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Original Research

L-Carvone from *Mentha spicata* L. Leaves Suppresses Oxidative Stress and Hypertrophy in the Isoproterenol-Induced Rat Model of Cardiac Hypertrophy

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Abstract

The effect of L-Carvone (a natural monoterpene from *Mentha spicata* L., leaves) in cardiac hypertrophy caused by isoproterenol administration was investigated. Male rats (Wistar) were divided into five groups: Control, diseased, diseased rats with losartan, diseased rats with low-dose L-Carvone, and high-dose L-Carvone. Rats were injected with isoproterenol (5 mg/kg) for 30 days to induce cardiac hypertrophy. Then, simultaneously with Losartan (15 mg/kg), L-Carvone was administered orally at a dosage of 25 mg/kg (low dose) and 100 mg/kg (high dose) treatment. The cardioprotective effect of L-Carvone was evaluated by examining the heart morphometric indices, and ECG analyses. Chronic isoproterenol administration resulted in changes in morphometric indices of the heart, ECG tracings, biochemical parameters such as tissue glucose, proteins, lipid profiles, serum cardiac markers, antioxidants, and histopathological integrity of the heart tissue. When compared with the isoproterenol group, L-Carvone administrated for 30 days ameliorated all these changes in rats significantly ($p < 0.05$). L-Carvone adequately averted chronic cardiac hypertrophy, most probably through its antioxidant potential.

Keywords: *M. spicata* L.; L-Carvone; Antioxidant; Cardioprotective; Antihypertrophic

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Introduction

Cardiac hypertrophy is a condition in which the heart muscle thickens. It can be caused by various factors such as pressure or volume stress, mutations of sarcomeric proteins, loss of contractile mass from prior infarction, and exercise. Its symptoms include arrhythmia (irregular heart rate or rhythm), chest pain, especially during activity, fatigue, fluttering or pounding feeling in the chest, heart murmur, lightheadedness or dizziness, fainting, shortness of breath, especially during activity. Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease in the United States. It affects between 0.2% and 0.5% of the global population. Most people with HCM have a normal life expectancy. However, HCM can lead to heart failure, atrial fibrillation, and sudden death due to ventricular arrhythmias. Isoproterenol (ISO), a β-adrenergic receptor agonist, is commonly used to treat cardiac hypertrophy (CH) as a model for studying oxidative stress and the downregulation of nuclear erythroid-like factor 2 (*Nrf2*) impaired antioxidant systems. To reduce oxidative stress correlated CH, therapies that activate *the Nrf2* gene and antioxidants have been reported [1,2]. Reductive stress (RS) refers to the decrease in the synthesis of reduced glutathione (GSH) and nicotinamide adenine dinucleotide hydrogen (NADH) due to endogenous mechanisms such as the one that constitutively inactivates or dysregulates the *Nrf2* gene associated antioxidants, hyperglycemia, and vigorous exercise [3,4]. Various kinds of medicinal plants have recovered the activities of certain serum cardiac enzymes as to the level of normal control groups. Acute and chronic hypertrophy decreases GSH level, diminishes the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), increases the level of malondialdehyde (MDA) in the heart, and diminishes the activities of other antioxidants such as glutathione reductase (GR), glutathione-S-transferase (GST), vitamin C and vitamin E. The destructive effects of hypertrophy and cardiac injury are mediated primarily by the outset of oxidative stress. Naturally, terpenoids had cardioprotective potential [2,5-7]. Carvone, chemically called 5-isopropenyl-2-methyl-2-cyclohexenone and from the monoterpene family, is found in many plants, including spearmint and caraway. Research has shown that carvone has various pharmacological effects such as antidiabetic, anti-inflammatory, anticancer, neurological, antimicrobial, antiparasitic, antiarthritic, anticonvulsant, and immunomodulatory effects [8] and phytochemicals in *Mentha* species significantly play a vital role in treating CH [7]. The antioxidant potential of carvone had been reported by several investigations [9,10]. Moreover, it has been found to modulate antiarrhythmic action by intracellular calcium signaling in rat hearts [11] and in obesity (high-fat diet-induced) mice [12]. Based on these data, we hypothesized that carvone could protect the cardiac tissue against injury caused by ISO administration. So, in this current study, we researched the *in vivo* action of L-Carvone on hypertrophied heart tissue of rats, by measuring hypertrophic indices, electrocardiography (ECG) tracings, and oxidative stress due to enzymatic aberrations.

Materials and Methods

Chemicals

Isoprenaline hydrochloride (Isoproterenol) was purchased from Sigma-Aldrich. Losartan, a standard drug, was bought commercially as Losarpen (25 mg) tablets from local pharmacy store, in India. All chemicals used for estimations were purchased from Himedia Laboratories Private Limited, India.

Extraction and preparation of the L-Carvone sample

Mentha spicata L. leaves were shade-dried and powdered coarsely. The results of phytochemical screening revealed qualitatively high constituents in hydroethanol (50% water: 50% ethanol) solvent. This ratio of hydroethanolic solvent was selected for further studies because partial polarity of the solvents makes the phytoconstituent of spearmint soluble [13]. The powdered sample was dissolved in a hydroethanolic solution (50:50) and kept in a rotatory shaker. After 72 hours, the whole leaf extract was obtained using the condensation method using a hot water bath [14]. L-Carvone was extracted from *M. spicata* leaves as previously described [10] using adsorption column chromatography. After 1 hour, when the eluate sample ran across the full column at 37°C and then, the eluted fractions numbering 3-9 were pooled together [10] and concentrated by hot water bath condensation process [14] until approximately 0.2-0.3 mL of liquid remained. After the condensation process, 2 mg of L-Carvone was obtained for 2 g of plant sample loaded in the column and used for further use. Later, the eluent crude sample was dispensed in a hydroethanolic solution (50:50) and stored in a refrigerator for further treatment. FTIR spectra were obtained from the Central Research Laboratory (CRL) of PSG College of Arts & Science, Coimbatore, India. FTIR spectra were recorded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} using ethanol as the solvent.

Animals

The study was certified by the Institutional Animal Ethics Committee (SCEA/NO.520/IAEC/2021), PSG Institute of Medical Science and Research (PSG IMS & R), Coimbatore, India, and male albino Wistar rats (150-250 g) were used for the study. The animals were

subjected to laboratory conditions for one week before beginning the experiments and were maintained at 25°C, 50–60% relative humidity, and a 12/12-hour light-dark cycle, with normal chow and water. The experimental protocol was performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India guidelines for the care of laboratory animals.

Induction and simultaneous treatment of the experimental rats

Treatments given to 30 rats for 30 days, in five groups with six rats in each group are:

Group I- A regular chow diet was given to a single group identified as the normal control group.

Group II- For the hypertrophic control group, ISO was suspended in saline (5mg/kg B.W.) and administered intraperitoneally [15].

Group III- Hypertrophic rats simultaneously received ISO (5 mg/kg B.W.) and losartan (15mg/kg B.W.) orally in saline [16].

Group IV- Hypertrophic rats simultaneously received ISO (5 mg/kg B.W.) and L-carvone (25 mg/kg B.W.) orally in hydroethanolic solution (50% water: 50% ethanol) [17].

Group V- Hypertrophic rats simultaneously received ISO (5 mg/kg B.W.) and L-carvone (100 mg/kg B.W.) orally in hydroethanolic solution (50% water: 50% ethanol) [17].

Blood sampling and tissue collection

At the end of 30 days, ISO injected groups simultaneously treated (losartan and L-Carvone), animals were sacrificed under mild anaesthesia. Blood was collected by the cardiac puncture method, and the clotted blood (30 minutes at normal room temperature) was centrifuged at 5000 rpm at 4° C for 20 minutes to obtain serum. Separated serum was stored at -20° C in microcentrifuge tubes for further analysis. After blood was collected, the heart tissue was excised and preserved in 10% formalin (neutral buffer) for histopathological analysis.

Electrocardiogram (ECG) examination of the CH

To check the status of the *in vivo* cardiac functions after 30 days of study, ECG was performed for 8 minutes to get an ECG graph in unanaesthetised rats at the conventional bipolar limb lead II using BITalino ECG Sensor and Open Signals[r]evolution software. The changes in the Q-R-S complex, R amplitude, R–R interval, and heartbeat rate (HR) were recorded [18].

Hypertrophic indices

The status of CH was evaluated using the hypertrophic indices, viz., body weight (BW), heart weight (HW), and Heart index (HW/BW ratio x 100) [19].

Biochemical analysis

Biochemical parameters such as Glucose was assayed using Glucose oxidase method (Arkray AUTOSPAN Liquid Gold Glucose Kit), Total Protein was assayed by Modified Biuret End Point Assay method (Arkray AUTOSPAN Liquid Gold Total Protein Kit), Albumin was assayed using Bromocresol green end point assay method (Arkray AUTOSPAN Liquid Gold Albumin Kit). Estimation of cholesterol was carried out by CHOD-PAP enzymatic end point assay method (Arkray AUTOSPAN Liquid Gold Cholesterol Kit), estimation of triglycerides using GPO-PAP enzymatic end point assay method (Arkray AUTOSPAN Liquid Gold Triglyceride Kit), estimation of HDL-C by using accelerator selective detergent enzymatic end point assay (Arkray AUTOSPAN Liquid Gold Direct HDL Cholesterol Kit), LDL cholesterol and VLDL cholesterol by Friedewald equation, estimation of AST by modified UV (IFCC) kinetic assay method (Arkray AUTOSPAN Liquid Gold AST Kit), estimation of ALT by modified UV (IFCC) kinetic assay method (Arkray AUTOSPAN Liquid Gold ALT Kit), determination of LDH activity by optimized DGKC kinetic assay method (Arkray AUTOSPAN Liquid Gold LDH Kit), estimation of serum CKMB, Troponin-I, NT-pro BNP, hs-CRP, C-peptide, homocysteine, sodium, potassium by Acculite CLIA test kit method, followed by phospholipids by precipitation method [20], liver tissue glycogen by anthrone method [21], calcium by precipitation method [22], pyruvate by DNPH method [23], lactate by colorimetric method [24], lipase estimation was done by titration method (phenolphthalein based indicator) [25], lipid peroxidation by MDA method [26], superoxide dismutase by NBT method [27], catalase by colorimetric method [28], glutathione peroxidase [29] by aromatic disulfide method, glutathione-S-transferase [30], glutathione reductase by Beutler method [31], vitamin C by DNPH method [32], vitamin E by simple modified method [33], total reduced glutathione by DTNB method [34].

Histopathological analysis

Rat hearts were excised and stored in 10% formalin in paraffin until the tissues were processed as transverse, 5 µm thick paraffin of left ventricular sections. Samples were then stained with Masson's trichrome staining (MTS) to investigate heart histological and fibrotic changes. Blue staining in the sections represents collagen accumulation. MTS was used to stain these sections, and all slides were magnified 40x for an analysis of the cellular architecture of the heart tissue.

Statistical analysis

For data processing, SPSS Statistics 23.0 software

(SPSS Inc., USA) was used to analyze data. Quantitative variables are expressed as mean standard deviation (SD). To calculate the difference between quantitative variables in more than two groups, ANOVA (F-test) and least significant difference (LSD) tests [18] were performed. Results were considered significant when p values were < 0.05. Microsoft Office Excel 2016 was used to plot bar graphs.

Results

FTIR Analysis of L-carvone

Figure 1 exhibits the characteristic features of the FTIR spectrum of L-Carvone components. The peaks revealed in table 1 represent the presence of different types of functional groups such as alkyl compounds (peak of 2924.09), aromatic compounds (peak of 1435.04), aromatic ethers (peak of 1242.16), strong alcohols (peak of 111.00), and aromatic benzene (peak of 802.39) in the FT-IR spectrum, confirming that the isolated L-Carvone is similar to that of the standard L-carvone compound.

In vivo ECG analysis

In figure 2, the ECG wave patterns indicate the occurrence of hypertrophic changes in ISO-induced animal groups with elevated Q-R-S intervals, R-R intervals, large Q-T intervals, and decreased heart rate, which

Table 1. FTIR frequency values and functional groups of isolated terpene compound L-Carvone.

S. No	Frequency (cm^{-1})	Reference Frequency (cm^{-1})	Functional Group	Compound	Intensity
1.	3618.46	3610	O-H	Phenol	Medium
2.	3332.99	3200	O-H	Phenol	Strong
3.	3078.39	3080	C-H	Vinyl	Medium
4.	2924.09	2925	C-H	Alkyl	Medium to strong
5.	2885.51	2870	C-H	Alkyl	Medium to strong
6.	2731.20	2720	C-H	Aldehyde derivates	Medium
7.	2615.47	2720	C-H	Aldehyde derivates	Medium
8.	2229.71	2230	C-N	Nitrile (Conjugated)	Medium
9.	2067.69	2140- 1900	C-N	Isothiocyanates	Medium
10.	1944.25	1900	C-N	Isothiocyanates	Medium
11.	1674.21	1675	sc=C	Alkenes	Medium
12.	1435.04	1450	C=C	Aromatic	Weak to strong
13.	1365.60	1350	N-O	Aromatic Nitro Compounds	Medium
14.	1242.16	1220- 1260	C-O	Aromatic ethers	Medium
15.	1141.86	1100- 1300	C-O	Esters	Two bands (distinct from ketones, which do not possess a C-O bond)
16.	1111.00	~1100	C-O	Secondary alcohols	Strong
17.	1056.99	1040- 1060	C-O	Primary alcohols	Strong and broad
18.	964.41	965	C-H	Alkene	Strong
19.	894.97	860- 900	C-H	Aromatic benzene	Strong
20.	802.39	800- 860	C-H	Aromatic benzene	Strong
21.	702.09	700- 750	C-H	Aromatic benzene	Strong
22.	686.66	670- 700	C-H	Alkenes	Strong
23.	570.93	540- 760	C-X	Chloroalkane	Weak to medium
24.	524.64	500- 600	C-X	Bromoalkane	Medium to strong
25.	478.35	~500	C-X	Iodoalkane	Medium to strong
26.	447.49	~500	C-X	Iodoalkane	Medium to strong

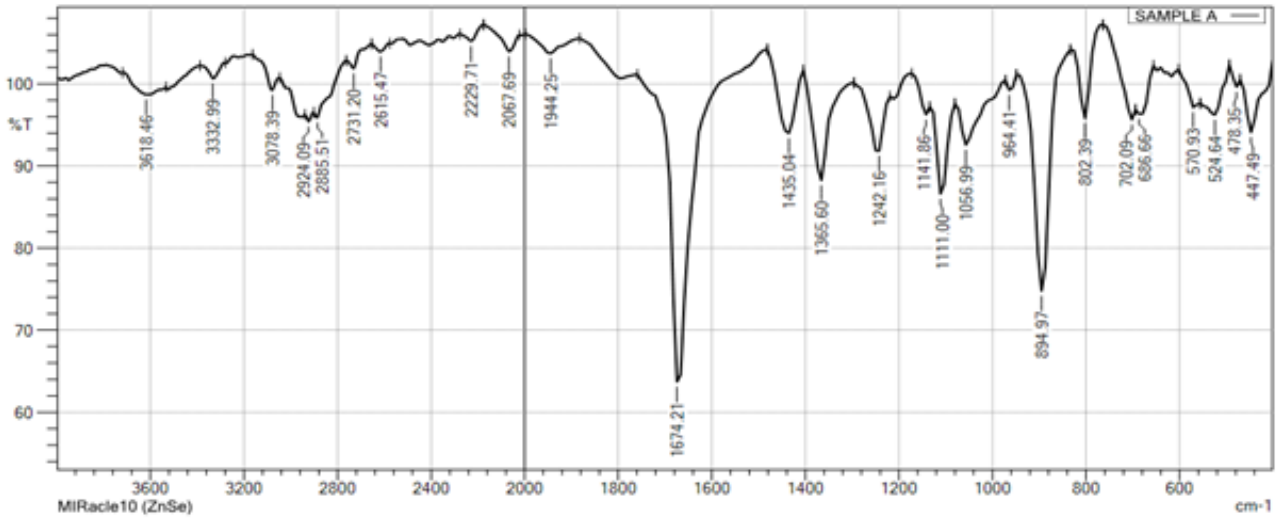

Figure 1. FTIR- spectrum of isolated terpenoid compound L-Carvone. Peaks obtained are the characteristic of the L-Carvone compound, similar to that of the commercial compound.

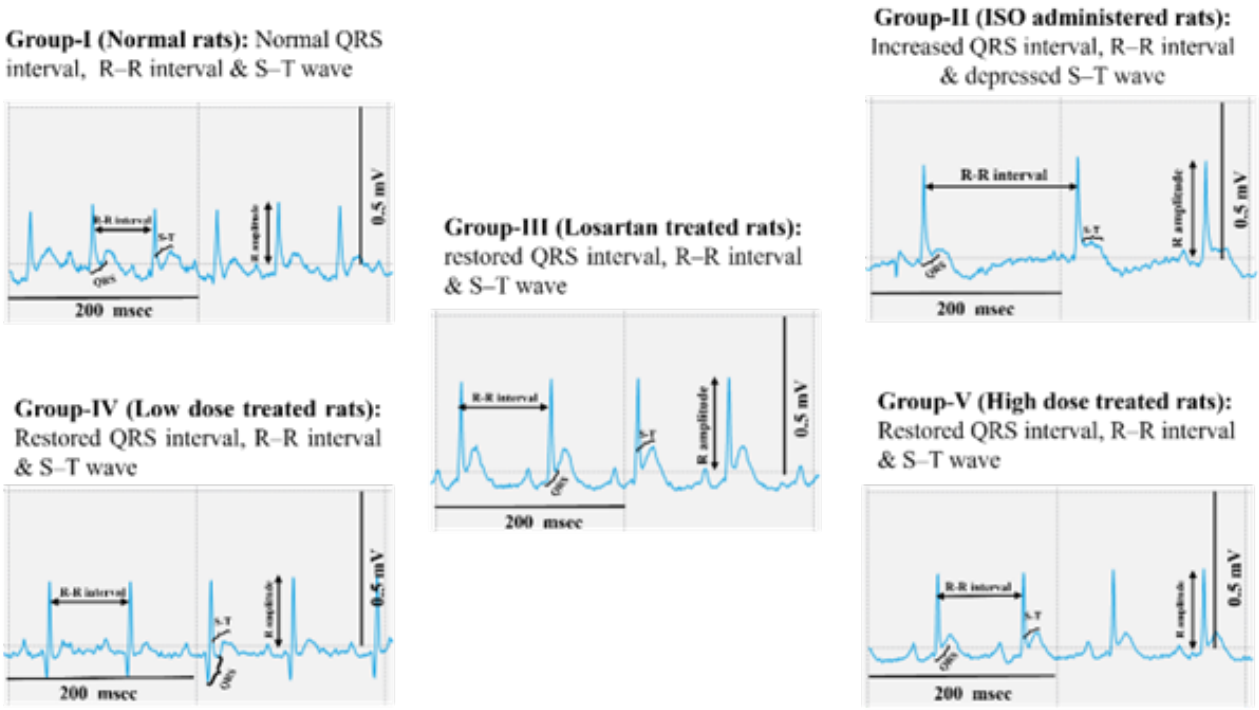

Figure 2. Graphical representation of the real-time ECG tracings of the cardiac ameliorative effect of L-Carvone from *M. spicata* L. leaves extracts. The prolonged QRS, ST wave depression, larger PR interval (LPR) accompanied by larger QT (LQT) interval, and increased R–R interval (IRR) are all indicators of cardiac hypertrophy. These alterations were reduced in rats treated with losartan and simultaneous treatment using L-Carvone abolished all typical characteristic ECG changes which is indicative of cardioprotective activity of the L-Carvone in restoring normal conduction system of the heart.

were restored in losartan and L-Carvone of high-dose (100 mg/kg) administered groups while L-Carvone of low-dose (25 mg/kg) showed lesser reverted effects compared with losartan-treated groups.

Hypertrophic Parameters

The ISO group (II) showed significantly increased hypertrophic indices [HW/BW; HW/Tail length and Heart index (HW/BW ratio x 100)] compared with the normal group. These hypertrophic indices reverted significantly in Losartan and L-Carvone plant extract-administrated rats as well (Table 2).

Results of L-Carvone on cardiac glucose, total protein, and albumin levels

ISO-administrated rats significantly increased tissue

Table 2. Effect of L-Carvone from *M. spicata* leaves extract in primary cardiac hypertrophic indices of rats.

Experimental groups	Body weight (g)	Heart weight (mg)	Heart weight/Tail length ratio	Heart index [HW/BW x 100]
Group I: Control	205.33 ± 3.78$^{a\ ns}$	870 ± 26.46^{a*}	48.72 ± 1.76$^{a*\#}$	408.94 ± 16.29$^{a*\#}$
Group II: ISO administered	202 ± 15.32	1148.67 ± 70.34	54.44 ± 1.28	533.85 ± 18.53
Group III: ISO + Losartan treated	210.00 ± 1.00$^{b\ ns}$	895.33 ± 33.12$^{b*\#}$	49.05 ± 1.24$^{b*\#}$	422.56 ± 33.45$^{b*\#}$
Group IV: ISO + Low-dose treated	195.33 ± 9.55$^{c\ ns}$	990.00 ± 88.89$^{c\ ns}$	56.02 ± 1.57$^{c\ ns}$	477.64 ± 44.75$^{c\ ns}$
Group V: ISO + High-dose treated	215.33 ± 13.28$^{d\ ns}$	1028.67 ± 23.09$^{d\ ns}$	50.55 ± 1.46$^{d*\#}$	430.33 ± 22.66$^{d*\#}$

All values are expressed as mean ± SD (n=3) and analyzed by ANOVA followed by an LSD test. * denotes $P < 0.05$ in comparison with normal control group while $^{\#}$ denotes $P < 0.05$ in comparison with ISO group

glucose, total protein, and tissue albumin levels compared with normal rats. It is identified that the simultaneous treatment using low (25 mg/kg) and high (100 mg/kg) doses of L-Carvone to ISO-induced rats have reverted these high levels of glucose, total protein, and albumin levels close to normal levels as similar to that of losartan group as shown in table 3**.**

Results of L-Carvone on the Cardiac Lipid Profile

Several tissue parameters such as total cholesterol, triglycerides, and phospholipids (PL) showed a significant increase in ISO-injected CH rats. These lipid types were significantly reduced near to the levels of normal rats after simultaneous treatment with L-Carvone than with simultaneous treatment with losartan (Table 3). HDL cholesterol was reduced in ISO-induced rats, which were then significantly reverted close to normal levels when treated with losartan and L-Carvone, of which the latter exhibited better effects, as shown in table 3. LDL and VLDL cholesterol levels were increased during CH, which then reverted close to normal levels significantly in losartan and L-Carvone treated groups.

Effects of L-Carvone on enzymatic changes in cardiac tissue in hypertrophied rats

The enzymes namely AST, ALT, LDH, CKMB, proteins such as troponin-I, NT-pro BNP, hs-CRP, and homocysteine activities in cardiac tissue were high in ISO-injected rats. Losartan- and L-Carvone administered groups restored the enzyme activities, among which L-Carvone revealed more effectiveness, as shown in table 3. C-peptide and lipase (lipid metabolic enzyme) were decreased significantly in ISO rats, which later reverted to normal levels significantly after simultaneous treatment with losartan and L-Carvone. Of which L-Carvone extract exhibited better effect (Table 3).

Results of L-Carvone on Other Important Biochemical Metabolic Parameters

ISO injection in rats resulted in significantly increased serum calcium, potassium, pyruvate, lactate, and liver tissue glycogen levels compared to normal rats. It is to be highlighted that the simultaneous treatment using low and high doses of L-Carvone extract to ISO-injected rats had significantly reduced the above-mentioned parameter levels to normal. Similarly, a beneficial effect was observed in a losartan-treated group as indicated in table 3. Likewise, the serum sodium level which was reduced due to ISO administration was reverted significantly to near normal levels by losartan and L-Carvone treatment.

Effects of L-carvone on changes in lipid peroxidation in hypertrophied rats

ISO increased lipid peroxidation (LPO) more than the control value to 0.8 nmol of malondialdehyde (MDA) formed/mg protein (Table 3). L-Carvone (25 mg/kg), treated group had a significant reduction in LPO levels but to a lesser extent than the control group. However, highly significant reduction in LPO levels were observed with a high L-Carvone dose (100 mg/kg).

Results of L-Carvone on changes in enzymatic antioxidants in hypertrophied rats

The enzymatic antioxidants such as SOD, CAT, GST, and GPx levels decreased by approximately 30–60% on ISO administration (Table 3). L-Carvone with the dose of 100 mg/kg significantly enhanced the levels of antioxidants, CAT, GST, and GPx. Notably enhanced SOD levels to near normal levels were seen even at 25 mg/kg dose of L-Carvone.

Results of L-carvone on changes in non-enzymic antioxidants in hypertrophied rats

The high dose of L-Carvone restored non-enzymatic antioxidants such as vitamin C, and vitamin E and

Table 3. Effect of L-carvone on biochemical parameters in rats with ISO-induced cardiac hypertrophy

Parameters	Control	ISO (5 mg/kg)	ISO + Losartan (15 mg/kg)	ISO + L-carvone (25 mg/kg)	ISO + L-carvone (100 mg/kg)
Tissue glucose (mg/g wet tissue)	88.17 ± 1.17	153.92 ± 0.26*	135.33± 2.59*	141.48 ± 0.76*#	105.38 ± 1.38*#
Total tissue protein (g/g wet tissue)	0.71 ± 0.01	1.53 ± 0.02*	0.94± 0.06*	1.36 ± 0.04*#	1.12 ± 0.04*#
Tissue albumin (g/g wet tissue)	0.70 ± 0.00	1.43 ± 0.02*	0.94 ± 0.05*	1.35± 0.03*#	1.11 ± 0.03*#
Total tissue cholesterol (mg/g wet tissue)	14.76 ± 0.38	20.1 ± 0.8*	13.74 ± 0.32*#	15.91 ± 0.2#	14.74 ± 0.74#
Tissue triglycerides (mg/g wet tissue)	47.21 ± 2.07	97.70 ± 1.90*	85.06 ± 1.20*	90.13 ± 3.55*#	47.13 ± 1.2*#
Tissue phospholipids (mg/g wet tissue)	85.00 ± 8.66	131.67 ± 5.77*	90.00 ± 8.66*#	98.33 ± 2.89*#	96.67 ± 2.89#
Tissue HDL-C (mg/g wet tissue)	2.78 ± 0.60	0.67 ± 0.26*	2.98 ± 0.40*	1.26 ± 0.40#	1.22 ± 0.37#
Tissue LDL-C (mg/g wet tissue)	6.91 ± 2.70	17.26 ± 2.30*	9.16 ± 4.64*	4.40 ± 2.91#	4.34 ± 1.98#
Tissue VLDL-C (mg/g wet tissue)	9.44 ± 0.41	19.54 ± 3.80*	19.31 ± 0.00*	28.05 ± 1.73*	12.76 ± 5.08*
Tissue AST (IU/g wet tissue)	61.25 ± 1.53	84.88 ± 2.15*	70.56 ± 1.28*	76.65 ± 3.77*#	71.86 ± 4.30*#
Tissue ALT (IU/g wet tissue)	31.09 ± 1.99	63.27 ± 2.58*	43.44 ± 2.83*	50.21 ± 2.70*#	36.75 ± 3.03#
Tissue LDH (IU/g wet tissue)	33.86 ± 0.87	52.62 ± 0.20*	46.55 ± 0.20*	40.30 ± 0.89*	38.09 ± 0.44*#
Liver glycogen (mg/100mg)	53.67 ± 1.23	99.83 ± 4.54*	55.17 ± 4.54*#	62.67 ± 1.18*	60.17 ± 4.54*
Serum CKMB (IU/L)	269.93 ± 0.66	340.43 ± 0.47*	135.43± 2.49*	300.10 ± 0.10*#	297.02 ± 0.24*#
Serum troponin-I (pg/ml)	38.61 ± 0.25	47.14 ± 0.34*	135.34± 2.58*	42.14 ± 0.34*#	36.41 ± 0.39*#
Serum NT-pro BNP (pg/µl)	3 899.74 ± 0.63	5 200.01 ± 0.60*	135.34± 2.58*#	4 399.68 ± 0.28*#	3 699.68 ± 0.30*#
Serum hs-CRP (mg/ml)	85.00 ± 0.15	94.00 ± 0.10*	88.00 ± 0.25*	90.00 ± 0.12*	88.00 ± 0.05*
Serum C-peptide (ng/µl)	178.00 ± 0.08	162.00 ± 0.25*	170.00 ± 0.18*	185.00 ± 0.05*	174.00 ± 0.13*
Serum homocysteine (µmol/L)	13.5 ± 0.30	18.2 ± 0.10*	14.00 ± 0.20*	16.4 ± 0.50*	15.2 ± 0.50*
Serum calcium (mg/dL)	10.41 ± 0.34	23.11 ± 2.14*	15.22 ± 2.70*#	16.42 ± 0.99*#	14.18 ± 1.71*#
Serum sodium (mEq/L)	157.00 ± 0.10	153.17 ± 0.29*	151.2 ± 0.20*	158.67 ± 0.31*	155.07 ± 0.06*
Serum potassium (mEq/L)	5.07 ± 0.11	6.12 ± 0.02*	6.01 ± 0.09*	6.03 ± 0.09*	6.00 ± 0.01*
Serum pyruvate (mg/dL)	1.56 ± 0.02	2.36 ± 0.07*	1.57 ± 0.02*	2.15 ± 0.08*#	1.56 ± 0.02*#
Serum lactate (mg/dL)	9.63 ± 0.40	18.69 ± 1.10	8.76 ± 0.52*	8.35 ± 0.52#	9.32 ± 0.48#
Serum lipase (IU/L)	32.52 ± 1.12	28.93 ± 2.01*	35.42 ± 3.91*	31.37 ± 3.11#	36.00 ± 4.91#
Serum LPO (nM/ml)	1.41 ± 0.29	2.42 ± 0.08*	2.14 ± 0.07*#	2.09 ± 0.13*	1.81 ± 0.08*

Tissue LPO (nM/g wet tissue)	1.07 ± 0.02	1.87 ± 0.05*	1.49 ± 0.03*	1.45 ± 0.02*	1.03 ± 0.02*
Serum SOD (mg/dL)	40.38 ± 0.50	33.66 ± 0.00*	37.56 ± 3.58*#	35.22 ± 2.69#	39.60 ± 1.19*
Tissue SOD (U/mg protein)	12.64 ± 0.60	10.90 ± 0.15*	11.66 ± 1.15*	11.13 ± 0.70*	11.98 ± 0.99*
Serum CAT (mg/dL)	32.88 ± 0.17	19.95 ± 0.23*	25.51 ± 0.47*	21.42 ± 0.24*	29.28 ± 0.56*
Tissue CAT (kat/g protein)	24.70 ± 0.31	5.56 ± 0.57*	12.43 ± 0.12*	11.12 ± 0.45*	22.90 ± 0.31*
Serum GPx (mg/dL)	20.37 ± 0.41	19.08 ± 0.31*	19.47 ± 0.86*#	19.28 ± 1.13*	19.98 ± 0.96#
Tissue GPx (nM of GSH oxidize/min/100mg protein)	21.08 ± 0.10	19.86 ± 0.79*	20.78 ± 0.51*#	20.18 ± 1.24*#	21.10 ± 0.07*
Serum GST (mg/dL)	1.44 ± 0.01	0.50 ± 0.04*	1.38 ± 0.02*#	0.78 ± 0.05#	1.34 ± 0.02*#
Tissue GST (nmol of *CDNB conjugated/min/100 mg protein)	1.30 ± 0.07	0.37 ± 0.02*	0.60 ± 0.09#	0.51 ± 0.02	0.97 ± 0.06#
Serum GR (mg/dL)	1.61 ± 0.17	1.10 ± 0.09*	1.38 ± 0.29*	1.15 ± 0.14*	1.47 ± 0.21*
Tissue GR (µkat/g protein)	1.10 ± 0.20	0.59 ± 0.30*	1.01 ± 0.19*	0.86 ± 0.41*	1.01 ± 0.19*#
Serum vitaminC (mg/dL)	1.38 ± 0.00	0.55 ± 0.01*	0.67 ± 0.01*#ECH	0.63 ± 0.01*	1.1 ± 0.03*#
Tissue vitaminC (µg/mg wet tissue)	1.93 ± 0.01	1.16 ± 0.02*	1.67 ± 0.02*	1.45 ± 0.04*#	1.93 ± 0.01*#
Serum vitaminE (mg/dL)	8.38 ± 0.78	5.9 ± 0.37*	6.93 ± 0.58*	6.50 ± 0.41*#	7.77 ± 0.55*#
Tissue Vitamin-E (µ/mg wet tissue)	10.39 ± 0.64	5.47 ± 0.55*	9.19 ± 0.23*#	7.2 ± 0.28*#	10.49 ± 0.57*#
Serum GSH (mg/dL)	21.95 ± 1.81	15.54 ± 0.72*	17.15 ± 2.42*#	15.54± 0.72*	19.74 ± 3.32*#
Tissue GSH (nM of GSH reduced/100 mg protein)	13.12 ± 1.96	10.50 ± 0.81	12.65 ± 1.62*#	10.15 ± 1.45*	11.37 ± 1.31*#

All values are expressed as mean ± SD (n=3) and analyzed by ANOVA followed by an LSD test. * denotes $P < 0.05$ in comparison with normal control group while # denotes $P < 0.05$ in comparison with ISO group

reduced glutathione activities to that of the control levels (Table 3). GSH was the most affected (71%) non-enzymatic antioxidant by ISO and treatment with high dose (100 mg/kg) L-Carvone significantly increased the GSH when compared with the losartan group; however, it was not effective at the low dose (25 mg/kg) (Table 3) .

Histopathological examination

Microscopic examination (×10) of Masson's Trichrome stained (MTS) heart tissue is represented in figure 3(a-e). Cirrhosis leads to a hyperdynamic state, a decrease in systemic vascular resistance, and an increase in cardiac output [35]. However, the cellular architecture was improved by L-Carvone treatment as evidenced by histopathological studies. It indicated that L-Carvone may act by renewing the cardiac cells by increasing the antioxidant activities.

Discussion

From this research study, it is evident that L-Carvone, a monoterpenoid compound, completely matches with its parent compound geraniol, which is a monoterpenoid, that has a protective effect against myocardial infarction (MI) through moderating MI-induced myocardial oxidative stress indicators (glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and *Keap1/Nrf2* pathway [2]. Myocardial enzymes are indicators of heart function, and their release into the bloodstream following ISO administration shows altered plasma membrane integrity caused by sarcolemma injury, which also causes membrane damage [36]. The contribution of dietary antioxidants from plants to the body's defense mechanism against oxidative stress is well known. They defend cells from oxidative stress and thus protect cells in chronic illnesses [37]. *M. spicata* L., thus has antioxidant properties and other health benefits. Although *M. spicata* L., with its primary phytoconstituent L-Carvone, a recommended compound [38] is known for its potential to reduce the risk of CH-causing factors such as diabetic complica-

tions and oxidative stress [39], research on the specific therapeutic effects on CH has not yet been conducted. ECG is employed as a screening approach for cardiac tissue activity. In figure 2, ECG patterns with changes in typical wave tracings have been identified. It represents the prolonged ventricular repolarization, impaired conduction of atrioventricular, and arrhythmias that have been initiated due to myocardial fibrosis associated with ischemia [40]. The main markers of myocardial fibrosis and the net increase in protein synthesis associated with cardiac hypertrophic phenotype are morphometric indices [41]. These types of CH histological markers were linked to an abnormal edematous intramuscular space, necrosis of cardiac myofibrils, and inflammatory cells invading injured tissues. In this study, rats administered ISO showed elevated heart index and HW/Tail length ratios compared with normal rats; however, after simultaneous treatment with L-Carvone, it reverted to normal condition [42]. Thus, animals given L-Carvone had general decreases in HW/BW and HW/Tail length ratios, as shown in table 2.

Constitutive effects of ISO result in inhibition of insulin secretion [43], as demonstrated in this study, which further burdens the cardiac system with energy demand [44]. This worsens the CH-inducing structural and functional changes in cardiac myocytes. These changes have been alleviated by the antihyperglycemic potential of L-Carvone.

Similarly, chronic ISO administration in animal studies reported an increment in the rates of protein synthesis and consequently, the increased total protein content of the heart tissue during CH [45]. The current study confirmed increase in total protein levels in ISO-injected rats. In rats that were given the L-Carvone, it was found to be either reduced or returned to normal values (Table 3), indicating that L-Carvone has the resilience to control the synthesis of protein in CH, as seen in many other clinical conditions such as cancer, obesity, diabetes, anxiety, depression, and in hepatic disorders [46].

LDH and lipid profile increase upon ISO administration due to myocardial damage [47]. Reversal of this myocardial damage and of LDH and lipid profile occurs after L-Carvone treatment. Several cardiac-specific markers i.e., CK-MB, troponin-I, NT-pro BNP, as well as inflammation-specific markers, hs-CRP, and C-peptide, were significantly reverted after simultaneous treatment with L-Carvone. The elevated AST [46] and ALT levels found in this study can be ascribed to the action of ISO because continuous ISO injection leads to necrosis of myocytes and cellular stress in many other organs. Due to the expansion of the edematous intramuscular space after cardiomyocyte swelling and damage, as well as the infiltration of the wounded tissues by protective inflammatory cells,

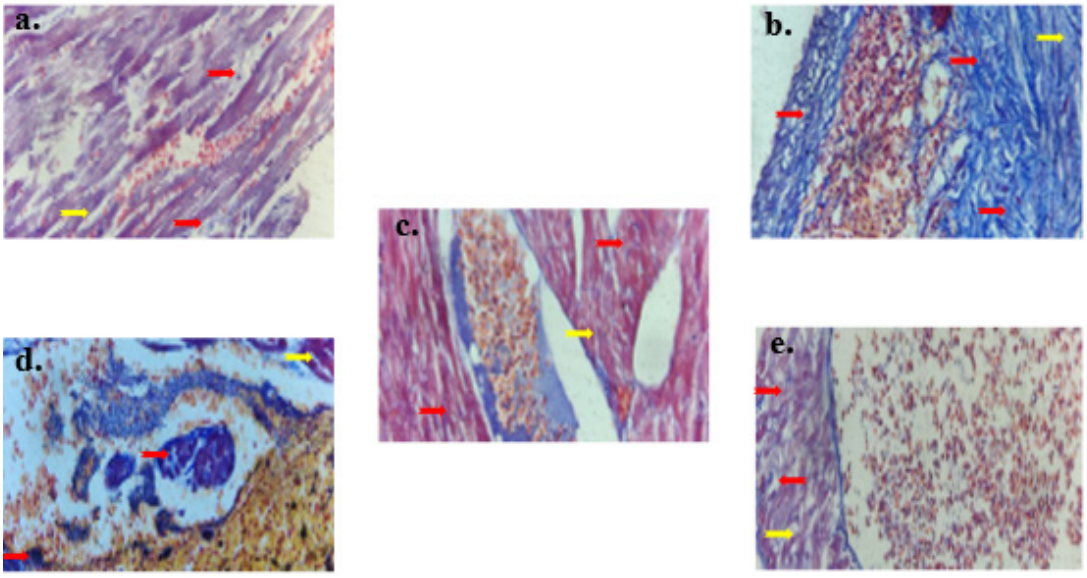

Figure 3. Masson's Trichrome-stained heart transverse sections of rat treated with (**a**) saline showing normal collagen deposition (red), normal contraction bandwidth (yellow) indicating healthy-looking cardiac muscle cells without significant collagen staining around the nuclei and minimal collagen staining indicating healthy cardiac tissue; (**b**) ISO showing an increased number of collagen deposition around the nuclei which appeared more crowded indicating fibrosis and potentially reflecting the hypertrophic response (red), necrosis of contraction band (yellow); (**c**) losartan showing reduced collagen deposition around the nuclei which also appeared less surrounded by collagen fibres (red) & necrosis of contraction band (yellow) suggesting a potential protective effect on the heart tissue; (**d**) low dose (25 mg/kg) showing mild to moderate repairment of collagen deposition (red) & necrosis of contraction band (yellow); (**e**) high dose (100 mg/kg) showing moderate to good repairment of collagen deposition around less crowded nuclei (red) & contraction band necrosis (yellow) indicating protective effect against hypertrophy (MTS, ×400).

the heart weight increases.
Left ventricular hypertrophy (LVH), which is a crucial factor in compromising contractile function and resulting in heart failure [48], manifests as a considerable increase in left ventricular mass with chronic strain. Simultaneous L-Carvone treatment (Table 3) is effective, may be due to its powerful free membrane-stabilizing capabilities brought on by its antioxidant and radical scavenging activity, which inhibit the excessive workload of the myocardium and prevent the leakage of cardiac marker enzymes, thus, PL salvaging myocardial tissues. After ISO injection, serum calcium, sodium, and potassium levels were reported to be similar to the study of Anandan *et al.,* 2015[49], which reported significant reversion after simultaneous treatment with L-Carvone.
The accumulation of thio-barbituric active reactive substance (TBARS) proves that increased PL degradation results in cell death [50]. The lipophilic cell membrane is damaged by excessive free radicals generated by ISO, shown by the enhanced lipid peroxidation in terms of TBARS. An important characteristic in the onset, progression, and occurrence of myocardial infarction (MI) and, associated consequences is an increase in plasma lipid peroxidation. As shown in the data in table 3, levels of free radical decreased after simultaneous treatment with L-Carvone when compared to diseased conditions.
Reduced plasma GSH concentration, an intracellular free-radical scavenger, is believed to be the result of its ability to combat free radicals under oxidative stress brought on by ISO. The fact that vitamins C and E are used more often to combat reactive oxygen species (ROS) is another explanation for their decreased levels [51]. All these antioxidant levels were increased after simultaneous treatment with L-Carvone when compared with the diseased condition (Table 3).
Histologically, as shown in figure 3 (a-e) rats administered ISO showed cardiac degeneration along with myocyte necrosis, apoptosis, and changes in cell membranes, most likely because of the altered glucose and lipid metabolisms. Inflammation and interstitial fibrogenesis originate from increased ROS, which in turn stimulate signaling networks linked to the deposition of extracellular matrix protein (collagen) [52,53]. These altered necrosis bands and collagen depositions were reverted after simultaneous treatment with L-Carvone. Histopathological study revealed that L-Carvone with its antioxidant activities was similar to the results of monoterpene compound 1,8-Cineole treated in an ISO-induced rat model [6]. The biochemical parameters in L-Carvone treated groups exhibited (Table 3) decreased levels of total cholesterols, triglycerides, LDL, VLDL, LDH, CK-MB, troponin-I, hs-CRP, calcium ions, sodium ions and increased levels of HDL, potassium, SOD, CAT, GPx, GST, GR, vitamin C, vitamin E, and GSH. These results precisely match with the research study on thymol, a monoterpene compound in various models of cardiovascular diseases including MI, drug-induced cardiotoxicity, atherosclerosis, hypertension, arrhythmias [7]. Thus, this study may contribute to the potential utilization of L-Carvone in future as a cardioprotective agent against CH, expanding the medicinal value of this natural monoterpenoid. Hence, L-Carvone is one of the most powerful contenders in phytochemicals of natural origin with several pharmacological properties displaying promising preventive and therapeutic properties against various hypertrophic human diseases.

Conclusion

This study shows that ISO-induced CH leads to oxidative stress, which further promotes the initiation and activation of various types of signaling cascades that develop structural and functional damages in different tissues. The current study discovered that simultaneous treatment with L-Carvone in rats exhibited significant protective effect on CH by modifying several antioxidant enzymes and reducing the histopathological injuries of heart tissue. Considering that L-Carvone exhibited cardioprotective potential, these results encourage further exploration and confirmation of oxidative damage preventive effect of L-Carvone in CH.
Further preclinical and clinical studies are needed to analyze and prove the activity of L-Carvone in the antioxidant defense mechanisms in the remission of CH, and in other cardiovascular disorders. Clinical trials with different methodologies are recommended to know the effects of dietary or supplementary L-Carvone dosages.
This current research can be extended by studies on restoration of *Nrf-2* metabolic signaling that leads to cardiac fibrosis. This may help to reveal many kinds of new therapeutic targets and treatment options in CH. Thus, the therapeutic properties of L-Carvone or *M. spicata L*., extract as a vital therapeutic agent responsible for reducing and reverting CH can be further explored.

Conflict of Interests

None.

Acknowledgments

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