Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221−1691.367687

Impact Factor® 1.51

L−carvone attenuates myocardial injury and dyslipidemia in rats with isoproterenol−induced cardiac hypertrophy

Anitha Nagarajan, Victor Arokia Doss✉

Department of Biochemistry, PSG College of Arts and Science, Coimbatore −641 014, Tamil Nadu, India

ABSTRACT

Objective: To explore the therapeutic efficacy of *L*-carvone from *Mentha spicata* L. leaf extracts against isoproterenol-induced cardiac hypertrophy in rats.

Methods: Isoproterenol (5 mg/kg) was injected intraperitoneally into rats for one month to induce cardiac hypertrophy. *L*-carvone (25 and 100 mg/kg) was administered orally to treat cardiac hypertrophy. The cardioprotective activity of *L*-carvone was evaluated by electrocardiogram, histopathological analysis as well as determination of biochemical parameters and enzymatic markers.

Results: *L*-carvone from *Mentha spicata* L. at 25 and 100 mg/kg ameliorated isoproterenol-induced cardiac hypertrophy, as evidenced by reduced QRS interval on electrocardiogram, and decreased heart weight and heart index. In addition, both doses of *L*-carvone markedly lowered the levels of glucose, total protein, low-density lipoprotein cholesterol, aspartate transaminase, alanine transaminase, lactate dehydrogenase, creatine kinase MB, troponin- Ⅰ, *N*-terminal pro-B type natriuretic peptide and triglycerides while increasing high-density lipoprotein cholesterol and lipase level (P<0.05). Moreover, *L*-carvone alleviated contraction band necrosis, and reorganized the myofibrils with normal striations and myocytes as well as normal nuclei in cardiac histoarchitecture of rats with isoproterenol-induced cardiac hypertrophy.

Conclusions: *L*-carvone from *Mentha spicata* L. leaf extract can restore abnormal cardiac function and may be further explored as a therapeutic agent against the deleterious effects of cardiac hypertrophy after further evaluation.

KEYWORDS: Cardiac hypertrophy; *L*-carvone; Dyslipidemia; Myocardial injury; Isoproterenol; *Mentha spicata*

1. Introduction

The number of cardiac deaths was 0.07 million by 2020. In the United States, the estimated direct cost of cardiovascular diseases (CVDs) rose from $103.5 billion in 1996-1997 to $226.2 billion in 2017-2018[1]. All mortality causes are independently associated with left ventricular hypertrophy[2]. Cardiovascular hypertrophy is a cellular response to the rise in biomechanical stress and is a versatile reaction to the tension or volume stress, and transformations of sarcomere and other proteins, causing loss of contractile mass due to localized necrosis. Many types of cardiac illness, including valve dysfunction, ischemia sickness, heart failure, and hypertension, are associated with hypertrophic expansion. Pressure overload-incited

Significance

Mentha spicata L. and its key phytoconstituent *L*-carvone are reported to reduce the risk factors including diabetic complications, oxidative stress, hyperlipidemia, hypertension, and structural and functional alterations in myocytes which can lead to cardiac hypertrophy. The present study demonstrated the therapeutic activity of *L*-carvone as an anti-hypertrophic agent against isoproterenol-induced cardiac hypertrophy by improving electrocardiogram characteristics, biochemical parameters, cardiac marker levels as well as cardiac histoarchitecture.

✉To whom correspondence may be addressed. E-mail: victordoss64@gmail.com

How to cite this article: Nagarajan A, Doss VA. *L*-carvone attenuates myocardial injury and dyslipidemia in rats with isoproterenol-induced cardiac hypertrophy. Asian Pac J Trop Biomed 2023; 13(1): 17-25.

Article history: Received 27 October 2022; Revision 24 November 2022; Accepted 21 December 2022; Available online 20 January 2023

concentric hypertrophy plays a compensating effect in certain cardiac diseases by lowering wall stress and oxygen utilization. Simultaneously, ventricular hypertrophy is the cause of expanded danger of harmful arrhythmia and cardiovascular breakdown[3]. A β-adrenergic receptor (β-AR) agonist isoproterenol produces concomitant activation of β-AR causing cardiac hypertrophy in animal models. The plant kingdom offers huge benefits for human beings and other living things on the earth. Several medicinal plant treatments have restorative effects on certain cardiac enzyme activities[4]. Generally, *Mentha spicata* (*M. spicata*) L. contains vital components. The major monoterpenoid found in *M. spicata* L. was *L*-carvone which is involved in reduction of the above risk factors and helps to secrete insulin[5]. Hence, this study aimed to evaluate the effect of *L*-carvone, an active compound of *M. spicata* L. on isoproterenol-induced cardiac hypertrophy.

2. Materials and methods

2.1. Chemicals and kits

Drugs and chemicals purchased from Hi-Media Laboratories, India were of analytical grade. Isoproterenol was purchased from Sigma-Aldrich. All kits used in the study were bought from Arkray Healthcare Pvt, Ltd., India.

2.2. Extraction of L-carvone

M. spicata L. leaves were shade dried and powdered coarsely. Powdered sample was dissolved in a hydroethanolic solution (50:50) and kept in a rotatory shaker for 72 h. After 72 h, the whole leaf extract was obtained by condensation method using hot water bath. Later, *L*-carvone was fractioned from *M. spicata* L. leaf crude extract by adsorption column chromatography[6]. Eluate fractions numbering 3-9 were pooled together and concentrated by hot water bath condensation process, dispensed in a hydroethanolic solution (50:50) and stored in a refrigerator for further treatment.

FTIR spectra were recorded on an FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} using ethanol as a solvent. FTIR spectra were obtained from the research center of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, India.

2.3. Animals

Male albino Wistar rats (150-250 g) were procured from animal house of PSG Institute of Medical Sciences and Research Coimbatore, India and subjected to the laboratory conditions of 25℃, a 12/12 h light-dark cycle, with 50%-60% relative humidity, and had free access to normal chow and water for one week before the experiments. The experimental protocols were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India for the care of laboratory animals.

2.3.1. Induction and treatment of experimental rats

Rats were separated into four groups of six animals in each group. Treatment was given for 30 d. Group Ⅰ received a regular chow diet and served as the normal control group. Group Ⅱ as the negative control was administered with isoproterenol [5 mg/kg body weight (b.w.)] intraperitoneally suspended in saline[7]. Isoproterenol-induced rats in group Ⅲ and Ⅳ were orally treated with a hydro-ethanolic solution of *L*-carvone (25 and 100 mg/kg b.w.), respectively[8].

2.3.2. Blood sampling and tissue collection

Rats were fasted before drawing the blood for blood sampling and tissue collection. After the experimental treatment at the end of 30 d, rats were sacrificed under anesthesia. By cardiac puncture method, the blood was collected and let stand for 30 min at room temperature and centrifuged at 5000 rpm at 4℃ for 20 min to obtain the serum. Separated plasma or serum was stored at −20℃ in microcentrifuge tubes for further work. Subsequently, the heart was excised and fixed in 10% neutral buffered formalin for histopathological study.

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

Compounds	Frequency (cm^{-1})	Reference frequency (cm^{-1})	Functional group	Intensity
Alkyl	2924.09	2925	C-H	Medium to strong
Aromatic	1435.04	1450	C=C	Weak to strong
Aromatic nitro compounds	1365.60	1350	N-O	Medium
Aromatic ethers	1242.16	1220-1260	C-O	Medium
Esters	1141.86	1100-1300	C-O	Two bands (distinct from ketones, which do not possess a C-O bond)
Secondary alcohols	1111.00	~1100	C-O	Strong
Primary alcohols	1056.99	1040-1060	C-O	Strong & broad
Alkene	964.41	965	C-H	Strong
Aromatic benzene	894.97	860-900	C-H	Strong
Aromatic benzene	802.39	800-860	C-H	Strong
Aromatic benzene	702.09	700-750	C-H	Strong

2.3.3. Electrocardiographic (ECG) analysis of cardiac hypertrophy

In unanesthetized rats, ECG analysis was performed using BITalino ECG Sensor equipment for 8 min to monitor the cardiac functions after 4 weeks at the conventional bipolar limb lead Ⅱ, *via* Open Signals [r]evolution software. The changes in pulse/heart rate (HR) QRS complex, R-amplitude, and R-R interval were recorded.

2.3.4. Indices of cardiac hypertrophy

Cardiac hypertrophy was assessed using the hypertrophic indices including heart weight (HW), body weight (BW), heart index (HW/BW×100) and heart weight/tibial length (HW/TL)[9].

2.3.5. Biochemical analysis

Serum glucose was assayed by using glucose oxidase method (AUTOSPAN Liquid Gold Glucose Kit). Modified Biuret End Point Assay (AUTOSPAN Liquid Gold Total Protein Kit) was used for determination of serum total protein. In addition, serum albumin was measured using Bromocresol green end point assay method (AUTOSPAN Liquid Gold Albumin Kit), cholesterol by CHOD-PAP enzymatic end point assay (AUTOSPAN Liquid Gold Cholesterol Kit), triglycerides by GPO-PAP enzymatic end point assay (AUTOSPAN Liquid Gold Triglyceride Kit), high-density lipoprotein cholesterol (HDL-C) by accelerator selective detergent enzymatic end point assay (AUTOSPAN Liquid Gold Direct HDL Cholesterol Kit), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) by Friedewald equation, aspartate transaminase (AST) by modified UV (IFCC) kinetic assay method (AUTOSPAN Liquid Gold AST Kit), alanine transaminase (ALT) by modified UV (IFCC) kinetic assay method (AUTOSPAN Liquid Gold ALT Kit), and lactate dehydrogenase (LDH) activity by optimized DGKC kinetic assay method (AUTOSPAN Liquid Gold LDH Kit), creatine kinase MB (CKMB), troponin-Ⅰ, and *N*-terminal pro-B type natriuretic peptide (NT-pro BNP) by Acculite CLIA test kit method, followed by estimations of serum phospholipids[10], lipase[11], and calcium[12], as well as tissue pyruvate[13] and lactate[14].

2.3.6. Histopathological analysis

Storage of excised heart was done in 10% formalin and then in paraffin, and the tissues were cut into 5 μm thin sections. Haematoxylin and eosin (H & E) were used to stain these sections. Later, all slides were evaluated for heart architecture under ×40 magnification.

2.4. Statistical analysis

Results were expressed as mean±standard deviation (SD). ANOVA and least significant difference (LSD) test[10] were used to analyze the significant difference between the groups using SPSS Statistics 23.0 software (SPSS Inc., USA). *P*-values less than 0.05 were considered significantly different.

2.5. Ethical statement

The study was approved by Institutional Animal Ethics Committee, (SCEA/NO.520/IAEC/2021) PSG Institute of Medical Sciences and Research Coimbatore, India.

3. Results

3.1. FTIR spectrum of L-carvone

Figure 1 shows the characteristic spectrum of *L*-carvone by FTIR spectroscopy. The compounds and their peaks are reported in Table

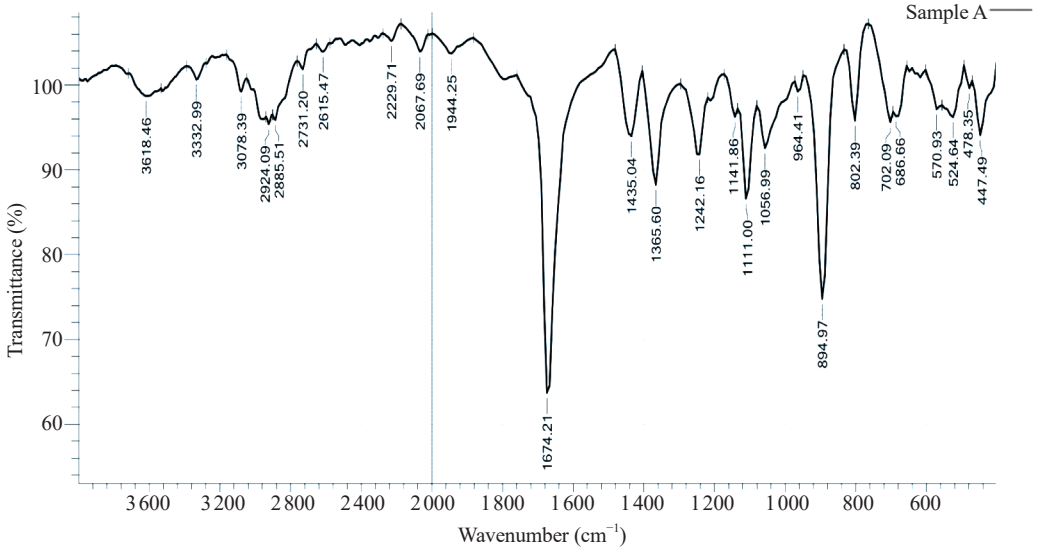

Figure 1. FTIR spectrum of isolated terpenoid compound *L*-carvone.

1. The results showed the presence of functional groups in *L*-carvone such as alkyl compound (2 924.09 cm^{-1}), aromatic compounds (1 435.04 cm^{-1}), aromatic ethers (1 242.16 cm^{-1}), secondary alcohols (1 111.00 cm^{-1}), and aromatic benzene (802.39 cm^{-1}), confirming that the isolated *L*-carvone was similar to standard *L*-carvone compound.

3.2. ECG analysis

As shown in Figure 2, the prolonged QRS interval, depressed S-T wave, and long PR interval accompanied by long QT interval and increased R-R interval were observed in rats with isoproterenol-induced cardiac hypertrophy. Treatment with *L*-carvone abolished all these typical characteristic ECG changes, indicating the cardioprotective activity of *L*-carvone.

3.3. Effect of L-carvone on indices of cardiac hypertrophy

As shown in Table 2, compared to the normal control group, the isoproterenol group showed significantly increased indices of cardiac hypertrophy including heart index and HW/TL ratio (P<0.05). *L*-carvone at a high dose significantly lowered the isoproterenol-induced indices of cardiac hypertrophy (P<0.05).

3.4. Effect of L-carvone on serum glucose, total protein, and albumin levels

Isoproterenol administration significantly increased the levels of serum glucose and total protein compared to the control group (P<0.05). Treatment with low and high doses of *L*-carvone reduced the increased levels of glucose and total protein to normal levels as indicated in Table 3 (P<0.05). Furthermore, isoproterenol reduced the level of serum albumin, which was increased by treatment with *L*-carvone with no significant difference (Table 3).

3.5. Effect of L-carvone on serum lipid profile

Lipid parameters including serum total cholesterol, triglycerides, and phospholipids were increased significantly in isoproterenol-induced cardiac hypertrophy groups. In contrast, *L*-carvone significantly decreased these parameters to near-normal levels (P<0.05) (Table 3). In

Table 2. Effect of *L*-carvone on indices of cardiac hypertrophy.

Indices	Control	Isoproterenol	*L*-carvone (25 mg/kg)	*L*-carvone (100 mg/kg)
Body weight (g)	203.33 ± 5.77	200.00 ± 17.32	193.33 ± 11.55	213.33 ± 15.28
Heart weight (mg)	870.00 ± 26.46	1 146.67 ± 72.34*	990.00 ± 88.88$^{\#}$	1 026.67 ± 23.09$^{*\#}$
Heart index	406.94 ± 18.29	531.85 ± 20.53*	475.64 ± 46.75	428.33 ± 24.66$^{\#}$
Heart weight/tibial length	48.72 ± 0.86	56.44 ± 1.28*	56.02 ± 1.58*	50.55 ± 0.56$^{*\#}$

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

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

Parameters	Control	Isoproterenol	*L*-carvone (25 mg/kg)	*L*-carvone (100 mg/kg)
Serum glucose (mg/dL)	89.09 ± 1.13	155.82 ± 1.45*	142.40 ± 0.93$^{*\#}$	105.38 ± 0.14$^{*\#}$
Serum total protein (g/dL)	2.37 ± 0.02	4.52 ± 0.11*	2.77 ± 0.04$^{*\#}$	2.72 ± 0.04$^{*\#}$
Serum albumin (g/dL)	2.20 ± 0.06	1.87 ± 0.05	2.09 ± 0.04	2.12 ± 0.10
Serum cholesterol (mg/dL)	75.04 ± 6.06	113.23 ± 4.75*	75.66 ± 5.80$^{\#}$	65.99 ± 8.19$^{\#}$
Serum triglycerides (mg/dL)	137.78 ± 7.70	197.78 ± 10.18*	188.89 ± 3.85$^{*\#}$	157.78 ± 3.85$^{\#}$
Serum phospholipids (mg/dL)	173.33 ± 5.77	203.33 ± 11.55*	198.33 ± 10.41*	181.67 ± 12.58$^{\#}$
Serum HDL-C (mg/dL)	24.01 ± 3.11	13.46 ± 0.50*	20.28 ± 1.62$^{\#}$	20.42 ± 1.75$^{\#}$
Serum LDL-C (mg/dL)	19.20 ± 1.11	20.39 ± 1.27*	11.85 ± 0.19$^{*\#}$	13.56 ± 0.23$^{*\#}$
Serum VLDL-C (mg/dL)	27.11 ± 0.20	49.78 ± 1.82*	36.00 ± 0.35$^{*\#}$	30.67 ± 0.11$^{*\#}$
Serum AST (IU/L)	57.68 ± 5.71	89.77 ± 6.30*	75.99 ±1.94$^{*\#}$	70.15 ± 3.69$^{*\#}$
Serum ALT (IU/L)	64.37 ± 2.55	89.44 ± 0.99*	69.55 ± 0.83$^{*\#}$	65.69 ± 1.69$^{\#}$
Serum LDH (IU/L)	38.88 ± 1.13	49.68 ± 1.57*	42.32 ± 1.16*	37.42 ± 1.09$^{*\#}$
Serum lipase (IU/L)	27.19 ± 0.10	20.83 ± 0.35*	21.41 ± 0.44$^{\#}$	26.04 ± 0.17$^{\#}$
Serum CKMB (IU/L)	269.83 ± 0.76	340.33 ± 0.57*	300.00 ± 0.20$^{*\#}$	297.03 ± 0.25$^{*\#}$
Serum troponin-Ⅰ (pg/mL)	38.60 ± 0.27	47.13 ± 0.35*	42.13 ± 0.35$^{*\#}$	36.40 ± 0.40$^{*\#}$
Serum NT-pro BNP (pg/μL)	3 899.73 ± 0.64	5 200.00 ± 0.70*	4 399.67 ± 0.29$^{*\#}$	3 699.67 ± 0.31$^{*\#}$
Serum calcium (mg/dL)	10.40 ± 0.35	23.00 ± 2.25*	16.43 ± 0.98$^{*\#}$	14.17 ± 1.70$^{*\#}$
Tissue pyruvate (mg/100 g)	0.59 ± 0.00	0.92 ± 0.03*	0.69 ± 0.04$^{*\#}$	0.45 ± 0.01$^{*\#}$
Tissue lactate (mg/100 g)	5.47 ± 0.23	6.25 ± 0.18*	6.04 ± 0.31$^{\#}$	5.24 ± 0.45$^{\#}$

All values are expressed as mean ± SD (n=3) and analyzed by ANOVA followed by LSD test. * denotes P<0.05 compared with the normal control group while $^{\#}$ denotes P<0.05 compared with the isoproterenol group. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; AST: aspartate transaminase; ALT: alanine transaminase; LDH: lactate dehydrogenase; CKMB: creatine kinase MB; NT-pro BNP: *N*-terminal pro-B type natriuretic peptide.

addition, HDL-C was significantly reduced in the isoproterenol group, which was restored to normal levels after treatment with *L*-carvone as shown in Table 3, whereas LDL-C and VLDL-C levels that were significantly elevated by isoproterenol administration were significantly decreased after treatment with *L*-carvone (P<0.05).

3.6. Effect of L-carvone on cardiac enzymes

According to Table 3, the activities of AST, ALT, LDH, CKMB, troponin-Ⅰ, and NT-proBNP were significantly increased in the isoproterenol group (P<0.05). *L*-carvone treatment prominently reduced these enzyme activities (P<0.05). In addition, isoproterenol administration decreased serum lipase level compared to the normal control group. Treatment with *L*-carvone could abrogate the reducing effect of isoproterenol on lipase level.

3.7. Effect of L-carvone on serum calcium as well as tissue pyruvate and lactate levels

As shown in Table 3, isoproterenol resulted in a significant increase in serum calcium, as well as tissue pyruvate and lactate levels. Treatment with low and high doses of *L*-carvone decreased these levels significantly (P<0.05).

3.8. Histopathological examination

H&E-stained heart tissues are represented in Figure 3. The isoproterenol group demonstrated separation of cardiac myofibrils, and lack of intact cardiomyocyte array that was represented by distorted striations. In contrast, treatment with 25 mg/kg of *L*-carvone resulted in mild to moderate rejuvenation of contraction band

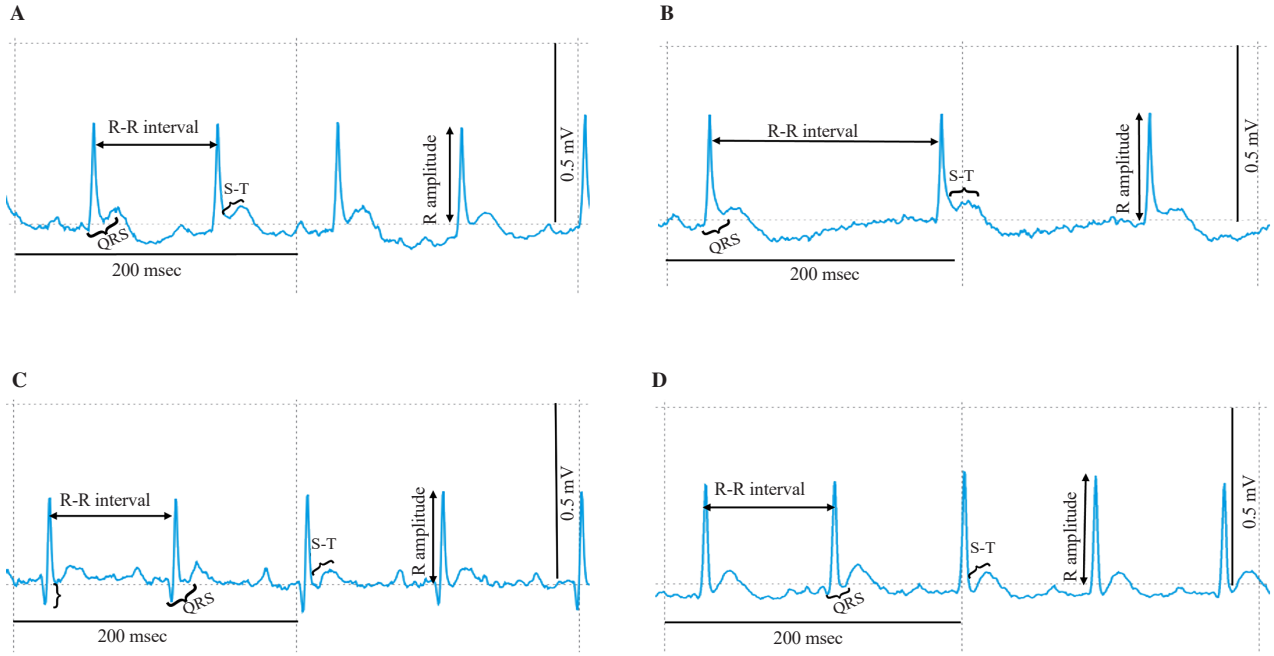

Figure 2. Graphical representation of the real time ECG tracings showing the effect of *L*-carvone from *Mentha spicata* L. leaf extracts. (A) The normal control group: normal QRS interval, R-R interval and S-T wave; (B) the isoproterenol group: increased QRS interval and R-R interval and depressed S-T wave; (C) the group treated with 25 mg/kg of *L*-carvone: restored QRS interval, R-R interval and S-T wave; (D) the group treated with 100 mg/kg of *L*-carvone: restored QRS interval, R-R interval and S-T wave.

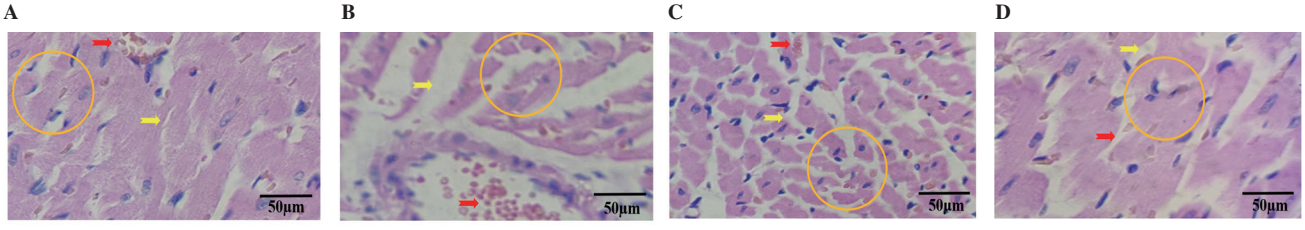

Figure 3. Histopathological analysis of heart tissues in isoproterenol administered rats treated with *L*-carvone. (A) The sections from the heart of the normal control group show normal contraction band (yellow arrow), organization of the myofibrils with normal striations and myocytes (red arrow), as well as normal nuclei (circle). (B) The isoproterenol group shows contraction band necrosis (yellow arrow), disorganization of the myofibrils with loss of striations, focal degenerating myocytes (red arrow), as well as loss of nuclei (circle). (C) The group treated with 25 mg/kg of *L*-carvone shows mild to moderate repairment of contraction band necrosis (yellow arrow), reorganization of the myofibrils with normal striations and myocytes (red arrow) as well as normal nuclei (circle). (D) The group treated with 100 mg/kg of *L*-carvone shows alleviated contraction band necrosis (yellow arrow), reorganization of the myofibrils with normal striations and myocytes (red arrow) as well as normal nuclei (circle) (scale bar=50 μm, H&E ×40).

necrosis as well as reorganized myofibrils with normal striations and myocytes. Moreover, 100 mg/kg of *L*-carvone improved the cardiac tissue architecture similar to normal cardiac tissues *via* alleviation of contraction band necrosis, and reorganization of normal striations in myofibrils and normal nuclei in myocytes.

4. Discussion

M. spicata L. and its key phytoconstituent *L*-carvone are reported to reduce the risk factors including diabetic complications[9,15], oxidative stress[15], hyperlipidemia[16], and hypertension[17], which can further lead to cardiac hypertrophy. However, their therapeutic activity as an anti-hypertrophic agent has not been known so far.

For evaluating heart function, ECG has been used[9]. According to the findings, ECG wave patterns illustrate isoproterenol-induced changes such as increased QRS, R-R, and long QT intervals. Improved ECG was noticed after treatment with *L*-carvone. Similar ECG tracings with altered wave patterns were reported earlier[18]. These alterations were induced by myocardial fibrosis, which may be the cause of ischemia. They are signs of poor atrioventricular conduction, extended ventricular repolarization, and arrhythmias with lowered heart rate (bpm). In contrast, the groups receiving *L*-carvone reversed isoproterenol-induced these modifications. The morphometric parameters are the key predictors of the cardiac hypertrophy phenotype, which is characterized by myocardial fibrosis and a net increase in protein production[19]. In our study, the isoproterenol-administered rats had increased heart index and HW/TL ratio, both of which were reduced after treatment with *L*-carvone.

When isoproterenol is administered, insulin production is inhibited[20], which causes hyperglycemia and additional energy demands on the cardiovascular system[21], and exacerbates ventricular hypertrophy by affecting structural and functional alterations in cardiac myocytes. These alterations were abrogated by *L*-carvone that regulated the activities of key enzymes involved in carbohydrate metabolism in diabetic rats induced by streptozotocin[15]. Additionally, chronic administration (85 mg/kg b.w.) of isoproterenol for 30 d induced cardiac hypertrophy in rats, and elevated serum total protein level and total protein content of the heart were reported[19] due to an increase in protein synthesis. *L*-carvone lowered the level of total protein, which was similar to the findings of Muruganathan *et al.*[15] in which oral treatment of carvone lowered elevated HbA1c levels while improving glycemic control, resulting in reduced protein glycation.

According to the findings, isoproterenol is the strongest binding partner of serum albumin (in a non-saturable manner), which causes adverse myocardial damage due to the decreased antioxidant potential of albumin[19] and is therefore thought to be a high-risk factor for cardiac hypertrophy, particularly in the case of left ventricular hypertrophy[22]. According to the study of Lasrado *et al.*[23], dry spearmint (*M. spicata* L.) extract has the potential to elevate the reduced albumin levels and the same result was seen in our study. Meeran *et al*. pointed out that lipid accretion in the bloodstream is a significant risk factor for myocardial infarction[24]. This could be induced by the quick free fatty acid mobilization from fat reserves, which is linked to cardiac deterioration. Additionally, one of the main causes of the elevated myocardial cholesterol level in isoproterenol-induced myocardial damage is accelerated lipid production. In this way, after treatment with *L*-carvone in isoproterenol-administered rats, lipid accumulation was totally reverted which may be by regulating insulin-induced genes as seen similarly in the study of Muruganathan *et al*[15]. Maintenance of cholesterol balance is vital in preventing CVDs[24]. The progression of CVDs is due to cholesterol accumulation triggered by atherogenesis. In the present study, *L*-carvone treatment lowered the increased level of cholesterol induced by isoproterenol. This may be due to the intervention of carvone in cyclic adenosine monophosphate (cAMP) pathway as mentioned in the study of Kang *et al*[25]. Furthermore, the expressions of genes involved in lipid production and transport in the liver *viz.*, *PPARγ*, *SCD1*, CD36 were found to be decreased by the action of carvone as reported by Alsanea and Liu[26]. A key strategy for preventing myocardial infarction is to keep cellular cholesterol in a stable state. Cholesterol content of the myocardium is increased in isoproterenol-administered rats as a result of increased uptake of LDL-C from the blood by the myocardial membranes. *L*-carvone treartment reversed these changes, similar to the findings of Zein *et al*[16]. Their study reported an increase in the level of the protein INSIG (insulin-induced gene) regulates the expression of sterol regulatory element-bound proteins, which regulates the LDL receptors expression, allowing the hepatocyte to remove cholesterol from LDL particles. Increased free cholesterol in the myocardium affects ionic permeability, membrane fluidity and the activities of membrane-bound enzymes that promote breakdown of phospholipids[27]. Thus, in isoproterenol-treated rats, cholesterol and phospholipid levels were increased and treatment with *L*-carvone significantly reduced the levels of cholesterol and phospholipid.

The increased triglyceride levels during myocardial infarction due to an increased fatty acid flow and ineffective removal of plasma VLDL from circulation[28], may be caused by a reduction in FFA oxidation. This may result in myocardial damage because reduced cardiac function causes irregular heartbeats and a loss of heart function synergy[24]. In line with a recent study[16] which revealed that the carvone group decreased the lipid profile further than the diabetic hyperlipidemic group. In this study, *L*-carvone had lower levels of cholesterol, triglycerides, and LDL-C levels. Thus, our results are consistent with a previous research showing that carvone prevents weight gain and fat deposition in the liver[26].

Low HDL-C levels and high blood cholesterol levels, especially LDL and VLDL-C, are significant risk factors for the development of CVDs[29]. Inhibiting the absorption of LDL by the artery wall and facilitating the transport of cholesterol to the liver, where it is processed and expelled from the body, are two of HDL-C's active roles in the reverse transport of cholesterol[30]. According to our research, *L*-carvone treatment lowered the risk of myocardial infarction by reducing the total cholesterol/HDL-C ratio in isoproterenol-induced myocardial infarction, as demonstrated by its efficiency in controlling the insulin-induced genes in diabetes-induced myocardial infarction[16]. Sasikumar and Devi[31] pointed out the possible β-adrenergic-induced stimulation of cAMP. This may bring positive inotrophic and chronotropic effects on the myocardium, resulting in regulation of lipid metabolism.

According to the findings of the present study, chronic isoproterenol administration causes cellular stress and necrosis in myocytes and other organs as well as elevated blood AST and ALT levels[32]. In streptozotocin induced diabetic rats[15] and rats with nicotine-induced liver toxicity[30], increased LDH levels were reversed after treatment with *M. spicata* L. Similar to these studies, in our study, *L*-carvone treatment ameliorated the increased serum LDH levels. Both reversible and irreversible ischemia is associated with cardiac troponin and NT-pro BNP[33]. We may understand that *L*-carvone prevented the leakage of cardiac enzymes (specific markers), and recovered myocardial tissues by preventing the myocardium's excessive workload because of its potent membrane-stabilizing properties. It has also been suggested that *L*-carvone may restore lipase secretion in cardiac cells by increasing lipolysis metabolism.

A previous study[34] showed that the intracellular lipid deposition may act as a substrate for lipid peroxidation, and the byproducts can degrade intracellular proteins and compromise the integrity of the biomembranes which impair ion transport. Intra and intercellular calcium (Ca^{2+}) play a very vital role in cardiac hypertrophy[35] which may be controlled by carvone treatment as seen in the work of Lasrado *et al*[23]. Isoproterenol injection decreased myocardial Ca^{2+} levels with a concominat increase in the serum. *L*-carvone treatment lowered the increased Ca^{2+} levels to normal levels, similar to the report of Lasrado *et al*[23]. Besides, *L*-carvone reduced Ca^{2+} entry through L-type Ca^{2+} channels and showed promising antiarrhythmic activity in the rat hearts[36].

Previous studies have suggested that ventricular hypertrophy and hypoxia cause elevated lactate levels[37]. In this study, isoproterenol significantly increased lactate levels in rats. *L*-carvone treatment can reverse this change. In histological study, myocardial degeneration was found in isoproterenol-treated rats, along with myocyte necrosis, apoptosis, and changed cell membranes, most likely due to altered glucose and lipid metabolisms[38–40]. Inflammation and interstitial fibrogenesis are the results of increased reactive oxygen species, which in turn stimulate signaling networks linked to the deposition of extracellular matrix protein (ideally collagen). The histological indicators of cardiac hypertrophy are related to the adverse oedematous intramuscular space and necrosis of cardiac myofibrils which is followed by inflammatory cell invasion of injured tissues. *L*-carvone treatment could ameliorate these histological changes. Muruganathan *et al*.[15] proved that the histopathological impairment and degenerative changes in the liver and pancreas observed in hyperglycemia were ameliorated after treatment with carvone in streptozotocin-induced diabetic rats. Dry spearmint extract in both *in vitro* and *in vivo* studies of Lasrado *et al*.[23] showed better results in various organs. Similarly, better results were seen in the histopathological appearance of the pancreas of the alloxan-induced diabetic hyperlipidemic rats[16]. *M. spicata* L. aqueous extract showed prominent improvement in hepatic histoarchitecture in nicotine-induced liver toxicity[30].

The study has some limitations. The anti-hypertrophic potential of *L*-carvone from *M. spicata* L. leaves was evaluated only after a month-long treatment. Hence, periodic studies with in-depth parameters are recommended for understanding the pathophysiology of cardiac hypertrophy more clearly.

In conclusion, the biochemical and physiological investigations prove the cardioprotective effects of *L*-carvone against isoproterenol-induced cardiac hypertrophy in rats through regulatory effects on glucose, protein, lipid profiles, and cardiac markers, which are the key fundamental elements for normal cardiac metabolism. Antihyperlipidemic or hypolipidemic capabilities of *L*-carvone are thought to be the cause of its protective effects on lipids and lipoproteins. The ECG and histopathological outcomes further corroborate the favorable cardioprotective effect of *L*-carvone. Overall, *L*-carvone may be further explored as a possible option for the treatment of cardiac hypertrophy and its complications. Further studies are needed to understand the underlying mechanisms of the cardioprotective action of *L*-carvone.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

We acknowledge the PSG Institute of Medical Sciences and Research and PSG Institutional Animal Ethics Committee, Coimbatore, India, and the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India for providing

animal ethical clearance. We express gratitude to the services rendered by Ponmani & Co Pvt. Ltd., Coimbatore for the supply of isoproterenol from Sigma-Aldrich, USA. We also want to thank Arkray Healthcare Pvt Ltd., India for providing us with the kits and Prakash Diagnostic Laboratory and Histotech services, Bangalore for the histopathological slides.

Funding

The authors received no extramural funding for the study.

Authors' contributions

VAD contributed to conceptional design. AN performed the data collection and analysis and drafted the article. Interpretation, critical revision and final approval of the manuscript was done by VAD.

References

[1] Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, et al. Heart disease and stroke statistics—2022 update: A report from the American Heart Association. *Circulation* 2022; **145**: 153-639.

[2] Movahed MR. Left ventricular hypertrophy is independently associated with all-cause mortality. *Am J Cardiovasc Dis* 2022; **12**(1): 38-41.

[3] Simpson LJ, Reader JS, Tzima E. Mechanical regulation of protein translation in the cardiovascular system. *Front Cell Dev Biol* 2020; **8**: 34.

[4] Shah SMA, Akram M, Riaz M, Munir N, Rasool G. Cardioprotective potential of plant-derived molecules: A scientific and medicinal approach. *Dose Response* 2019; **17**(2). doi: 10.1177/1559325819852243.

[5] Menyiy MN, Mrabti HN, Omari NE, Bakili AE, Bakrim S, Mekkaoui M, et al. Medicinal uses, phytochemistry, pharmacology, and toxicology of *Mentha spicata*. *Evid Based Complement Alternat Med*; 2022. doi: 10.1155/2022/7990508.

[6] Elmastaş M, Dermirtas I, Isildak O, Aboul-Enein HY. Antioxidant activity of S-Carvone isolated from spearmint (*Mentha spicata* L. Fam Lamiaceae). *J Liq Chromatogr Relat Technol* 2006; **29**(10): 1465-1475.

[7] Ennis IL, Escudero EM, Console GM, Camihort G, Dumm CG, Seidler RW, et al. Regression of isoproterenol-induced cardiac hypertrophy by Na^+/H^+ exchanger inhibition. *Hypertension* 2003; **41**(6): 1324-1329.

[8] Muruganathan U, Srinivasan S, Indumathi D. Antihyperglycemic effect of carvone: Effect on the levels of glycoprotein components in streptozotocin-induced diabetic rats. *J Acute Dis* 2013; **2**(4): 310-315.

[9] Dharaniyambigai K, Doss VA. Identification of serum predictors of *n*-acetyl-l-cysteine and isoproterenol induced remodelling in cardiac hypertrophy. *Turk J Biol* 2021; **45**: 323-332.

[10]Mahajan L, Saxena S, Sarma PU. Phosphorus compounds: Their discovery in biological world. *Indian J Clin Biochem* 2018; **33**(3): 243-245.

[11]Swain HS, Das BK, Upadhyay A, Ramteke MH, Kumar V, Meena DK, et al. Stocking density mediated stress modulates growth attributes in cage reared *Labeo rohita* (Hamilton) using multifarious biomarker approach. *Sci Rep* 2022; **12**: 9869.

[12]Solanki A, Agarwal P. Comprehensive analysis of changes in clinically significant divalent serum cation levels during automated plateletpheresis in healthy donors in a tertiary care center in North India. *Asian J Transfus Sci* 2015; **9**: 124-128.

[13]Moraes FD, Rossi PA, Figueiredo JSL, Venturini FP, Cortella LRX, Moraes G. Metabolic responses of channel catfish (*Ictalurus punctatus*) exposed to phenol and post-exposure recovery. *An Acad Bras Cienc* 2016; **88**(2): 865-875.

[14]Ahmadi F, Lee WH, Oh YK, Park K, Kwak WS. Fruit and vegetable discards preserved with sodium metabisulfite as a high-moisture ingredient in total mixed ration for ruminants: Effect on *in vitro* ruminal fermentation and *in vivo* metabolism. *Asian Australas J Anim Sci* 2020; **33**: 446-455.

[15]Muruganathan U, Srinivasan S. Beneficial effect of carvone, a dietary monoterpene ameliorates hyperglycemia by regulating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. *Biomed Pharmacother* 2016; **84**: 1558-1567.

[16]Zein N, Shehata M, Amer AM. Carvone's hypoglycemic and hypolipidemic potent activity *via* regulation insulin-induced genes in diabetic hyperlipidemic rats. *Biointerface Res Appl Chem* 2023; **13**(3): 206.

[17]Rajeshwari T, Raja B. Antioxidant and free radical scavenging effect of *D*-carvone in hypertensive rats. *In vivo* and *in vitro* study. *Int Lett Nat Sci* 2015; **8**: 6-12.

[18]Mesquita TRR, de Jesus ICG, dos Santos JF, de Almeida GKM, de Vasconcelos CML, Guatimosism S, et al. Cardioprotective action of *Ginkgo biloba* extract against sustained β-adrenergic stimulation occurs *via* activation of M_2/NO pathway. *Front Pharmacol* 2017; **8**: 220.

[19]Lobo RO, Shenoy CK. Myocardial potency of bio-tea against isoproterenol induced myocardial damage in rats. *J Food Sci Technol* 2015; **52**(7): 4491-4498.

[20]Zhang J, Knapton A, Lipshultz SE, Weaver JL, Herman EH. Isoproterenol-induced cardiotoxicity in Sprague-Dawley rats correlation of reversible and irreversible myocardial injury with release of cardiac troponin T and roles of iNOS in myocardial injury. *Toxicol Pathol* 2008; **36**: 277-288.

[21]Chang GR, Chen WK, Hou PH, Mao FC. Isoproterenol exacerbates hyperglycaemia and modulates chromium distribution in mice fed with a high fat diet. *J Trace Elem Med Biol* 2017; **44**: 315-321.

[22]Moon KH, Song IS, Yang WS, Shin YT, Kim SB, Song JK, et al. Hypoalbuminemia as a risk factor for progressive left-ventricular hypertrophy in haemodialysis patients. *AMJ Nephrol* 2000; **20**(5): 396-401.

[23]Lasrado JA, Trinker D, Ceddia MA, Herrlinger KA. The safety of a dry spearmint extract *in vitro* and *in vivo*. *Regul Toxicol Pharmacol* 2015; **71**(2): 213-224.

[24]Meeran MFN, Jagadeesh GS, Selvaraj P. Thymol attenuates altered lipid metabolism in β-adrenergic agonist induced myocardial infarcted rats by inhibiting tachycardia, altered electrocardiogram, apoptosis and cardiac hypertrophy. *J Funct Foods* 2015; **14**: 51-62.

[25]Kang W, Choi D, Park S, Park T. Carvone decreases melanin content by inhibiting melanoma cell proliferation *via* the Cyclic Adenosine Monophosphate (cAMP) pathway. *Molecules* 2020; **25**(21): 5191.

[26]Alsanea S, Liu D. BITC and S-Carvone restrain high-fat diet-induced obesity and ameliorate hepatic steatosis and insulin resistance. *Pharm Res* 2017; **34**(11): 2241-2249.

[27]Dhivya V, Priya LB, Chirayil HT, Sathiskumar S, Huang CY, Padma VV. Piperine modulates isoproterenol induced myocardial ischemia through antioxidant and anti-dyslipidemic effect in male Wistar rats. *Biomed Pharmacother* 2017; **87**: 705-713.

[28]Kannan MM, Quine SD. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. *Metabolism* 2013; **62**: 52-61.

[29]Gunathilake KDPP, Wang Y, Rupasinghe HV. Hypocholesterolemic and hypotensive effects of a fruit-based functional beverage in spontaneously hypertensive rats fed with cholesterol-rich diet. *J Funct Foods* 2013; **5**: 1392-1401.

[30]Ben Saad A, Rjeibi I, Alimi H, Ncib S, Bouhamda T, Zouari N. Protective effects of *Mentha spicata* against nicotine-induced toxicity in liver and erythrocytes of Wistar rats. *Appl Physiol Nutr Metab* 2018; **43**(1): 77-83.

[31]Sasikumar CS, Devi S. Effect of 'Abana' pretreatment on isoproterenol-induced hyperlipidemia in rats. *Indian J Pharm Sci* 2001; **63**: 101-104.

[32]Bruneval P, Yunge L, Hüttner I. Isoproterenol induced fatty change in rat myocardium: Freeze fracture study of the sarcolemma at sites of lipid droplet-membrane appositions. *J Submicrosc Cytol* 1985; **17**: 335-343.

[33]Myhre PL, Røsjø H, Sarvari SI, Ukkonen H, Rademakers F, Engvall JE, et al. Cardiac troponin T and NT-proBNP for detecting myocardial ischemia in suspected chronic coronary syndrome. *Int J Cardiol* 2022; **361**: 14-17.

[34]Jayachandran KS, Vasanthi HR, Rajamanickam GV. Antilipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction. *Mol Cell Biochem* 2009; **327**: 203-210.

[35]Nagarajan A, Doss VA. Antihypertrophic effect of menthol from *Mentha* x *piperita*—Cardiac hypertrophy review. *Nat Prod J* 2022; **12**. doi: 10.2174/2210315512666220429110704.

[36]Silva GBA, Souza DS, Menezes-Filho JER, Silva-Neto JA, Cruz JS, Roman-Campos DR, et al. (-)-Carvone modulates intracellular calcium signaling with antiarrhythmic action in rat hearts. *Arq Bras Cardiol* 2022; **119**(2): 294-230.

[37]Dong S, Qian L, Cheng Z, Chen C, Wang K, Hu S, et al. Lactate and myocardiac energy metabolism. *Front Physiol* 2021; **12**: 715081.

[38]Jemai H, Sayadi S. Heart histopathology and oxidative features in diabetic rats and protective effects of Oleuropein. *Adv Biosci Biotechnol* 2015; **6**: 383-389.

[39]Zhang GX, Kimura S, Nishiyama A, Shokoji T, Rahman M, Yao L, et al. Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovasc Res* 2005; **65**: 230-238.

[40]Jodalen H, Lie R, Rotevatn S. Effect of isoproterenol on lipid accumulation in myocardial cells. *Res Exp Med* 1982; **181**: 239-244.

Publisher's note