

EFFECTS OF CORIANDRUM SATIVUM EXTRACT AND SIMVASTATIN IN ISOPROTERENOL INDUCED HEART FAILURE IN RATS

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EFEKTI EKSTRAKTA KORIJANDERA I SIMVASTATINA NA IZOPROTERENOL-INDUKOVANU SRČANU INSUFICIJENCIJU KOD PACOVA

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ABSTRACT

Heart failure is a syndrome, caused due to structural and functional cardiac abnormalities, characterized by changes in the hemodynamic and neurohumoral mechanisms. It is becoming a major health burden worldwide. More effective therapies are desperately needed. Coriandrum sativum (C. sativum), a traditional spice crop has been known to possess many biological and medicinal properties. The present study was designed to investigate the cardioprotective efficacy of C. sativum in rat model of isoproterenol induced heart failure. Heart failure was produced by injecting isoproterenol subcutaneously (85 mg/kg twice at an interval of 24 h). Oral efficacy of seed extract was assessed on hemodynamic profile, antioxidant enzyme activities, lipid peroxidation, lipid profile, atherogenic indices, mRNA and protein expression of endothelin receptors (ET_A and ET_B) and histopathology. Treatment of heart failure rats with C. sativum orally (1g/kg b.wt) improved the altered hemodynamics, restored the cardiac antioxidant enzymes armory, attenuated oxidative stress, improved lipid profile, lowered atherogenic indices, decreased the levels of ET_A and ET_B receptor mRNA and protein, and restored the cardiac morphology. In conclusion, our results suggest C. sativum to be a cardioprotective agent in heart failure, possibly by the virtue of its ability to alleviate oxidative stress, improve lipid profile and endothelial dysfunction.

Keywords: Coriandrum sativum, oxidative stress, heart failure, endothelial dysfunction, endothelin receptors

SAŽETAK

Insuficijencija srca je sindrom uzrokovan strukturnim i funkcionalnim abnormalnostima srca, a karakteriše se promenama hemodinamskih i neurohumoralnih mehanizama. Insuficijencija srca predstavlja veliki zdravstveni problem širom sveta i zbog toga su neophodne efikasnije terapije. Koriander (C. sativum) je tradicionalno začinsko bilje za koje se zna da poseduje mnoga biološka i terapijska svojstva. Cilj ove studije bio je da ispita kardioprotektivne efekte C. sativuma na modelu srčane insuficijencije izazvane izoproterenom kod pacova. Srčana insuficijencija izazvana je subkutanom injekcijom izoproterenola (85 mg/kg dva puta u intervalu od 24 h). Efikasnost per os primenjenog ekstrakta semena korijandera ispitivana je na hemodinamski profil, aktivnost antioksidacionih enzima, peroksidaciju lipida, lipidni profil, aterogeni indeks, mRNK, proteinsku ekspresiju endotelinih receptora (ETA i ETB) i histopatologiju. C. sativumom primenjen oralno (1 g/kg TM) doveo je do poboljšanja hemodinamskih parametara, obnovio je srčane antioksidacione enzime, snizio oksidacioni stres, poboljšao lipidni profil, smanjio aterogeni indeks, smanjio nivoe ETA i ETB receptora mRNA i proteina i obnovio srčanu morfologiju. Naši rezultati ukazuju da se C. sativum može koristiti kod srčane insuficijencije kao kardioprotektivni agens, najverovatnije zbog njegove sposobnosti da ublaži oksidacioni stres, poboljša lipidni profil i endotelnu disfunkciju.

Ključne reči: korijander, oksidacioni stres, srčana insuficijencija, endotelna disfunkcija, endotelinski receptori

ABBREVIATIONS

AC- Atherogenic Coefficient	ISO- Isoproterenol
AIP- Atherogenic Index of Plasma	LDL- Low-density lipoproteincholesterol
C. sativum- Coriandrum sativum	MDA- Malondialdehyde
DBP- Diastolic blood pressure	ROS- Reactive Oxygen species
GPx- Glutathione peroxidase	SBP- Systolic blood pressure
GR- Glutathione reductase	SOD- Superoxide dismutase
GSH- Glutathione	TBA- Thiobarbituric acid
HDL-C- High-density lipoprotein-cholesterol	TC- Total cholesterol
HF- Heart failure	TCA- Trichloroacetic acid
HR- Heart rate	TG- Triglycerides;
MAP- Mean arterial pressure	vLDL-C- Very low-density lipoprotein-cholesterol

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INTRODUCTION

Heart failure (HF) is a clinical syndrome, caused due to structural and functional cardiac abnormalities, characterized by changes in the hemodynamic and neurohumoral mechanisms that result in inability of the heart to pump blood efficiently. Epidemiological studies suggest high prevalence of cardiovascular diseases out of which heart failure accounts for more than 23 million cases worldwide (1). Major risk factors include ischemic heart disease, hypertension, coronary artery disease, cardiomyopathies, valvular and congenital heart disease, arrhythmias, pericardial disease, and cardiotoxic substances (2). Despite many technological advances in the field of cardiovascular diseases prevention, the incidence and prevalence of HF continue to increase (3).

In the recent years, considerable progress has been made to understand the mechanisms underlying heart failure. Evidence suggests the role of reactive oxygen species (ROS) in the pathogenesis of cardiovascular diseases (4). Increased production of ROS causes peroxidation of cell membrane lipids resulting in cardiac oxidative stress. Lipids are known to regulate cardiac function and altered lipid profile or high cholesterol levels are established predictors of atherogenesis and cardiovascular mortality (5, 6). Reduced oxidative stress and cholesterol levels has been associated with cardioprotection mechanisms (7). Vascular endothelin receptors are a class of G-protein coupled receptors that mediate vascular smooth muscle contraction and are known to play an important role in cardiovascular system regulation (8). Several experimental studies have suggested the role of ET_A and ET_B receptor systems to be highly dysregulated in chronic heart failure (8, 9).

Various treatment modalities for heart failure are available which include aldosterone inhibitors, angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), beta-blockers, diuretics, calcium channel blockers and digoxin. Statins or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors is a standard drug used for the treatment of heart failure by the virtue of its lipid lowering properties and hence has shown to reduce the incidence and mortality rate in HF patients (10). Many pleiotropic properties of statins beyond cholesterol reduction include its antioxidant, anti-inflammatory properties, improvement in endothelial dysfunction, release of endothelial progenitor cells, and a number of anti-tumor activities (11, 12). Despite many beneficial effects, statins have been reported to cause many adverse effects in the long term (13) which limits the use of statins to a very narrow range. Hence, the need to find an alternate strategy for the prevention and treatment of heart failure is very important.

Investigations are being carried out to study the role of herbal products for the treatment of various diseases as they are considered safe with minimal or no side effects. *C. sativum*, an important spice crop is mainly used in flavouring foods and has also been used as a medicinal plant for the treatment of various diseases (14). *C. sativum* is known

to possess hypolipidemic (15), hypocholesterolemic (16) and antioxidant (17) properties. A study conducted in rats has also shown the cardioprotective action of *C. sativum* against myocardial necrosis (18).

Till date, no work has been done regarding the cardioprotective aspects of *C. sativum* in HF model. Therefore, in the light of above inferences, we designed this study to evaluate the therapeutic and prophylactic potential of *C. sativum* on hemodynamic changes, oxidative stress, lipid profile and endothelin receptors, in comparison with simvastatin against isoproterenol induced HF in wistar rats.

MATERIALS AND METHODS

Animals

Studies were performed on male Wistar rats (150–200 g, 6–8 weeks old). All experiments were conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee. All rats had access to water and rodent chow ad libitum.

Coriandrum sativum seed extract

Dried coriander seeds (*C. sativum*) were powdered and mixed with distilled water to make aqueous suspension. It was administered to rats orally at a dose of 1g/kg b.wt. in accordance with Lal et al. (15)

Induction of heart failure (HF) and experimental groups

HF was induced by subcutaneous injections of isoproterenol (85 mg/kg) dissolved in normal saline, daily for two consecutive days at an interval of 24h. These animals were kept for 15 days for the development of HF model (19). Animals were randomly divided into 8 groups with 7 rats in each. Group I (Control): Control rats were fed normal pellet diet. Group II (HF): Rats were given ISO (85 mg/kg, subcutaneously) once at an interval of 24 h for two consecutive days and were kept for 15 days. Group III: (Therapeutic simvastatin): Rats were treated as in group II, in addition they received simvastatin (10 mg/kg; oral gavage) for another 15 days. Group IV (Therapeutic *C. sativum*): Rats were treated as in group II, in addition they received *C. sativum* 1g/kg; oral gavage) for another 15 days. Group V (Prophylactic simvastatin): Rats were pretreated with simvastatin (10 mg/kg; oral gavage) for 15 days and at the 15th day subcutaneously injected with ISO (85 mg/kg) for 2 consecutive days and were continued with simvastatin treatment for another 15 days. Group VI (Prophylactic *C. sativum*.): Rats were pretreated with *C. sativum* (1g/kg; oral gavage) for 15 days and at the 15th day subcutaneously injected with ISO (85 mg/kg) for two consecutive days and were continued with coriander treatment for another 15 days. Group VII (Simvastatin): Rats were treated with simvastatin (10 mg/kg; oral gavage) for 15 days. Group VIII (*C. sativum*): Rats were treated with *C. sativum*. (1 g/kg; oral gavage) for 15 days.



Hemodynamic Measurements

Rats were anaesthetized with intraperitoneal injection of urethane at a dose of 1 gm/kg b.wt. Disappearance of pedal reflexes indicated adequate anesthesia. Body temperature of the rat was maintained at 37°C to 38°C. Tracheostomy was performed to allow free air breathing without any obstruction. Femoral artery was cannulated by a polyethylene catheter filled with heparin solution (500 IU/mL, v/v), for recording arterial blood pressure (ABP). The catheter was attached to a 23-gauge needle connected through a three way stopcock to a pressure transducer (Statham-P23D, Oxnard, California). Femoral vein of the other limb was cannulated for injecting drugs. Before recording ABP, the catheter was flushed with heparinized saline solution (500 IU/mL, v/v) to prevent the formation of any blood clot that might interfere with the normal recording of ABP. The pressure recording system was calibrated with a mercury manometer before each experiment. ABP was measured after 20 minutes of stabilization. SBP, DBP, MAP, and HR were recorded on Power Lab data-acquisition system (4SP, AD Instruments, Australia) with a computerized analysis program (Lab Chart 7, AD Instruments).

Post-mitochondrial supernatant preparation and biochemical estimations

Left ventricle was removed quickly, cleaned of extraneous material and immediately perfused with ice-cold saline (0.85% NaCl) and were then homogenised in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%) using a Potter–Elvehjen homogeniser. The homogenate was filtered through muslin cloth and centrifuged at 800 g for 5 min at 4°C in a REMI cooling centrifuge to separate the nuclear debris. Aliquot obtained was centrifuged at 12,000 rpm for 20 min at 4°C to obtain the PMS, which was used as a source of enzymes. All biochemical estimations were completed within 24 h of animal sacrifice.

Estimation of glutathione

Glutathione (GSH) level was assessed by the method of Jollow et al. (20). A quantity of 1 ml of 10% PMS mixed with 1 ml of 4% sulphosalicylic acid, incubated at 4°C for 1 h, and then centrifuged at 4°C at 1200 g for 15 min. The reaction mixture of 3 ml was composed of 0.4 ml of supernatant, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml dithio-bis-2-nitrobenzoic acid (4 mg/ml). The yellow colour developed was read immediately at 412 nm on the spectrophotometer (Lambda EZ201; Perkin Elmer). GSH concentration was calculated as μmol GSH conjugates/mg tissue.

Assay for glutathione peroxidase activity

Activity of Glutathione peroxidase (GPx) was calculated by the method of Mohandas et al. (21). The total volume of 2 ml was composed of 0.1 ml EDTA (1mM), 0.1 ml sodium azide (1mM), 1.44 ml phosphate buffer (0.1 M, pH 7.4), 0.05 ml glutathione reductase (1 IU/ml is equivalent to 1 mol GSSG reduced/min per ml), 0.05 ml GSH (1mM),

0.1 ml NADPH (0.2mM), 0.01 ml H_2O_2 (0.25mM) and 0.1 ml of 10% PMS. The depletion of NADPH at 340nm was recorded at 25°C. Enzyme activity was calculated as μmol NADPH oxidised/ min per mg protein with a molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Glutathione reductase activity

GR activity was determined by the method of Carlberg and Mannervik (22). The reaction mixture consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5mM), 0.05 ml GSH (1mM), 0.1 ml NADPH (0.1mM) and 0.1 ml of 10% PMS, in a total volume of 2 ml. Enzyme activity was quantified at 25°C by measuring the disappearance of NADPH at 340 nm and was calculated as μmol NADPH oxidized/min per mg protein using a molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Assay for catalase activity

Catalase activity was assessed by the method of Claiborne (23). In short, the reaction mixture consisted of 0.05 ml PMS, 1.0 ml of H_2O_2 (0.019 M), 1.95 ml phosphate buffer (0.1 M, pH 7.4), in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm, and the change in absorbance was calculated as μmol H_2O_2 consumed/min per mg protein.

Measurement of superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured by the method of Marklund and Marklund (24). The reaction mixture consisted of 2.875 ml Tris–HCl buffer (50mM, pH 8.5), pyrogallol (24mM in 10mM-HCl) and 100 ml PMS, in a total volume of 3 ml. Enzyme activity was measured at 420nm and was expressed as units/mg protein. One unit of enzyme is defined as the enzyme activity that inhibits the autooxidation of pyrogallol by 50%.

Measurement of lipid peroxidation (LPO)

Assay for lipid peroxidation (LPO) was done according to the method of Wright et al. (25). The reaction mixture consisted of 0.05 ml serum sample, 0.73 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml ascorbic acid (100mM) and 0.02 ml ferric chloride (100mM) in a total volume of 1 ml. This mixture was then incubated at 37°C in a shaking water bath for 1 h. Reaction was stopped by the addition of 1 ml trichloroacetic acid (10%). Following the addition of 1 ml thiobarbituric acid (TBA) (0.67%), all the tubes were placed in a boiling water bath for 20 min. The tubes were shifted to an ice bath, and then centrifuged at 2500 g for 10 min. Amount of malondialdehyde (MDA) formed in the serum samples was assessed by measuring the optical density of the supernatant at 532 nm against blank using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Lipid profile

Serum total cholesterol (TC), triglycerides (TG), low-density lipoproteincholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and high-density lipopro-



tein-cholesterol (HDL-C) were determined by commercially available spectrophotometric assay kits (Monozymes, India).

Atherogenic indices

Atherogenic indices were calculated as described by Ikewuchi and Ikewuchi (26) using the formulae:

$$\text{Cardiac Risk Ratio (CRR)} = \text{TC} / \text{HDL-C}$$

$$\text{Atherogenic Coefficient (AC)} = (\text{TC} - \text{HDL-C}) / \text{HDL-C}$$

$$\text{Atherogenic Index of Plasma (AIP)} = \text{Log (TG / HDL-C)}$$

(The values of TC, TG and HDL-C were converted to mmol/l for calculation of atherogenic indices).

RNA isolation and quantitative real-time reverse transcription-polymerase chain reaction

Total tissue RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction method using Trizol reagent (Invitrogen, USA) following the manufacturer's instructions. The tissue was homogenized in trizol (0.2 g tissue per 2 ml trizol) with a Polytron tissue homogenizer. Chloroform extraction, isopropanol precipitation, and 75% (vol/vol) ethanol washing of precipitated RNA were subsequently performed. The obtained RNA was resolved in diethyl pyrocarbonate (DEPC) treated water. Quantity and quality of RNA extracted was analyzed by NanoDrop (ND-3300, NanoDrop Technologies, USA) and agarose gel electrophoresis. One microgram total RNA was reverse transcribed using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas Life Sciences, USA) as per the manufacturer's recommendations.

Real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using the Light cycler SYBR Green RT-PCR kit (Roche, U.S.A) with the help of specific primers for endothelin receptor A (ET_A) and endothelin receptor B (ET_B), and normalized to Glyceraldehydes 3-phosphate dehydrogenate (GAPDH) following the standard protocol. Analysis was performed using $\Delta\Delta C_t$ method as described earlier (27). The sequence of the primers used is shown in Table 1.

Western blotting

Total protein was extracted from the heart left ventricle. Protein concentration was determined by Bradford as-

say. Denaturation of proteins was performed in 2X Laemmli sample buffer by heating to 95°C for 5 min, followed by a quick spin. Total protein (40 µg) in each sample was separated by 10% SDS polyacrylamide gel electrophoresis and transferred to methanol-activated PVDF membrane in Tris-Glycine buffer containing 20% of methanol. Membranes were blocked by TBST with BSA and incubated with rat polyclonal antiserum raised against rat ET_AR (1: 200) and rat ET_BR (1: 100), followed by incubation with horseradish peroxidase conjugated secondary antibody. β-actin was used as an internal control. Immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) detection system. Densitometric analysis was performed using image-analysis software to determine the protein level. The band intensities of target proteins were expressed relative to β-actin and normalized to the percentage of control.

Statistical Analysis

The results were presented as mean ± standard error of the mean (SEM). All the data was analyzed by analysis of variance (ANOVA) followed by Tukey multiple comparison tests, for the analysis between the groups. The minimum criterion for statistical significance was set at P < 0.05 for all comparisons.

Histopathological Examination

The heart (left ventricle) was fixed in 10% formalin solution for histopathological analysis. The sections were examined under light microscope, and photomicrographs were taken. Tissues were embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E). The sections were examined under light microscope, and photomicrographs were taken.

RESULTS

Arterial blood pressure and Heart rate

HF rats showed a significant (p<0.05) decrease in SBP, DBP, HR and MAP as compared to the control group. However, both therapeutic and prophylactic treatment with *C. sativum* and simvastatin significantly (p<0.05) increased SBP, DBP, HR and MAP compared to the HF group. No significant changes were observed in *C. sativum* and simvastatin only treated groups. The results are as shown in Figure 1.

Enzymatic and non-enzymatic antioxidant levels

Table 2 demonstrates the activities of different antioxidant enzymes in response to *C. sativum* and simvastatin treatment in ISO induced rats. ISO administration significantly reduced cardiac GSH (p<0.05) levels in HF rats as compared to the control rats. Activities of GR (p<0.05), GPx (p<0.05), SOD (p<0.05) and catalase (p<0.05) were also found to be decreased in ISO induced rats when compared with the control group. Therapeutic and prophylactic treat-

Table 1: Primer sequences of ET_A receptor, ET_B receptor and GAPDH genes for Real Time PCR.

Gene	Primer Sequence
ET _A receptor (sense)	5'-ATCGCTGACAATGCTGAGAG-3'
ET _A receptor (antisense)	5'-CCACGATGAAAATGGTACAG-3'
ET _B receptor (sense)	5'-GAAAAGAGGATTCCACCTG-3'
ET _B receptor (antisense)	5'-ACGAACACGAGGCATGATAC-3'
GAPDH (sense)	5'-GCCATCAACGACCCCTTCATTG-3'
GAPDH (antisense)	5'-TGCCAGTGAGCTTCCCGTTC-3'

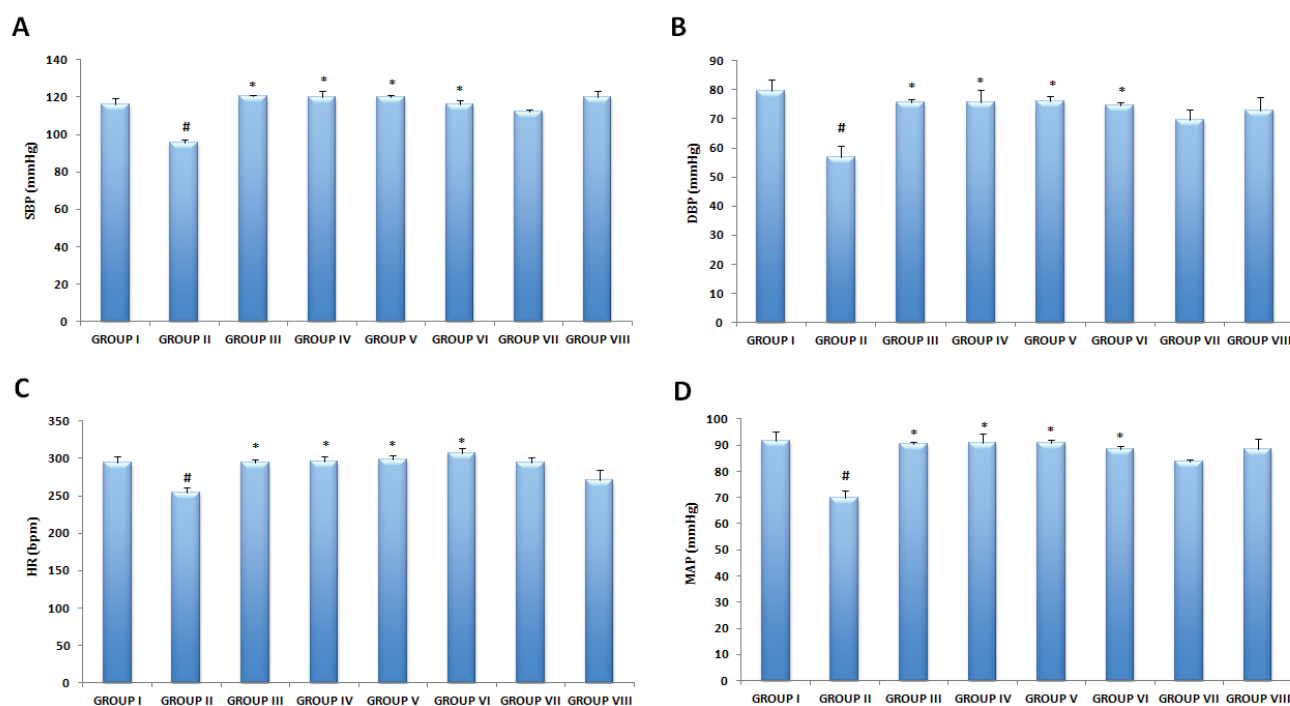


Fig 1: Hemodynamic Parameters

Figure 1: Hemodynamic parameters: (A) Systolic blood pressure (SBP), (B) Diastolic blood pressure (DBP), (C) Heart rate (HR), (D) Mean arterial pressure in Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results represent mean \pm SEM of seven animals per group. Results obtained are significantly different from control group ($\#p < 0.05$). Results obtained are significantly different from HF group ($*p < 0.05$).

ment with *C. sativum* and simvastatin however increased the level of GSH ($p < 0.05$) and activities of GR ($p < 0.05$), GPx ($p < 0.05$), SOD ($p < 0.05$) and catalase ($p < 0.05$), compared to the HF group. However, no significant difference was found in the *C. sativum* and simvastatin only treated group, when compared to the control group.

Lipid peroxidation

In comparison with the control group, levels of MDA increased significantly ($p < 0.05$) in HF rats. Administration of *C. sativum* and simvastatin both therapeutically and prophylactically showed significant ($p < 0.05$) reduction in MDA levels when compared with the HF group. *C. sati-*

Table 2: Effect of simvastatin and *Coriandrum sativum* treatment on cardiac glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase levels in isoproterenol induced rats.

Treatment groups	GSH ($\mu\text{mol GSH/g tissue}$)	GPx ($\mu\text{mol NADPH oxidized/min/mg protein}$)	GR ($\mu\text{mol NADPH oxidised/min/mg protein}$)	Catalase ($\mu\text{mol H}_2\text{O}_2$ consumed/min/mg protein)	SOD (units/mg protein)
Group I	4.24 \pm 0.32	7.20 \pm 0.72	9.18 \pm 0.58	88.40 \pm 2.40	3.90 \pm 0.38
Group II	1.77 \pm 0.14 [#]	3.52 \pm 0.24 [#]	4.50 \pm 0.42 [#]	42.23 \pm 1.98 [#]	1.52 \pm 0.22 [#]
Group III	3.25 \pm 0.20 [*]	5.19 \pm 0.33 [*]	6.54 \pm 0.24 [*]	67.18 \pm 3.20 [*]	2.69 \pm 0.20 [*]
Group IV	3.28 \pm 0.25 [*]	5.28 \pm 0.27 [*]	6.62 \pm 0.57 [*]	68.12 \pm 2.33 [*]	2.72 \pm 0.24 [*]
Group V	3.46 \pm 0.19 [*]	6.29 \pm 0.32 [*]	8.53 \pm 0.54 [*]	71.97 \pm 2.44 [*]	3.52 \pm 0.18 [*]
Group VI	3.48 \pm 0.28 [*]	6.37 \pm 0.30 [*]	8.62 \pm 0.32 [*]	72.23 \pm 2.09 [*]	3.57 \pm 0.15 [*]
Group VII	4.22 \pm 0.31	7.23 \pm 0.42	9.20 \pm 0.44	88.57 \pm 1.11	3.92 \pm 0.24
Group VIII	4.25 \pm 0.29	7.28 \pm 0.50	9.23 \pm 0.51	88.73 \pm 2.78	3.94 \pm 0.21

Results represent mean \pm SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*.); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ($\#p < 0.05$). Results obtained are significantly different from HF group ($*p < 0.05$).



Table 3: Effect of simvastatin and *coriandrum sativum* L. treatment on lipid profile of isoproterenol induced rats.

Treatment groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	vLDL (mg/dl)
Group I	118.45 ± 1.48	93.18 ± 3.10	47.18 ± 0.82	42.16 ± 0.73	21.30 ± 0.84
Group II	164.1 ± 2.43 [#]	163.33 ± 3.73 [#]	22.5 ± 0.34 [#]	81.25 ± 3.42 [#]	29.56 ± 0.89 [#]
Group III	148.73 ± 4.05 [*]	146.63 ± 2.80 [*]	30.33 ± 0.98 [*]	71.43 ± 2.13 [*]	25.43 ± 0.91 [*]
Group IV	150.66 ± 1.87 [*]	143.85 ± 3.71 [*]	28.5 ± 1.78 [*]	72.46 ± 2.05 [*]	25.85 ± 0.46 [*]
Group V	132.88 ± 1.86 [*]	111.4 ± 2.0 [*]	43.1 ± 0.84 [*]	50.06 ± 1.2 [*]	23.1 ± 0.45 [*]
Group VI	133.33 ± 2.60 [*]	109.03 ± 2.63 [*]	42.33 ± 0.55 [*]	51.36 ± 0.95 [*]	23.28 ± 0.32 [*]
Group VII	116.91 ± 1.46	90.7 ± 2.79	48.3 ± 0.67	40.36 ± 0.4	20.33 ± 0.38
Group VIII	117.56 ± 0.60	91.61 ± 3.28	47.96 ± 0.71	41.93 ± 0.62	21.0 ± 0.64

Results represent mean ± SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ([#]p < 0.05). Results obtained are significantly different from HF group (^{*}p < 0.05).

vum and simvastatin alone treated groups did not have any significant effect on the serum MDA levels (Figure 2).

Lipid profile

HF rats showed a significant (p<0.05) elevation in TC, TG, LDL-C, vLDL-C levels and a significant (p<0.05) reduction in the level of HDL-C respectively, compared to control rats. Therapeutic and prophylactic treatment with *C. sativum* and simvastatin significantly (p<0.05) decreased the levels of TC, TG, LDL-C, vLDL-C and increased (p<0.05) HDL-C levels when compared to HF rats. Treatment with *C. sativum* and simvastatin alone did not show any significant effect on the lipid profile (Table 3).

Atherogenic indices

Atherogenic indices were assessed by calculating Cardiac risk ratio (CRR), Atherogenic coefficient (AC) and Atherogenic index of plasma (AIP). A significant (p<0.05) increase in CRR, AC and AIP was observed in HF rats. However, therapeutic and prophylactic treatment with *C. sativum* and simvastatin showed a significant (p<0.05) reduction in CRR, AC and AIP. *C. sativum* and simvastatin per se treatment showed no significant changes in atherogenic indices when compared with the control group (Table 4).

Expression of ET_A and ET_B receptor mRNA levels

Quantitative real-time PCR was used to measure the mRNA levels of ET_A and ET_B receptors. Relative mRNA levels of ET_A receptors in the left ventricle of HF rats were found to be increased significantly (p<0.05) as compared with the control group. Both therapeutic and prophylactic treatment with *C. sativum* and simvastatin showed a significant (p<0.05) decrease in the levels of ET_A receptor mRNA, when compared to the HF group (Figure 3A). Further, mRNA levels of ET_B receptor were also found to be significantly (p<0.05) upregulated in HF group, compared to the normal control group. However, in comparison with the HF group therapeutic and prophylactic treatment with *C. sativum* and simvastatin significantly (p<0.05) downregulated the expression of ET_B receptor mRNA (Figure 3B). No significant change was observed

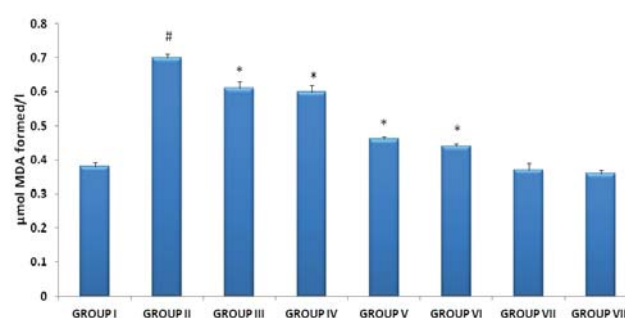


Figure 2 Serum MDA levels

Figure 2: Serum Malodialdehyde (MDA) levels. Results represent mean ± SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ([#]p < 0.05). Results obtained are significantly different from HF group (^{*}p < 0.05).

Table 4: Effect of simvastatin and *C. sativum* treatment on Atherogenic Indices of isoproterenol induced rats.

Treatment groups	Cardiac Risk Ratio (CCR)	Atherogenic Coefficient (AC)	Atherogenic Index of Plasma (AIP)
Group I	2.51 ± 0.03	1.51 ± 0.03	-0.06 ± 0.016
Group II	7.30 ± 0.17 [#]	6.30 ± 0.17 [#]	0.50 ± 0.008 [#]
Group III	4.92 ± 0.15 [*]	3.92 ± 0.15 [*]	0.32 ± 0.017 [*]
Group IV	5.41 ± 0.42 [*]	4.41 ± 0.42 [*]	0.34 ± 0.023 [*]
Group V	3.08 ± 0.07 [*]	2.08 ± 0.07 [*]	0.053 ± 0.013 [*]
Group VI	3.14 ± 0.04 [*]	2.14 ± 0.04 [*]	0.052 ± 0.004 [*]
Group VII	2.37 ± 0.04	1.37 ± 0.04	-0.09 ± 0.014
Group VIII	2.45 ± 0.04	1.45 ± 0.04	-0.07 ± 0.015

Results represent mean ± SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ([#]p < 0.05). Results obtained are significantly different from HF group (^{*}p < 0.05).



in the ET_A and ET_B receptor mRNA levels of *C. sativum* and simvastatin alone treated groups.

Expression of ET_A and ET_B receptor protein levels

Protein levels of ET_A and ET_B receptor was evaluated by western blotting. Expression level of ET_A receptor protein was found to be significantly ($p < 0.05$) upregulated in HF rats, when compared to the untreated control rats. However, in comparison with the HF rats, treatment with *C. sativum* and simvastatin both therapeutically and prophylactically reduced their levels significantly ($p < 0.05$) as observed in Figure 4A. Levels of ET_B receptor protein was also found to be increased significantly ($p < 0.05$), in comparison with

the control group. Therapeutic treatment decreased the levels of ET_B receptor protein significantly ($p < 0.05$) in *C. sativum* and simvastatin groups accompanied by further downregulation of ET_B receptor protein in prophylactic *C. sativum* and simvastatin groups significantly ($p < 0.05$). The results are shown in Figure 4B. No significant change was assessed in the ET_A and ET_B receptor protein levels of *C. sativum* and simvastatin alone treated groups, when compared with the normal group.

Histopathology

Histological sections from left ventricle of control rats showed normal morphology. However, HF rats showed

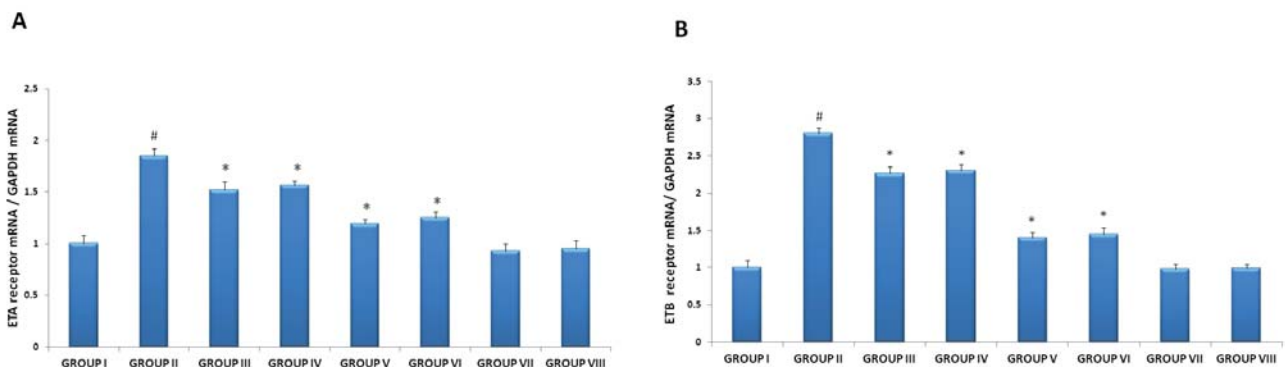


Figure 3: Real Time PCR results of ET_A and ET_B receptor mRNA in the LV (A) Relative mRNA levels of ET_A receptor, (B) Relative mRNA levels of ET_B receptor. Results represent mean \pm SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ($\#p < 0.05$). Results obtained are significantly different from HF group

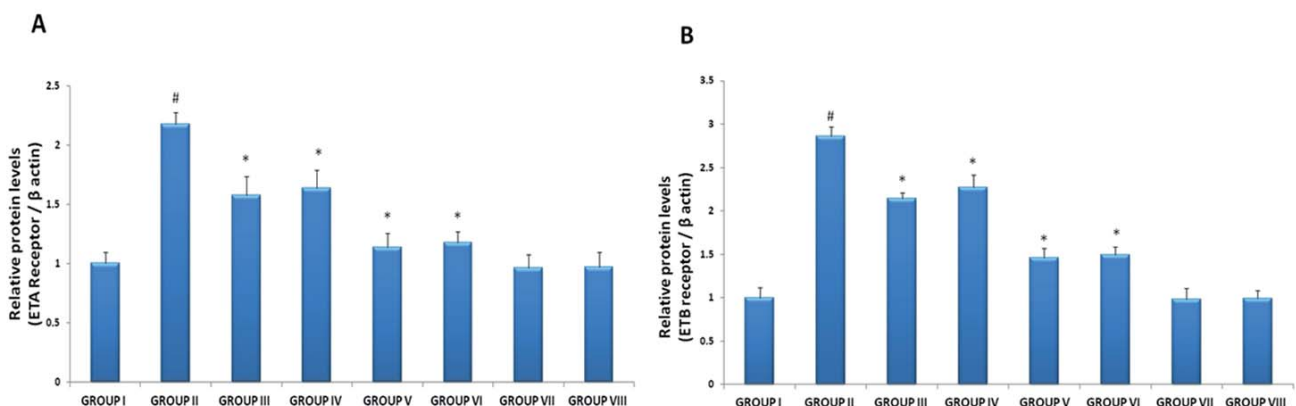
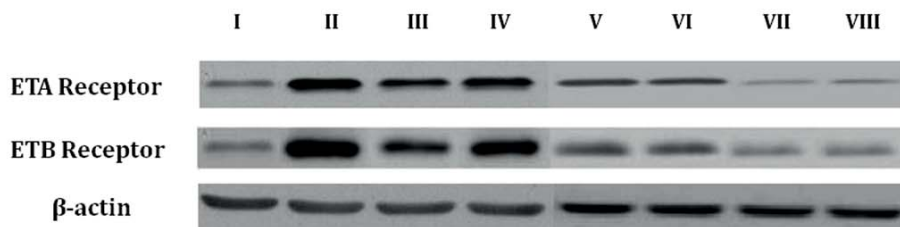


Figure 4: Western blot results of ET_A and ET_B receptor proteins in the LV

Figure 4: Western blot results of ET_A and ET_B receptor proteins in the LV. (A) ET_A receptor protein expression level, (B) ET_B receptor protein expression level. Results represent mean \pm SEM of seven animals per group. Group I (Control); Group II (HF); Group III (Therapeutic simvastatin); Group IV (Therapeutic *C. sativum*); Group V (Prophylactic simvastatin); Group VI (Prophylactic *C. sativum*); Group VII (Simvastatin per se); Group VIII (*C. sativum* per se). Results obtained are significantly different from control group ($\#p < 0.05$). Results obtained are significantly different from HF group ($^*p < 0.05$).

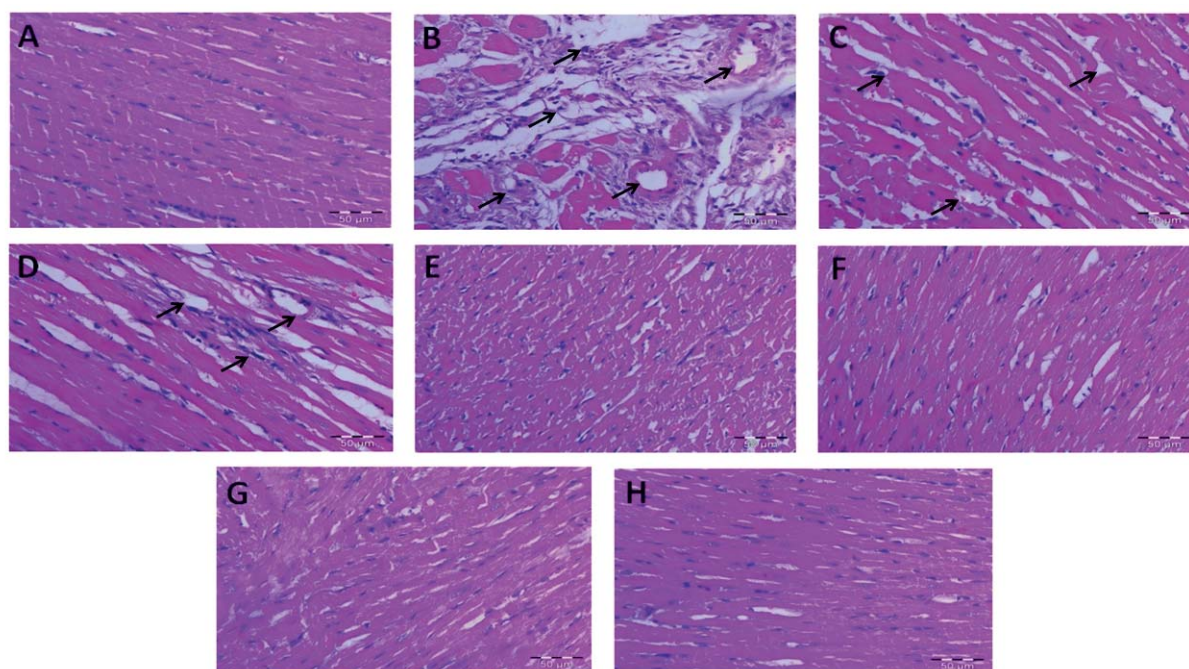


Figure 5: Histopathological changes in the myocardium

Figure 5: Histopathological changes in the myocardium (A) Control group showing normal architecture of cardiac myofibers (B) HF group showing alteration in myocardial structure with interstitial oedema, myocyte necrosis and inflammatory cell infiltration (C) Therapeutic simvastatin (D) Therapeutic *C. sativum* showing mild necrosis and inflammatory cell infiltration (E) Prophylactic simvastatin (F) Prophylactic *C. sativum* showing slight myocardial degeneration (G) Simvastatin per se (H) *C. sativum* per se. Specimens stained with hematoxylin and eosin.

distortion in the myocardial structure, myocyte necrosis with interstitial oedema and leucocyte infiltration. Therapeutic treatment with *C. sativum* and simvastatin showed partial improvement in myocardial morphology. However, prophylactic *C. sativum* and simvastatin treated groups showed marked improvement in the myocyte necrosis, interstitial oedema and leucocyte infiltration thereby restoring the normal myocardial morphology. No change in the myocardial architecture was observed from the histopathological sections of *C. sativum* and simvastatin alone treated groups (Figure 5).

DISCUSSION

The results of our present study clearly demonstrate the cardioprotective effect of *Coriandrum sativum* in isoproterenol induced HF rats. Isoproterenol (ISO), a synthetic catecholamine and a β -adrenergic receptor agonist induces myocardial necrosis leading to left ventricular hypertrophy. Mechanisms underlying ISO induced heart failure include oxidative stress due to excessive production of free radicals from oxidative metabolism of catecholamines, which result in both structural and functional myocardial injury (28, 29). Moreover, calcium overload and hypoxia followed by coronary hypotension and myocardial hyperactivity may be the cause for morphological alterations that results in myocardial damage (30). ISO induced heart failure serves as an excellent experimental model to evaluate the cardioprotective efficacy of various herbal and synthetic compounds.

Statin, a hypolipidemic drug is used in the treatment of chronic heart failure due to its ability to lower cholesterol levels, improve endothelial function, prevent thrombus formation and modulate inflammatory responses (31). Despite a number of beneficial properties, statins have been reported to cause detrimental effects like muscle toxicity, myopathy, rhabdomyolysis and liver damage (13). Hence, the development of alternate therapeutics which are safe and cost-effective is a good approach for the future. In this regard, herbal and natural products are being studied at the molecular and cellular level to understand their mechanism of action. In the present study, statins were used to compare its cardioprotective actions with the herbal drug *C. sativum*.

Pharmacological analysis of *C. sativum* seeds have revealed the presence of polyphenols (rutin, chlorogenic acid and caffeic acid derivatives), flavonoids (quercetin, isoquercetin, keampferol, rhamnetin and apigenin) and β -carotenoids (32, 33). The oil of *Coriandrum sativum* seeds is rich in α -pinene, β -pinene, camphor, citronellol, p-cymene, geraniol, limonene, linalool, myrcene, α -phellandrene, terpinene, monoterpenoid glycosides and their derivatives (33). Many of these compounds are known to prevent oxidative stress by inhibiting free radical generation in the cellular system, when obtained through diet (33). Flavonoids are known for their anti-inflammatory, antitumor and antioxidant activities (34). Hence, these constituents in combination or independently may be responsible for the cardioprotective properties of *C. sativum*.

Hemodynamic changes have been reported in cardiovascular diseases (35, 36). Our present study showed



significantly lower SBP, DBP, HR and MAP in HF rats as reported earlier (37). Therapeutic and prophylactic treatment with *C. sativum* and simvastatin, however attenuated the decline in SBP, DBP, HR and MAP, thus showing an improvement in altered hemodynamic profile.

Oxidative stress has been well implicated in the pathophysiology of cardiac remodeling and progression of heart failure (37). Increased production of reactive oxygen species damages the cell membrane lipids and causes lipid peroxidation. MDA is one of the final products of lipid peroxidation and a reliable marker for the assessment of oxidative stress (38). The enzymatic antioxidants are mainly involved in scavenging free radicals and reactive oxygen species. Previous studies have demonstrated that isoproterenol administration increases oxidative stress and depletes the level of cardiac GSH and antioxidant armory in the heart (39, 40). Our present study also suggested increased MDA formation and reduced GSH, GPx, GST, GR, catalase and SOD levels in HF rats. *C. sativum* treatment reduced MDA levels and improved the cardiac GSH content and antioxidant enzyme activities as reported previously (41) suggesting its free radical scavenging properties against cardiac oxidative stress in HF rats.

Lipids are known to play an important role in heart failure. Lipids provide structure and stability to cell membranes and changes in lipid profile may contribute to cell death resulting in myocardial ischemia. Altered lipid profile has been well established in cardiovascular diseases (42). Our present study demonstrated a significant increase in serum LDL, vLDL, total cholesterol, triglycerides and decrease in HDL levels in HF rats as reported previously (19). The mechanism of action of isoproterenol on fat cells is believed to be mediated by the cAMP cascade, where isoproterenol activates adenylate cyclase, thereby increasing the cAMP formation. Subsequently, cAMP promotes lipolytic activity by activating cAMP-dependent protein kinase that phosphorylates lipase resulting in the hydrolysis of stored triacylglycerol, which contributes to hyperlipidemia (43, 44). Therapeutic and prophylactic treatment with *C. sativum* however reduced the levels of total cholesterol, triglyceride, LDL, vLDL and increased HDL levels. Thus, *C. sativum* administration modulated the level of lipid and lipoproteins by decreasing lipid uptake and enhancing lipid breakdown suggesting the hypolipidemic property of *C. sativum* thus conferring cardioprotective effect. *C. sativum* has also known to inhibit the enzyme HMG CoA reductase which is the key enzyme in the pathway of cholesterol biosynthesis in liver (45). Simvastatin treatment in HF rats also showed a similar effect.

Atherogenic indices are powerful indicators of the risk of cardiovascular disease which is assessed by evaluating CRR, AC and AIP, the higher the value the higher the risk of developing heart disease. AIP has been reported to be a significant predictor of atherosclerosis (46). In our present study, HF rats showed a significant increase in CRR, AC and AIP. However, therapeutic treatment with *C. sativum* and simvastatin decreased CRR, AC and AIP which was further reduced by prophylactic treatment of *C. sativum*

and simvastatin signifying their role in reducing the probability of cardiovascular pathogenesis.

Vascular endothelium has an important role in cardiovascular system regulation. Endothelin (ET-1) secreted from vascular endothelial cells is a potent vasoconstrictor, that has affinity for two types of receptors namely ET_A and ET_B receptor. Both these receptors co-exist on vascular smooth muscle cells (VSMC) that mediates vasoconstriction. In addition ET_B receptors present on vascular endothelial cells (VEC) contributes to vasodilatation and ET-1 clearance (47). It has been suggested that in cardiovascular diseases, vasodilator ET_B receptors may switch their phenotype to contractile ET_A receptors (48, 49) and that the increased expression of ET_B receptors is directly correlated with the degree of ischemic heart disease (50). In the present study mRNA levels of ET_A and ET_B receptors was found to increase significantly in HF rats as compared to the control rats followed by increased expression of ET_A and ET_B receptor protein levels. Our data is consistent with the earlier studies (9). Therapeutic and prophylactic treatment with *C. sativum* and simvastatin reduced ET_A and ET_B receptor mRNA levels as compared with the HF treated rats. Their protein levels were also found to be significantly lower as compared with the HF rats.

C. sativum cardioprotective efficacy was further supported by histopathological studies. Histological findings revealed distorted myocardial architecture, cardiac myocyte hypertrophy, fibrosis and myocardial necrosis in isoproterenol induced rats. Therapeutic and prophylactic treatment with *C. sativum* and simvastatin alleviated isoproterenol induced myocardial changes and left ventricular hypertrophy. Hence, our results provide strong evidence indicating the cardioprotective efficacy of *C. sativum* against isoproterenol induced heart failure.

In conclusion, *C. sativum* may serve as an alternative herbal drug in protection from heart failure. The cardioprotective action of *C. sativum* may be due to its ability to improve the hemodynamic profile, alleviate oxidative stress, lower cholesterol levels, improve endothelial dysfunction and restore the cardiac morphology.

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