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ORIGINAL ARTICLE

Decalepis hamiltonii and its Bioactive Compounds Protects Isoproterenol-Induced Myocardial Oxidative Stress in Rats

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ABSTRACT

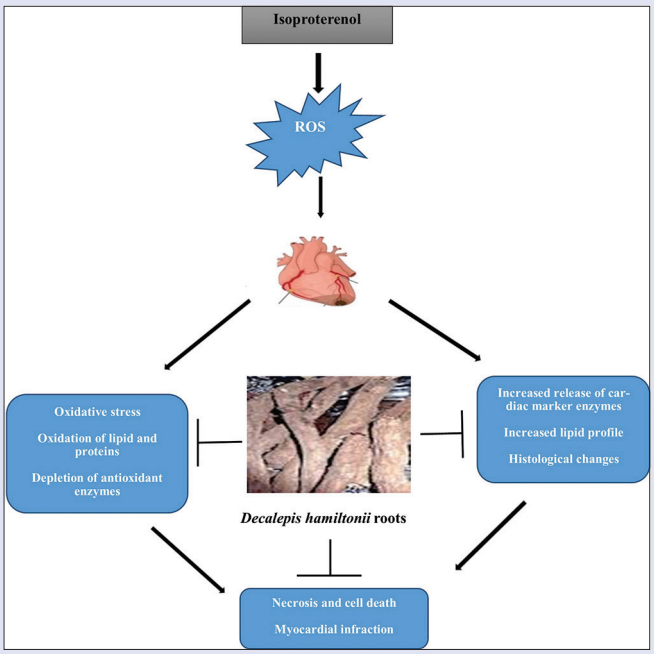
Background: *Decalepis hamiltonii* (*Dh*), a climbing shrub belonging to the family *Apocynaceae*, is consumed for its health-promoting properties. **Objective:** The present study was designed to evaluate the cardioprotective potential of *Dh* extract and its bioactive compounds on region-specific responses against isoproterenol (ISO)-induced myocardial injury in male Wistar rats. **Materials and Methods:** Rats were orally supplemented with ellagic acid (50 mg/kg body weight [BW]), 4-hydroxy isophthalic acid (30 mg/kg BW), and *Dh* extract (100 mg/kg BW) for 30 days, and they were subsequently administered (intraperitoneally) with ISO (150 mg/kg BW) for the last 2 days. **Results:** ISO-treated rats showed a significant increase in serum lipid profile, markers of cardiac damage, and decreased antioxidant status. Supplemented groups showed improved lipid profile, reduced serum marker enzymes, and enhanced antioxidant status in ISO-administered rats. Histopathological studies further confirmed the protective effect of *Dh* extract against ISO-induced myocardial infarction (MI). **Conclusion:** Our results demonstrated that *Dh* extract and its compounds efficiently ameliorated ISO-induced MI in rats.

Key words: *Decalepis hamiltonii*, isoproterenol, myocardial infarction, oxidative stress, serum lipid profile

SUMMARY

- Effect of *Decalepis hamiltonii* (*Dh*) extract and its bioactive compounds against isoproterenol (ISO)-induced myocardial infarction was investigated
- The ISO-treated rats showed increased oxidative stress and myocardial damage as evidenced by different biochemical parameters and histoarchitecture
- Pretreatment of *Dh* extract and its compounds diminished the ISO-induced oxidative stress and improved the antioxidant status
- Our results confirm that Dh and its compounds offer cardioprotective activity.

Abbreviations used: Dh: *Decalepis hamiltonii*; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid; ISO: Isoproterenol; MI: Myocardial infarction; LV: Left ventricle; RV: Right ventricle; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase.



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INTRODUCTION

Myocardial infarction (MI) is one of the leading causes of mortality and morbidity, worldwide.[1] It occurs as a consequence of the imbalance between myocardial oxygen demand and coronary blood supply. It is characterized by structural and functional modifications in the myocardium, resulting in contractile dysfunction, ventricular dilatation, and subsequent heart failure.[2]

Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist that causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle. The pathophysiological and morphological changes observed in ISO-administered rats are similar to MI in humans.[3] Further, it undergoes autoxidation generating highly toxic reactive oxygen species (ROS) that stimulate lipid peroxidation, leading to both structural and functional myocardial injury. Therefore, ISO-induced myocardial injury serves as a well-standardized model for human MI to study the beneficial effects of drugs on cardiac function.[4]

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In recent years, the therapeutic potential of medicinal plants has gained interest as antioxidants in reducing free radical-induced tissue injury. Among conventional herbs, *Decalepis hamiltonii* (*Dh*) (*Apocynaceae*) has been used as a common ingredient in many of the Ayurvedic preparations. The roots possess potent antioxidant properties, which could be responsible for various health benefits.[5] The aqueous root extract is a cocktail of many polyphenolic compounds which include ellagic acid (EA), 4-hydroxy isophthalic acid (4-HIA), 14-aminotetradecanoic acid, 4-(1-hydroxy-1-methyl ethyl)-1-methyl-1,2-cyclohexanediol, 2-hydroxymethyl-3-methoxybenzaldehyde, and 2,4, trihydroxybicyclo -octan-3-one.[6] Studies have shown that *Dh* root extract exhibits antiatherosclerotic, antidiabetic, hepatoprotective, and neuroprotective activities.[7] We also reported that EA and 4-HIA are the major phenolic compounds in the *Dh* extract, which exhibited protective effects against peroxyl-induced oxidative stress in the rat erythrocytes. However, to our knowledge, limited reports are available regarding the cardioprotective effects of *Dh* extract and its constituents.

Therefore, the present study was hypothesized to investigate the cardioprotective effects of *Dh* extract and its compounds in ameliorating ISO-induced MI in rats. The hypothesis was examined by assessing the biochemical markers of cardiac injury as well as antioxidant enzyme activities and markers of oxidative stress in the left ventricle (LV) and right ventricle (RV) regions of the heart. Further, histopathological studies were also carried out to understand the cardioprotective effects of *Dh* extract.

MATERIALS AND METHODS

Chemicals

EA, 4-HIA, epinephrine, reduced glutathione (GSH), glutathione reductase, thiobarbituric acid (TBA), t-butyl hydroperoxide, guanidine hydrochloride, reduced nicotinamide dinucleotide phosphate (NADPH), reduced nicotinamide adenine dinucleotide, nitro blue tetrazolium (NBT), and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were procured from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade.

Preparation of aqueous root extract

Dh roots were collected from Savanadurga Forest, Magadi Taluk, Ramanagara District, India. The plant material was identified at the Department of Botany, Bangalore University, Bengaluru. A voucher specimen was deposited in the herbarium (BUB, No. 2223). The aqueous root extract was prepared according to the method of Ravikiran *et al.*[8] Briefly, the outer fleshy part was separated from the inner woody core using a scalpel, dried at 40°C, and finely powdered. The aqueous extract of the outer fleshy part (100 g) was prepared by dissolving the powder in 1 L of warm water (50°C) and allowing it to stand for 24 h and filtered through Whatman no. 1 paper. The filtrate was lyophilized, weighed (17.5 g), and stored at 4°C until further use.

Animal maintenance

Adult male Wistar rats weighing 200–220 g were obtained from the Central Animal Facility, IISc, Bengaluru. The animals were placed three per cage in polypropylene cages fitted with stainless steel wire-mesh bottoms, maintained at a temperature of 28°C ± 1°C, relative humidity of 77% ± 1%, and under 12-h dark and light cycle. All animals had free access to food (Amruth Feeds, Bengaluru) and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) (BUB/IAEC/TRK/05/2015), Department of Zoology, Bangalore University, Bengaluru, and complied with the guidelines of the Committee for the Purpose of control and Supervision of Experiments on Animals.

Dosage fixation

Different doses of EA (25, 50, 75, and 100 mg/kg body weight [BW]), 4-HIA (10, 20, 30, 40, and 50 mg/kg BW), and *Dh* extract (50, 100, 150, and 200 mg/kg BW) were screened to determine the dose-dependent effect in ISO-induced myocardial-infarcted rats. The effective doses were assessed based on the activities of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), levels of antioxidant enzymes, and markers of oxidative stress. EA (50 mg/kg BW), 4-HIA (30 mg/kg BW), and *Dh* extract (100 mg/kg BW) were found to be most effective in reducing the biochemical markers and enhancing the antioxidant status. Hence, these doses were fixed for further studies.

Experimental design

A total of 40 rats were randomly divided into eight groups.

- Group I: Normal controls rats (normal saline p.o)
- Group II: ISO-treated
- Group III, IV, and V: Rats pretreated with EA, 4-HIA, and *Dh* extract, respectively
- Group: VI, VII, and VIII: Rats pretreated with EA, 4-HIA, and *Dh* extract, respectively, with ISO.

EA, 4-HIA, and *Dh* extract were prepared in normal saline and administered at a dosage of 50 mg/kg BW (EA), 30 mg/kg BW (4-HIA), and 100 mg/kg BW (*Dh* extract), respectively, using an intragastric tube for 30 days. Group II rats were given normal saline for 27 days and injected intraperitoneally (i.p.) with ISO at a dosage of 150 mg//kg BW consecutively on the 28th and 29th day at an interval of 24 h.[9] Group VI, VII, and VIII received the compounds and *Dh* extract for 30 days followed by ISO (150 mg/kg BW i.p.) at an interval of 24 h for the last 2 days.

Tissue preparation

After completion of the experimental protocol, the animals were sacrificed on the 30th day under diethyl ether anesthesia followed by cervical dislocation.[10] The heart was excised and LV and RV regions were separated, washed with ice-cold saline, and weighed. The tissue homogenates were prepared in phosphate buffer (50 mM, pH 7.4) and centrifuged at 2000 ×*g* for 10 min (Superspin RV/FM, Plasto Crafts). The homogenates were used for the estimation of malondialdehyde (MDA), superoxide radical (SOR), and thiols (SHs). The remaining homogenate was centrifuged at 2000 ×*g* rpm at 4°C for 10 min. The supernatant obtained was used for antioxidant enzyme assays and protein carbonyl (PC) estimation.

Biochemical assays in the serum

Blood was collected by cardiac puncture under ether anesthesia and allowed to clot for 30 min at RT. The serum was separated by centrifugation at 2500 rpm for 15 min. Total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels as well as the activities of LDH and CK-MB were determined in the serum using commercially available kits (Code No. 731 LS102-25, Span Diagnostics Ltd., India).

Measurement of lipid peroxidation

MDA content was measured according to the procedure of Ohkawa *et al.*[11] using 1,1,3,3-tetramethoxypropane as the standard. Briefly, 100 µL of homogenate was added to 200 µL of 8.1% Sodium dodecyl sulphate (SDS); 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% aqueous TBA solution were added; and the solution was made up to 4 mL with distilled water (d.w.). The solution was heated on boiling water bath for 60 min and cooled and 1 mL of d.w was added. 5 mL butanol and

pyridine (15:1) was added and then centrifuged at 4000 rpm for 10 min. The absorbance of the orange layer was read at 532 nm.

Measurement of protein oxidation

PC content was measured according to the procedure of Levine *et al.*[12] Briefly, 100 µL of supernatant was incubated with 0.5 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl for 60 min in the dark. Protein was precipitated using 0.5 mL of 20% Trichloroacetic acid (TCA) and then centrifuged at 10,000 ×*g* for 3 min at 4°C. The supernatant was discarded and the pellet was washed with 1:1 ethyl acetate/ethanol twice by centrifugation (3400 ×*g* for 5 min) to remove DNPH. The pellet was dissolved after washing in 1.5 mL of 6 M guanidine hydrochloride in phosphate buffer (pH 6.5). Absorption was read at 370 nm in a spectrophotometer (Model SL 159, ELICO). The concentration of carbonyl groups was determined from the extinction coefficient of 22,000 Mcm^{-1} and expressed as nmol/mg protein.

Measurement of superoxide radical

SOR levels were measured according to the method of Das *et al.*[13] Briefly, 200 µL of homogenate was incubated with 80 µL of 0.1% NBT in an oscillating water bath for 1 h at 37°C. Termination of the assay and extraction of the reduced Nitroblue tetrazolium (NBT) were carried out by centrifuging the samples for 10 min at 200 ×*g* and then resuspending the pellets with 1 mL of glacial acetic acid. The absorbance was measured at 560 nm and converted to µmol diformazan using a standard curve generated from nitroblue diformazan. Final results were expressed as µmol diformazan/mg protein.

Determination of total thiol, protein thiol, and nonprotein thiol levels

The SH groups were determined according to the procedure of Sedlak and Lindsay.[14] For total thiol (T-SH), briefly, aliquots of 250 µL of the homogenate were mixed in 5 mL test tubes with 750 µL of 0.2 M Tris buffer (pH 8.2) and 50 µL of 0.01 M DTNB. The mixture was made up to 5 mL with 3950 µL of absolute methanol. A reagent blank and a sample blank were prepared in the same manner. The reaction mixture was centrifuged at 3000 ×*g* for 15 min, and absorbance of the supernatants was read in a spectrophotometer at 412 nm. Molar extinction coefficient at 412 nm was 13,100/M/cm in both T-SH and nonprotein thiol (NP-SH) procedures.

For NP-SH, aliquots of 250 µL of the homogenates were mixed in 5 mL test tubes with 200 µL d.w. and 50 µL of 50% TCA. The tubes were shaken intermittently for 10 min and centrifuged at 3000 ×*g* for 15 min. 200 µL of the supernatant was mixed with 400 µL of 0.4 M Tris buffer (pH 8.9). 10 µL DTNB was then added, and absorbance was read within 5 min at 412 nm against a reagent blank. The protein thiol (P-SH) groups were calculated by subtracting the NP-SH from T-SH.

Antioxidant enzymes activities

Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich.[15] Briefly, 100 µL of the supernatant was added to 880 µL of carbonate buffer (0.05 M, pH 10.2) and 0.l mM ethylenediaminetetraacetic acid. 20 µL of 30 mM epinephrine in 0.05% acetic acid was added to the mixture, and absorbance was followed for 5 min at 480 nm in a spectrophotometer. The amount of enzyme that resulted in 50% inhibition of epinephrine auto-oxidation was defined as one unit.

Catalase (CAT) activity was measured according to the method of Aebi.[16] Briefly, 100 µL of the supernatant with an equal volume of absolute alcohol was incubated for 30 min at 0°C–8°C following which 10 µL of Triton X-100 was added. A known volume of this was taken in an equal volume of 0.066 M hydrogen peroxide (H_2O_2) in phosphate buffer, and the decrease in absorbance was measured at 240 nm for a min in a spectrophotometer. An extinction coefficient of 43.6 Mcm^{1} was used to determine enzyme activity, one unit of which was equal to µmol of H_2O_2 degraded/min/mg protein.

Glutathione peroxidase (GPx) activity was measured following the method of Flohé and Günzler.[17] Briefly, the reaction mixture consisted of 500 µL of phosphate buffer, 100 µL of 0.01 M GSH, 100 µL of 1.5 mM NADPH, and 100 µL of glutathione reductase. 100 µL of supernatant was added to the reaction mixture and incubated at 37°C for 10 min. 50 µL of 12 mM t-butyl hydroperoxide was added to 450 µL of cell extract reaction mixture and measured at 340 nm for 180 s in a spectrophotometer. Molar absorptivity of 6.22×10^3 M/cm was used to determine enzyme activity. One unit of activity is equal to mM NADPH oxidized/min/mg protein.

Histopathology

The LV and RV tissues were fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed by embedding in paraffin and were sectioned, stained with hematoxylin and eosin, and examined under a light microscope.

Statistical analysis

All the data were represented as the mean ± standard error. The changes in serum lipid profile, LDH, and CK-MB between the groups were analyzed by one-way ANOVA. The changes in antioxidant enzyme activities and markers of oxidative stress were analyzed by two-way ANOVA. Significant "*F*" ratios between the group means were further subjected to Tukey's test. Probability (*P*) values at <0.05 were considered statistically significant.

RESULTS

Body weight, heart weight, and heart weight/body weight ratio

As represented in Table 1, no significant changes in BWs were noticed in ISO-administered rats when compared to their respective controls. *Dh* + ISO group exhibited a significant decrease in BWs over the ISO group. The heart weight (HW) and HW/BW ratio were recorded as an index of cardiac hypertrophy and were significantly increased in ISO-induced rats with respect to the normal control rats. A significant decrease in the HW and HW/BW ratio was evident in the supplemented

Table 1: Effect of ellagic acid, 4-hydroxy isophthalic acid, and *Decalepis hamiltonii* extract on body weight, heart weight, and heart weight to body weight ratio in isoproterenol-treated rats

Groups	BW	HW	BW/HW
Control	256±1.85	0.67±0.01	0.26±0.01
ISO	252±0.04	0.99±0.02*	0.39±0.01*
EA	250±2.02	0.65±0.01#	0.25±0.03#
4-HIA	252±2.84	0.64±0.01#	0.25±0.03#
Dh	248±1.20	0.62±0.01#	0.24±0.03#
EA + ISO	248±1.45	0.90±0.05	0.35±0.02
4-HIA + ISO	249±2.08	0.92±0.01#	0.36±0.01
Dh + ISO	245±0.33	0.85±0.01#	0.36±0.01#

Data are expressed as mean±SE of five animals/group and analyzed by one-way ANOVA followed by Tukey's test. *Significance between the sedentary control and ISO-treated group; #Comparison of ISO-treated group with other experimental groups; *,#*P*<0.05 were considered significant. BW: Body weight; HW: Heart weight; ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid; Dh: *Decalepis hamiltonii*; SE: Standard error; LDH: Lactate dehydrogenase; CK-MB: Creatine kinase-MB

rats over the ISO alone-treated rats. However, pretreatment of *Dh* extract to ISO-administered rats significantly reduced the HW and HW/BW ratio by 14% and 8%, respectively, over the ISO alone-treated rats.

Serum lipid profile

The serum lipid profile changes in the control and the ISO-administered rats are represented in Table 2. ISO-treated rats showed a significant increase in TC, TG, and LDL with a concomitant decrease in HDL levels compared to control rats. Pretreatment of EA, 4-HIA, and *Dh* extract to ISO-treated rats reduced the serum lipid profile changes compared to ISO alone-treated rats. Maximum reduction was evident in *Dh* + ISO group in TC, TG, LDL, and HDL levels. Supplementation of EA, 4-HIA, and *Dh* extract significantly decreased the lipid profile changes over the ISO-treated rats.

Cardiac marker enzymes

Table 3 depicts the effects of *Dh* extract and bioactive compounds on the activities of cardiac marker enzymes (LDH and CK-MB) in the normal and ISO-treated rats. There is a significant increase in the levels of LDH (51%) and CK-MB (50%) in the ISO-treated rats relative to the control rats. However, pretreatment of EA, 4-HIA, and *Dh* extract in ISO-administered rats reduced the serum levels of these enzymes. Pretreatment of *Dh* extract and the compounds significantly decreased the levels of these enzymes to the normal levels.

Antioxidant enzyme activities

The antioxidant enzyme activities were significantly decreased in the ISO-treated rats as represented in Figure 1. Region-specific changes were evident for SOD and CAT activities. *Dh* + ISO group exhibited higher SOD activity by 41% and 40% (LV and RV) compared to ISO alone-treated rats. A significant increase in CAT (LV - 57%, RV - 56%) activity was noticed in supplemented rats treated with ISO. A notable feature of GPx activity is that insignificant changes were noticed between the regions. However, activity was significantly enhanced only in EA + ISO (LV - 14%, RV - 27%) and *Dh* + ISO (LV - 23%, RV - 36%) groups over the ISO alone-treated rats. Supplemented rats showed increased antioxidant enzyme activities compared to ISO alone-treated rats.

Markers of oxidative stress

The levels of oxidative stress markers (MDA and PC) were significantly elevated in the rats treated with ISO compared to the control rats. The MDA and PC contents were decreased maximally in the *Dh* + ISO-treated rats in LV (60% and 51%) and RV (65% and 50%) over the ISO alone-treated rats. Supplementation of the EA, 4-HIA, and *Dh* extract was found to be more effective in curtailing lipid peroxidation and protein oxidation [Figure 2a and b]. However, insignificant changes were observed between the regions.

Table 2: Effect of ellagic acid, 4-hydroxy isophthalic acid, and *Decalepis hamiltonii* extract on changes on serum lipid profile in isoproterenol-treated rats

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Con	84.26±2.44	91.57±0.59	38.42±1.99	27.85±2.18
ISO	135.95±2.62*	123.93±3.10*	25.48±2.27*	85.69±1.60*
EA	79.17±3.24#	84.33±2.48#	49.44±1.98	14.17±2.45#
4-HIA	82.43±2.09#	85.76±2.54#	48.33±2.23#	16.51±3.10#
Dh	75.68±1.09#	79.48±0.75#	51.45±1.99#	8.25±1.22#
EA + ISO	109.26±1.27#	112.64±3.06#	41.98±1.78#	25.24±1.98#
4-HIA + ISO	113.46±5.25#	108.41±2.2	39.21±2.36	25.53±1.82#
Dh + ISO	96.06±1.59#	99.51±2.89#	44.17±3.16	13.57±1.95#

Values are expressed as mean±SE and were analyzed through one-way ANOVA followed by Tukey's test. *Significance between the sedentary control and ISO-treated group; #Comparison of ISO-treated group with other experimental groups; *,#P<0.05 were considered significant. TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid; Dh: *Decalepis hamiltonii*; SE: Standard error

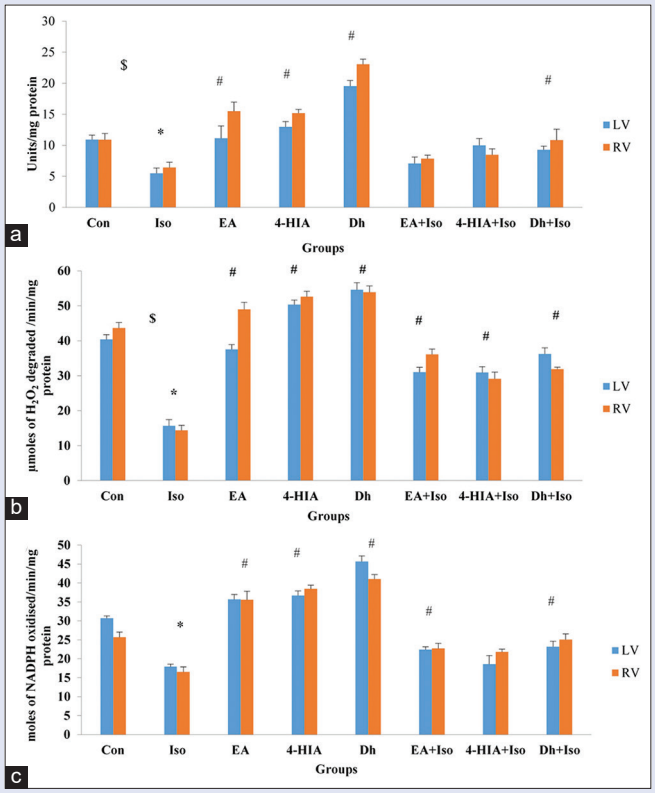

Figure 1: The levels of SOD (a), CAT (b) and GPx (c) in the control and experimental groups. The data are expressed as mean ± SE from six independent animals and analyzed by two-way ANOVA followed by Tukey's test. *Significance between the control and ISO alone treated group. #Comparison of ISO alone treated the group with other experimental groups. $indicates the significance between the regions. *#$Probability values $P < 0.05$ were considered significant. SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; SE: Standard error; ISO: Isoproterenol

Table 3: Effect of ellagic acid, 4-hydroxy isophthalic acid, and *Decalepis hamiltonii* extract on lactate dehydrogenase and creatine kinase-MB activities in isoproterenol-treated rats

Groups	Con	ISO	EA	4-HIA	Dh	EA + ISO	4-HIA + ISO	Dh + ISO
LDH	95.14±2.75	193.6±2.49*	95.84±1.71#	95.47±2.39#	85.63±3.24#	156.03±2.13#	166.59±3.17#	123.46±1.88#
CK-MB	85.17±2.65	189.93±3.12*	75.71±1.69#	80.56±2.33#	59±1.75#	142.95±3.48#	151.58±2.75#	133.26±2.02#

LDH and CK-MB activities. Values are expressed as mean±SE and were analyzed through one-way ANOVA followed by Tukey's test. *Significance between the sedentary control and ISO-treated group; #Comparison of ISO-treated group with other experimental groups; *,#P<0.05 were considered significant. ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid; Dh: *Decalepis hamiltonii*; SE: Standard error; LDH: Lactate dehydrogenase; CK-MB: Creatine kinase-MB

SOR levels were measured in the myocardium as it is one of the major free radicals produced during physiological reactions. The levels were significantly higher in the LV compared to RV in all the experimental groups. As reported in Figure 2c, there was a significant increase in SOR levels in the LV (47%) and RV (42%) in ISO alone-treated rats. Notably, both supplemented controls and supplemented rats administered with ISO showed reduced radical generation.

The T-SH, NP-SH, and P-SH levels exhibited insignificant changes between the regions. In ISO-administered rats, a significant decrease in the levels of SHs in LV (T-SH - 48%, NP-SH - 52%, P-SH - 44%) and RV (T-SH - 60%, NP-SH - 26%, P-SH - 81%) was observed when compared to the controls. T-SH levels were significantly enhanced in the *Dh* + ISO-treated and EA + ISO-treated rats. However, NP-SH levels were significantly upregulated only in *Dh* + ISO-treated rats. A remarkable aspect of the P-SH levels is that insignificant changes were noticed in the supplemented groups treated with ISO. Pretreatment of EA, 4-HIA, and *Dh* extract increased the levels of T-SH, NP-SH, and P-SH in comparison with ISO alone-treated rats [Table 4].

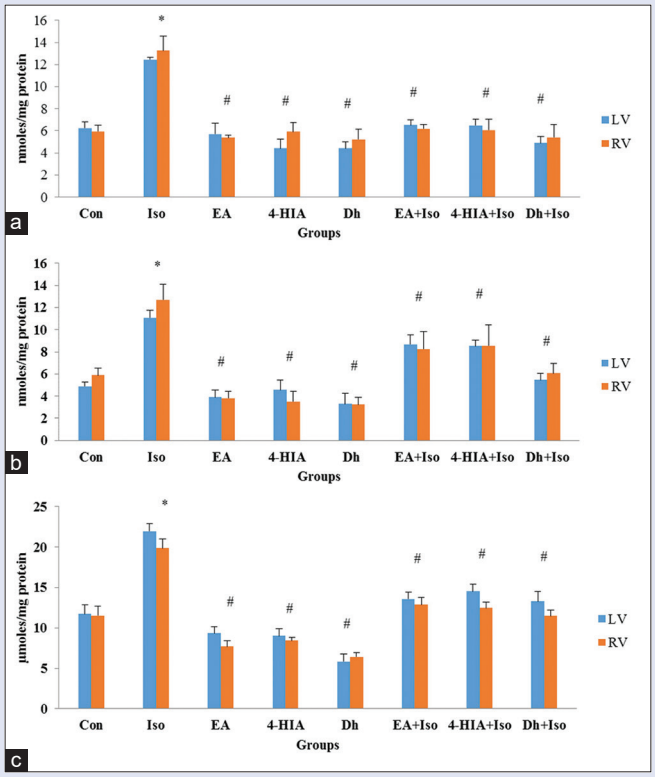

Figure 2: The levels of MDA (a), PC (b), and SOR (c) in the control and experimental groups. The data are expressed as mean ± SE from six independent animals and analyzed by two-way ANOVA followed by Tukey's test. *Significance between the control and ISO alone treated group. #Comparison of ISO alone-treated group with other experimental groups. *#Probability values $P < 0.05$ were considered significant. MDA: Malondialdehyde; PC: Protein carbonyl; SOR: Superoxide radical; SE: Standard error

Histopathological changes

The histopathology of the LV and RV regions of the control rats showed normal myocardial architecture with no evidence of necrosis and inflammation. However, extensive membrane damage, focal myocardial necrosis, and infiltration of the inflammatory cells were observed in both the regions of ISO alone-treated rats. Pretreatment with *Dh* extract offered better protection compared to EA and 4-HIA against the deleterious effects of ISO in terms of lesser extent of infiltration and myonecrosis. Supplemented groups revealed normal myocardial histoarchitecture [Figures 3 and 4].

DISCUSSION

Cardiac diseases have been linked to oxidative stress, which is due to the reactions of free radicals with proteins, lipids, and DNA. Antioxidants from plant sources have been considered as an effective treatment for cardiovascular diseases. The root extract of *Dh* is consumed for various health benefits and is a cocktail of many antioxidant compounds.[6] Studies have reported that aqueous root extract has protective effects against neurotoxicity and age-related oxidative stress.[8] Therefore, the present study was undertaken to investigate the cardioprotective potential of *Dh* extract and its bioactive compounds in ameliorating ISO-induced MI in rats. LV and RV regions were selected due to the differences in morphology and function as well as in the distribution of antioxidant defense systems.

In our study, BW changes were insignificant among the experimental groups and are in line with previous reports.[18] However, we observed a significant increase in HW and HW/BW ratio in ISO alone-treated rats indicating cardiac hypertrophy. Several studies also reported that increased HW and HW/BW ratio in ISO-induced myocardial-infarcted rats might be due to edematous intramuscular space, increased protein and water content, necrosis, and penetration of inflammatory cells to damaged cardiac tissues.[10,19] Supplementation of *Dh* extract significantly reduced the HW and HW/BW ratio, thereby attenuating cardiac hypertrophy in ISO-administered rats.

Hyperlipidemia is one of the leading causes of cardiovascular diseases. Altered lipid metabolism plays an important role in the development of atherosclerosis and in the progression of MI.[20] In the present

Table 4: Effect of ellagic acid, 4-hydroxy isophthalic acid and *Decalepis hamiltonii* extract on total thiol, nonprotein thiols, protein thiol activities in isoproterenol-treated rats

Parameters	Regions	Groups							Dh + ISO
		CON	ISO	EA	4-HIA	Dh	EA + ISO	4-HIA + ISO	
T-SH	LV	234.59±8.0	121.62±7.67*	368.31±6.56#	293.65±12.02#	394.91±5.06#	146.47±5.36#	131.43±9.74	174.97±5.49#
	RV	247.98±12.4	98.53±4.77	354.46±6.42	312.32±6.55	413.91±5.75	138.77±6.23	114.41±6.15	163.42±5.00
NP-SH	LV	140.1±9.54	68.64±8.49*	132.01±9.17#	166.39±9.52#	181.75±11.23#	76.96±5.28	62.62±5.96	99.54±9.45#
	RV	95.40±7.91	69.90±9.54	128.41±12.29	147.18±11.65	229.93±15.00	56.59±8.36	55.81±8.44	111.98±14.89
P-SH	LV	94.49±4.56	52.97±8.87*	236.30±15.73#	127.26±15.32#	213.15±11.49#	69.51±9.76	65.14±11.02	64.77±10.89
	RV	152.58±11.20	28.62±10.72	226.05±7.80	165.14±10.31	183.98±16.49	82.18±13.60	58.59±14.55	64.78±10.54

T-SH: Total thiols; NP-SH: Nonprotein thiols; P-SH: Protein thiols. Values are expressed as mean±SE and were analyzed through two-way ANOVA followed by Tukey's test. *Significance between the sedentary control and ISO-treated group; #Comparison of ISO-treated group with other experimental groups; *,#$P<0.05$ were considered significant. ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid; Dh: *Decalepis hamiltonii*; SE: Standard error; LV: Left ventricle; RV: Right ventricle

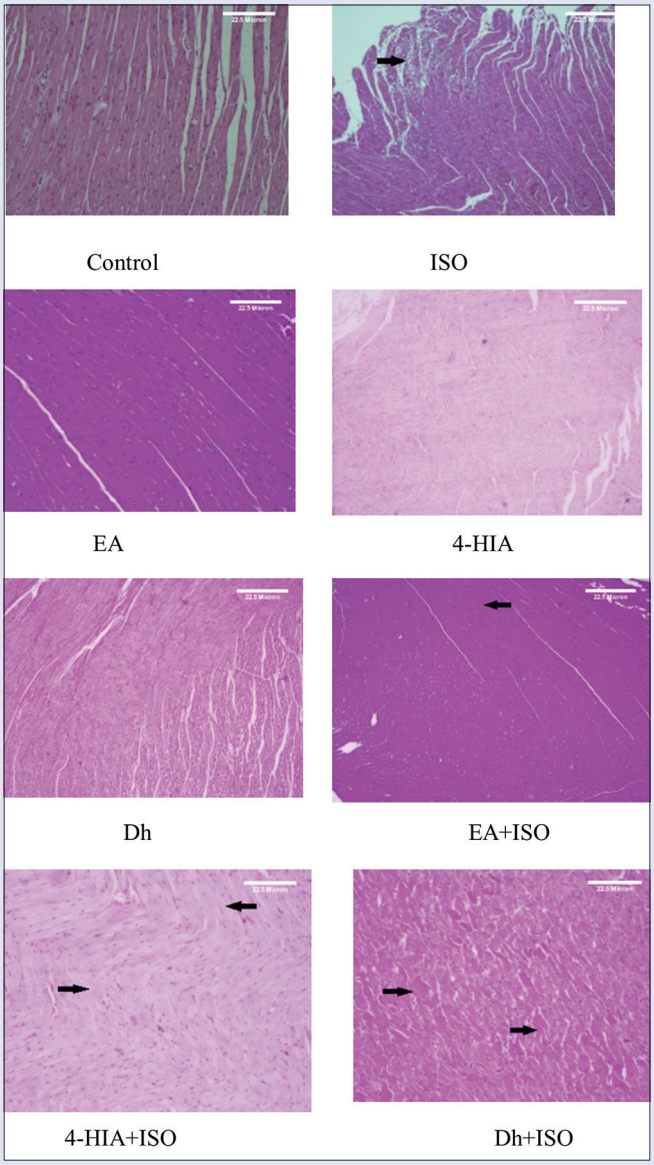

Figure 3: Photomicrographs of histopathological changes of LV of control, supplemented, and ISO-treated rats. Control, ISO, EA, 4-HIA, *Decalepis hamiltonii* extract, EA + ISO, 4-HIA + ISO, and *Decalepis hamiltonii* + ISO. Tissues were stained with hematoxylin and eosin, visualized under a light microscope at ×100. Arrow indicates infiltration of inflammatory cells and myocardial necrosis. LV: Left ventricle; ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid

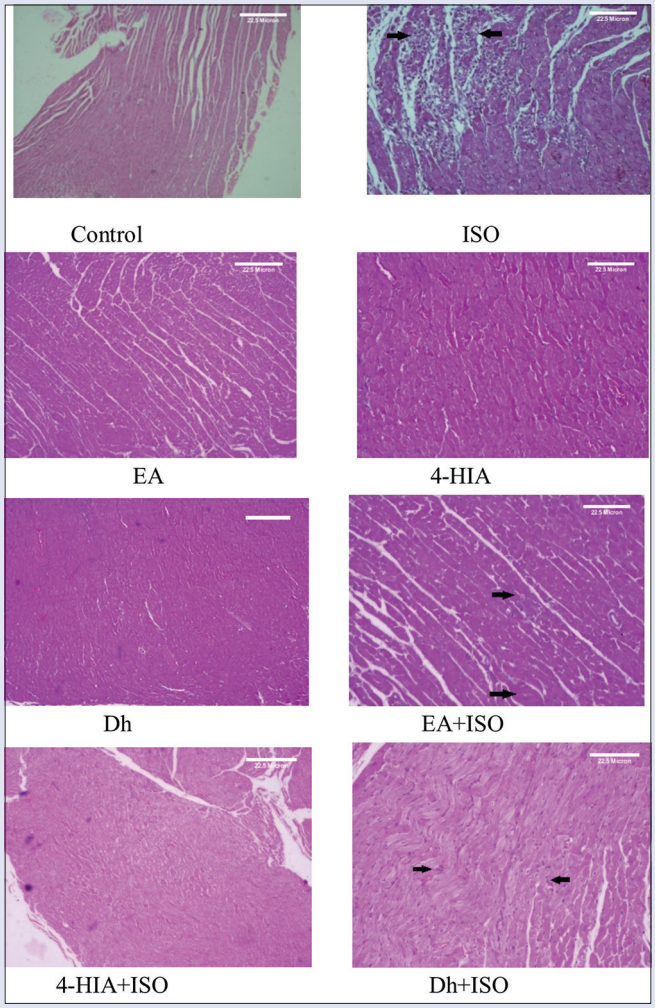

Figure 4: Photomicrographs of histopathological changes of RV of control, supplemented, and ISO-treated rats. Control, ISO, EA, 4-HIA, *Decalepis hamiltonii* extract, EA + ISO, 4-HIA + ISO, and *Decalepis hamiltonii* + ISO. Tissues were stained with hematoxylin and eosin, visualized under a light microscope at ×100. Arrow indicates infiltration of inflammatory cells and myocardial necrosis. RV: Right ventricle; ISO: Isoproterenol; EA: Ellagic acid; 4-HIA: 4-hydroxy isophthalic acid

study, ISO administration significantly elevated lipid profile markers and decreased HDL levels. This may be attributed to enhanced lipid biosynthesis by cyclic-AMP and decreased activity of lipoprotein lipase in the myocardium. Our results are consistent with the previous studies of Abbas.[21] Pretreatment with EA, 4-HIA, and *Dh* extract improved ISO-induced hyperlipidemic damage, suggesting their lipid-lowering effects. Several reports have shown that pretreatment with plant extracts and natural compounds prevent ISO-induced hyperlipidemia.[22,23]

LDH and CK-MB are the reliable diagnostic markers to assess the severity of ISO-induced MI.[24] These marker enzymes were elevated in the serum following ISO treatment, which indicates damage to myocardial tissues. Our findings on increased levels of these enzymes are similar to the earlier reports.[25] Administration of EA, 4-HIA, and *Dh* extract with ISO resulted in a significant decrease in the concentration of these enzymes into the circulation which could be due to antioxidant and free radical scavenging activities of the compounds and the extract, suggesting that *Dh* extract and its compounds maintained the membrane integrity, thereby reducing the leakage of these enzymes.

Oxidative stress is considered to be one of the mechanisms of ISO-induced MI. Antioxidants constitute the first line of cellular defense system that limits the toxicity associated with free radicals. SOD, CAT, and GPx are the important antioxidant defense enzymes involved in the dismutation of SORs and H_2O_2. ISO treatment resulted in decreased activities of these antioxidant enzymes, indicating severe oxidative damage. The results of the present study are in line with previous reports.[26] RV exhibited higher antioxidant enzyme activities compared to the LV may be due to the structural differences and enhanced free radical scavenging activity.[27] Pretreatment of EA, 4-HIA, and *Dh* extract significantly upregulated the activity of these enzymes compared to the ISO alone-induced rats. The increased activity in the supplemented rats may be attributed to the modulation of nuclear factors, such as Nrf2, activator protein-1, and

NFκB, which in turn bind to the antioxidant response elements in the regulatory region of the target genes and induces the transcription of antioxidant enzymes.[28] Previous studies from our laboratory and others have demonstrated that supplementation of *Dh* extract and EA improves the antioxidant status in the different regions of the brain.[8,29]

Lipid peroxidation is an important pathological event in myocardial necrosis, leading to the accumulation of lipid hydroperoxides, causing irreversible damage to the myocardial membrane.[30] In the present study, MDA levels were significantly increased in the ISO alone-treated rats, which indicates enhanced ROS generation by auto-oxidation of ISO, thereby initiating lipid peroxidation.[31] Earlier reports also showed elevated MDA levels in ISO alone-treated rats.[24] Proteins are also prone to oxidation by free radicals attacking amino acid side chains to produce carbonyl groups.[32] Our results indicate higher oxidation of proteins in the ISO alone-treated rats, suggesting severe damage to the myocardium. However, pretreatment of EA, 4-HIA, and *Dh* extract resulted in reduced levels of MDA and PC in ISO-treated rats, indicating that the compounds and extract possess antilipid peroxidative and antiprotein oxidation properties. Previous studies have also demonstrated that supplementation of *Dh* extract and its bioactive compounds decreased the lipid peroxidation and protein oxidation products in the rat liver and brain.[33,34]

SOR is one of the major free radicals generated during oxidative phosphorylation found to higher in the LV compared to RV. Increased radical generation in ISO alone-treated rats is attributed to the oxidation of ISO, causing the cardiac tissues more prone to oxidative damage. This is in accordance with the previous reports of Dhivya *et al.*,[35] where increased ROS generation was observed in ISO-induced MI in rats. The SOR levels were significantly reduced in the supplemented as well as ISO-treated groups. This modulatory effect of *Dh* extract and its constituents could be due to scavenging of free radicals, thus increasing SOD activity as evident in the supplemented groups.

SH levels were determined as they are the main constituents of intracellular nonprotein sulfhydryl groups involved in many cellular functions, such as drug metabolism and detoxification of free radicals.[36] In the present study, a significant decrease in the levels of SHs was observed in the ISO alone-treated rats, indicating decline in the antioxidant status in the myocardium. Decreased SH levels may be due to its increased utilization to enhance the activity of GPx. Administration of *Dh* extract and its bioactive compounds significantly elevated the levels of SHs in the ISO-treated rats by maintaining the SH groups in the reduced state and enhancing the antioxidant status.[37]

Protective effects of EA, 4-HIA, and *Dh* extract on myocardial histoarchitecture were further confirmed by light microscopic studies. Histopathological examination of LV and RV regions of ISO alone-treated rats showed edema, infiltration, and myocardial necrosis. Several studies have reported similar histopathological changes in the ISO-treated rats.[38] These changes may be due to increased oxidative stress and alterations in lipid metabolism, thereby causing the structural modifications. However, pretreatment with *Dh* extract followed by ISO administration exhibited improved myocardial architecture in terms of mild necrosis with the presence of some inflammatory cells compared to ISO *per se*-administered rats. These results further confirmed the cardioprotective potential of *Dh* extract.

CONCLUSION

The present study revealed the cardioprotective potential of *Dh* extract and its bioactive compounds against ISO-induced MI by modulating cardiac marker enzymes, lipid profile, and antioxidant status. The *Dh* extract was found to be more effective than individual bioactive compounds. However, further studies are warranted to establish the modulation of NFκB and Nrf2 signaling pathways by *Dh* extract to evaluate its protective effect in treating cardiovascular diseases.

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Conflicts of interest

There are no conflicts of interest.

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