**) elevations in TC, TG, LDL, and VLDL, accompanied by a marked decrease in HDL levels compared to control (Table **7**). Pretreatment with both extracts improved the lipid profile dose-dependently, with 400 mg/kg doses showing the strongest hypolipidemic activity. The combination group also significantly corrected dyslipidemia. These improvements indicate enhanced lipid regulation, reduced lipid peroxidation, and stabilization of cardiovascular metabolic pathways.

Table 7. Effect of extracts on serum lipid profile

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	165.45 ± 3.11	125.11 ± 3.69	79.11 ± 4.56	55.56 ± 5.54	35.11 ± 2.57
ISO	255.33 ± 8.54###	256.11 ± 7.65###	25.34 ± 5.82###	181.11 ± 9.65###	52.56 ± 3.86###
EENS 200	145.11 ± 5.61 NS	165.31 ± 7.45 NS	69.46 ± 5.07 NS	45.11 ± 5.45 NS	23.7 ± 3.43 NS
EENS 400	132.34 ± 5.56***	121.44 ± 2.69**	68.04 ± 7.11***	48.38 ± 5.01**	23.22 ± 1.53**
EESM 200	109.11 ± 8.67###	201.56 ± 7.73 NS	57.67 ± 6.92 NS	36.74 ± 7.51 NS	41.35 ± 1.52 NS
EESM 400	117.65 ± 5.7***	133.11 ± 5.18***	66.69 ± 3.45***	49.3 ± 6.35**	29.13 ± 1.06**
Combination	145.45 ± 5.56***	146.23 ± 11.86**	61.42 ± 8.59*	50.42 ± 10.9**	27.29 ± 2.37*
T. arjuna	159.5 ± 3.68***	131.56 ± 9.34***	71.23 ± 5.74 NS	53.37 ± 4.44***	32.55 ± 1.65***

Effect on Oxidative Stress Markers

ISO significantly increased LPO and decreased GSH, SOD, CAT, and GPx ($p < 0.001$) (Table **8**). Pretreatment with the extracts improved all antioxidant parameters, with 400 mg/kg doses showing the highest activity. ISO generates excess ROS, causing lipid peroxidation and depletion of endogenous antioxidants; therefore, the restoration of antioxidant enzyme levels by the extracts indicates potent free-radical scavenging activity. The enhanced GSH concentration and improved enzymatic antioxidant activity reflect direct oxidative protection, and the combination extract demonstrated synergistic antioxidant potential.

Table 8. Effect of extracts and *T. arjuna* tablets on myocardial oxidative stress markers during ISO-induced cardiotoxicity in rats

Experimental Group	LPO	GSH	SOD	CAT	GPx
Group I Vehicle control	118.54 ± 2.65	32.54 ± 1.54	16.41 ± 0.54	0.85 ± 0.05	8.57 ± 0.12
Group II Disease control (85 mg kg⁻¹)	275.74 ± 5.1###	7.65 ± 0.32###	7.21 ± 0.42###	0.23 ± 0.05###	1.88 ± 0.29###
Group III (EENS 200 mg/kg + ISO)	108.13 ± 1.43#	29.11 ± 1.32#	17.43 ± 0.12##	0.87 ± 0.05 NS	9.96 ± 0.47###
Group IV (EENS 400 mg/kg + ISO)	174.76 ± 1.65***	22.23 ± 0.79***	14.11 ± 0.52***	0.54 ± 0.02***	6.17 ± 0.14***
Group V (EESM 200 mg/kg + ISO)	107.4 ± 1.62###	35.11 ± 0.22 NS	17.94 ± 0.10 NS	0.89 ± 0.32 NS	9.09 ± 0.15 NS
Group VI (EESM 400 mg/kg + ISO)	132.6 ± 1.55***	30.43 ± 0.6***	15.63 ± 0.61***	0.76 ± 0.04***	8.54 ± 0.09***
Group VII (EENS + EESM 100 mg/kg + ISO)	126.73 ± 1.57**	29.76 ± 1.54**	14.90 ± 0.57**	0.71 ± 0.02**	8.54 ± 0.13**
Group VIII (*T. arjuna* + ISO)	159.54 ± 1.54***	31.65 ± 1.81***	15.98 ± 0.42***	0.81 ± 0.01***	7.54 ± 0.11***

NS – Not significant, #: $p < 0.05$ vs Control, ##: $p < 0.01$ vs Control, ###: $p < 0.001$ vs Control. *: $p < 0.05$ vs ISO, **: $p < 0.01$ vs ISO, ***: $p < 0.001$ vs ISO.

Histopathological Examination

Histopathological evaluation of cardiac tissue revealed normal myocardial architecture in Group I, characterized by intact muscle fibers, clear striations, and absence of edema or inflammatory infiltration. In contrast, ISO-treated Group II showed marked pathological alterations, including dilation of cardiac muscle fibers, breaks in muscle strands, myofibrillar loss, vacuolization,

and inflammatory cell infiltration, confirming severe myocardial injury (Figure **2**). Pretreatment with EENS and EESM at both doses (Groups III–VI) demonstrated dose-dependent protection, with reduced myofibrillar degeneration, decreased vacuolization, and better preservation of myocardial continuity compared to ISO control. The combination group (Group VII) exhibited improved myocardial integrity with minimal congestion and reduced structural disruption, indicating synergistic cardioprotection. The *Terminalia arjuna*–treated group (Group VIII) also displayed partial preservation of muscle fibers with decreased vacuolization and improved tissue organization, consistent with its established cardioprotective potential.

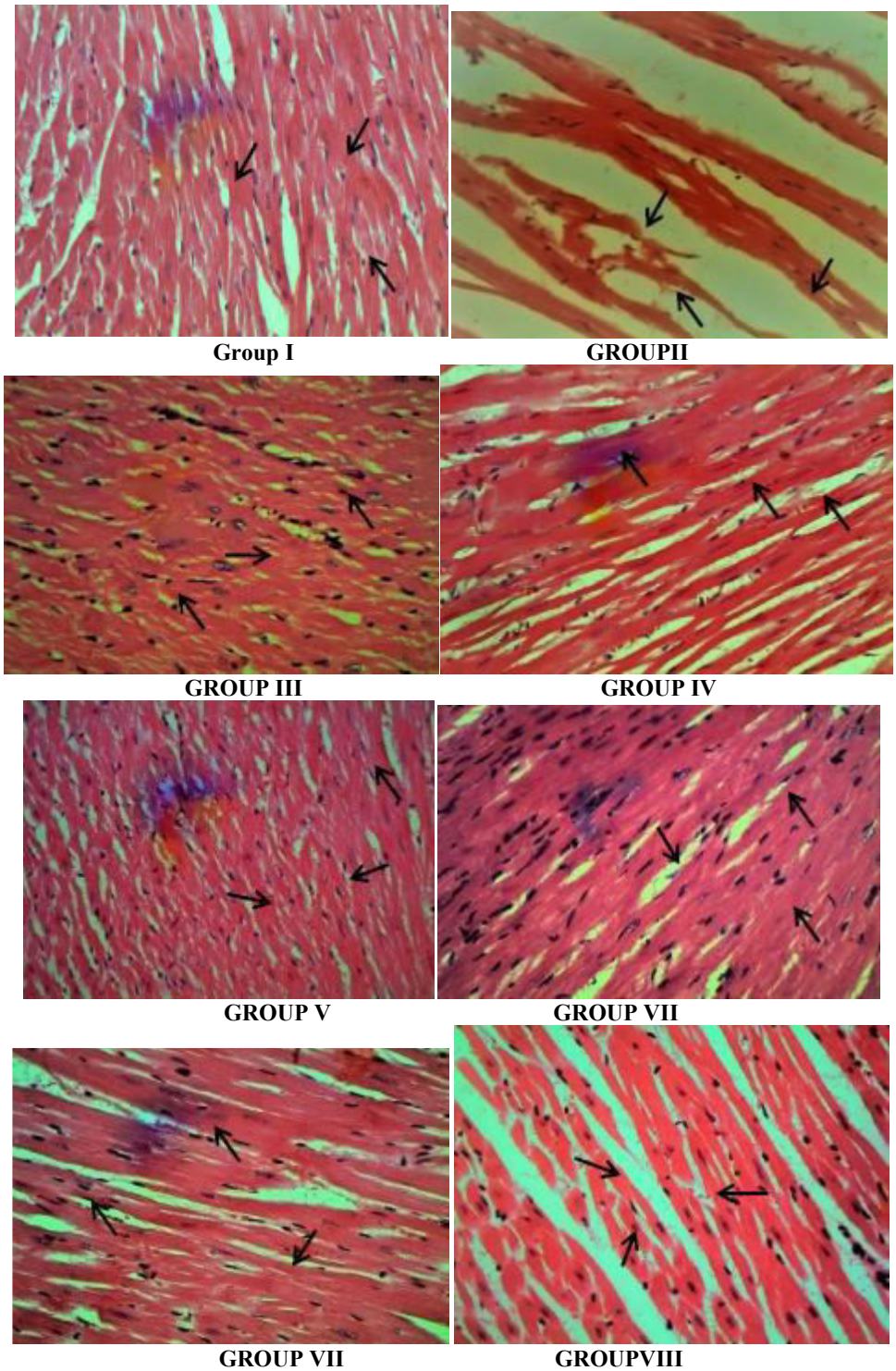

Figure 2. Histopathology study

CONCLUSION

The present study demonstrates that ethanolic seed extracts of *Nigella sativa* (EENS) and *Solanum melongena* (EESM) exert significant cardioprotective effects against isoproterenol-induced myocardial injury in rats. Pretreatment with both extracts effectively mitigated ISO-induced alterations in cardiac biomarkers, lipid profile, oxidative stress markers, ECG parameters, and myocardial histoarchitecture. These protective effects are largely attributed to the high content of polyphenols, flavonoids, and other antioxidant phytoconstituents present in both seeds. Notably, the combination of EENS and EESM produced superior

outcomes compared to individual treatments, indicating a synergistic interaction that enhances antioxidant efficiency and myocardial defense mechanisms. Overall, the findings establish both extracts individually and in combination as promising natural cardioprotective agents.

ABBREVIATIONS

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CAT: Catalase; CK-MB: Creatine kinase–myocardial band; CMC-Na: Carboxymethyl cellulose sodium; CVD: Cardiovascular disease; ECG: Electrocardiogram; EENS: Ethanolic extract of *Nigella sativa* seeds; EESM: Ethanolic extract of *Solanum melongena* seeds; GPx: Glutathione peroxidase; GSH: Reduced glutathione; HDL: High-density lipoprotein; ISO: Isoproterenol; LDH: Lactate dehydrogenase; LDL: Low-density lipoprotein; LPO: Lipid peroxidation; MI: Myocardial infarction; NS: *Nigella sativa*; PUFA: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SM: *Solanum melongena*; TA: *Terminalia arjuna*; TC: Total cholesterol; TG: Triglycerides; VLDL: Very-low-density lipoprotein.

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