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Original Article

Effect of Carvacrol on Catalase Activity and mRNA Expression Following Left Ventricular Hypertrophy in Rats

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A B S T R A C T

Background and Aims: Cardiac hypertrophy is a compensatory augmentation response of the heart due to pressure overload that can lead to heart failure. Carvacrol is considered as the major compound of many plants, that possesses strong antioxidant properties. The present study aimed to evaluate effect of carvacrol on catalase activity and mRNA expression following left ventricular hypertrophy in rats.

Materials and Methods: In the current study, male Wistar rats were divided into four groups (n=7): Intact animals served as the control group (C), some rats were subjected to supra renal aortic banding without any treatment (H) in order to induce left ventricular hypertrophy, and some rats were pretreated with different doses of carvacrol (25, 50 and 75 mg/kg/day in H+C25, H+C50 and H+C75 groups respectively). Serum and cardiac catalase (CAT) activity was determined by the biochemical methods. CAT mRNA expression was assessed using real-time polymeras chain reaction.

Results: In H+C50 and H+C75 groups, the CAT activity was significantly higher in the left ventricular tissues (47.5±20 and 42±13.6, vs. H 22.4±17 U/mg protein respectively). Serum CAT activity was increased in H+C50 and H+C75 groups ($p<0.001$), and CAT mRNA expression was increased in H+C50 group ($p<0.05$), as well.

Conclusions: The findings of this study suggest that Carvacrol may protect the heart against left ventricular hypertrophy via augmentation of CAT mRNA expression and activity.

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Introduction

Left ventricular hypertrophy (LVH) is regarded as a primary independent risk factor for the worldwide morbidity and mortality within patients suffering from hypertension. Cardiac hypertrophy is classically deliberated as an adaptive and compensatory response to boost the heart output. Nonetheless, extended cardiac hypertrophy causes heart failure, which its mechanisms are broadly unknown [1-4]. Oxidative stress can be mentioned as the main component of the production of cardiovascular diseases (CVD) such as hypertension, atherosclerosis, LVH, heart failure [5]. The extent of tissue damage depends on the balance between the generation of reactive oxygen species (ROS) and the antioxidant defense system. ROS are derived from many origins including mitochondrial respiratory chain, xanthine oxidase, uncoupled nitric oxide synthase, and NADPH oxidase [6]. However, there are some pieces of evidence supporting the idea that the mitochondrial respiratory chain is an essential source of intracellular ROS production and concurrently, a major target for the damaging effects of ROS. To defend cells from ROS, mitochondria contain an elaborate protection system to detoxify free radicals as well as to repair free radical-induced damage [7, 8].

Recent studies suggest that the hypertrophy process is correlated with expansion in intracellular oxidative stress. Although the exact sources of free radicals in cardiac hypertrophy are less known, some evidence showed that electron transport chain of mitochondria and the phagocytic-type NADPH oxidase are recognized as the main sources of ROS generation in cardiomyocytes [9-11]. Catalase is an essential antioxidant enzyme that detoxifies H_2O_2 into oxygen as well as water and also restricts deleterious effects of ROS. Increase of H_2O_2 production has been demonstrated in serum of the patients suffering from essential hypertension [12, 13].

Investigations have displayed that plant extracts or their active ingredients (such as monoterpenes) are rich in antioxidants which exert a protective effect on the cardiovascular diseases. Therefore, there is an increasing interest in the use of natural antioxidants. Carvacrol is introduced as a main monoterpenic phenol (2-methyl-5-isopropylphenol) that exists in essential oils of *Labiatae* including *Origanum* [14], *Satureja* [15], *Thymbra* [16], and *Thymus revolutus Celak* [17]. Cardiovascular effects of Carvacrol such as vasorelaxation and hypotension [18, 19] as well as its strong antioxidant properties [20] have been previously reported.

Since a high prevalence of cardiovascular diseases is observed, it is reasonable to use plant materials such as Carvacrol that has potent antioxidant properties to provide the body antioxidants. The author's previous study has revealed that Carvacrol administration decreases the hypertrophic markers such as heart weight/body weight and Atrial natriuretic peptide (ANP) mRNA level in rats undergoing aortic banding-induced HLV. Although it has been presented that Carvacrol has an anti-

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oxidative activity, no reports have been stated on its antioxidant effects in cardiac hypertrophy. Therefore, the present study aimed to evaluate the serum and tissue enzymatic activity as well as mRNA levels of catalase (CAT) in the rats' hypertrophied heart.

Materials and Methods

Experimental groups

Male Wistar rats, weighing 170-200 g, were obtained from animal house of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Animal handling and all the related procedures were approved by Shahid Sadoughi University Committee on animal researches. All the animals were randomly divided into five groups (n=7 in each group): In group H, cardiac hypertrophy was induced by the abdominal aortic banding [21] without any treatments. In the treated groups, Carvacrol (Merck, Germany) administration was started 7 days prior to the aortic banding that continued until the end of the experiments (2 weeks after surgery). Carvacrol was given intraperitoneally at the doses of 25, 50 and 75 mg/kg/day in H+C25, H+C50 and H+C75 groups respectively. Intact animals served as the control group(C) (n=7 in each group).

Serum and tissue preparation and measurement of catalase activity

On the fourteenth day after aortic banding, blood samples were collected from the right atrium. Serum was separated by centrifugation for 20 min. at 4000 g and stored at –20°C. The hearts were rapidly isolated and washed by ice-cold isotonic saline and then, stored at -80°C. CAT activity was measured according to the method of Aebi. Left ventricular tissues were homogenized in a lysis buffer (Triton X-100, NaCl, Tris, EDTA, SDS, EGTA, HEPES, and PMSF) using a homogenizer (T10Bhomogenizer; Germany) and centrifuged for 45 min (13000 rpm at 4°C). Briefly, the supernatant was diluted with 0.85 volumes of phosphate buffer (50mM, pH 7.0), and incubated at room temperature for 10 min. The reaction was initiated by adding 0.05 ml of 50 Mm H_2O_2/phosphate buffer (50mM, pH 7.0). The absorbance was read at 240 nm for 3 minutes.

Real-Time Polymerase Chain Reaction (RT-PCR) analysis of mRNA level

RNA extraction

To evaluate the catalase mRNA expression in the left ventricles, the heart was rapidly removed and placed on ice. The whole RNA was extracted from the left ventricular tissue via RNx-PLUS solution (CinnaGen, Iran) according to the manufacturer's protocol. The RNA concentration was determined measuring the absorbance at 260 nm by NanoDrop spectrophotometer (Model 2000, Thermo Scientific, Germany).

cDNA synthesis

First-strand cDNA synthesis was done, using RevertAid™M-MuLV Reverse transcriptase (Fermentas, USA) in the total volume of 20 µL.

RT-PCR reaction

CDNA of experimental groups was tested by master mix containing SYBR green (Takara, Japan) with specific primers under real time RT-PCR reaction (Rotor Gene system - Qiagen, USA). Beta-actin was considered as a

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reference gene. Sequences of primers are summarized in table1.

Statistical analysis

The study data was analyzed according to ΔΔCt method. In order to statistically analyze the study data and compare the differences among experimental groups, one-way ANOVA followed by Tukey' post- hoc comparisons test was used. The data were expressed as Mean± SEM and p value of less than 0.05 was considered as the significance level.

Table 1. Sequences of reverse and forward primers used for RT-PCR

Gene	Gene primer sequences
Beta-actin	F:5′-AACCCTAAGGCCAACCGTGAAAAGAT-3′ R:5′-ACCGCTCGTTGCCAATAGTGATG-3′
Catalase	F:5′-CTGACGTCCACCCTGACT-3′ R:5′-GGCAGCTATGTGAGAGCC-3′

Results

As shown in figure 1, the results of biochemical assessments demonstrated that the catalase activity was significantly increased in left ventricular tissue of H+C50 and H+C75 groups compared to group H (47.5±20 and 42±13.6 respectively vs. 22.4±17 U/mg protein, p<0.001). The changes of tissue catalase activity was not proved to be significant in group H+C25 compared to group H.

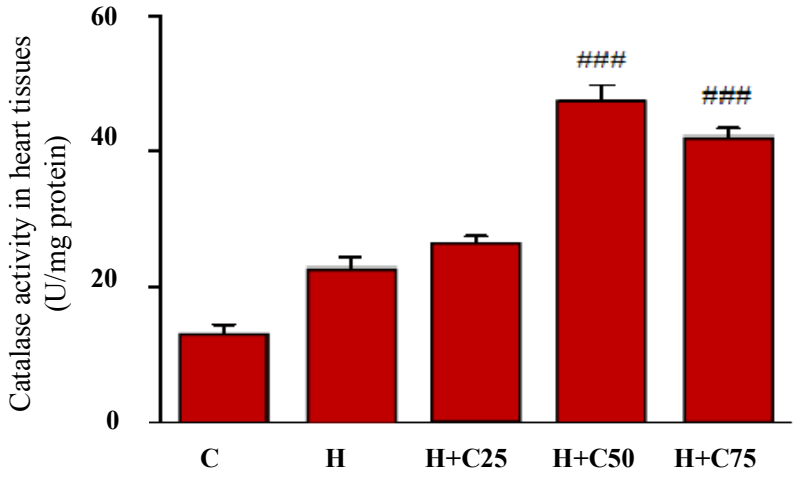


Fig. 1. Catalase activities of left ventricular tissue in the control group (C) hypertrophied without treatment (H) groups and rats that received Carvacrol (H+C) at doses of 25, 50 and 75mg/kg/day. Data are presented as Mean ± SEM, (n=7 in each group).
###p<0.001 indicate the significant difference as compared to group H according to one way ANOVA followed by Tukey' s post test

The measurement of serum catalase activity in experimental groups showed that catalase activity in serum of group H increased to 23±13 U/ml, which was significant in comparison to group C (p<0.01). In the C+H50 and H+C75 groups, serum catalase activity was increased to

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53.3±1.3 and 43±0.9 U/ml, respectively, which increased significantly in these group as compared to the group has indicated in figure 2 ($p<0.001$).

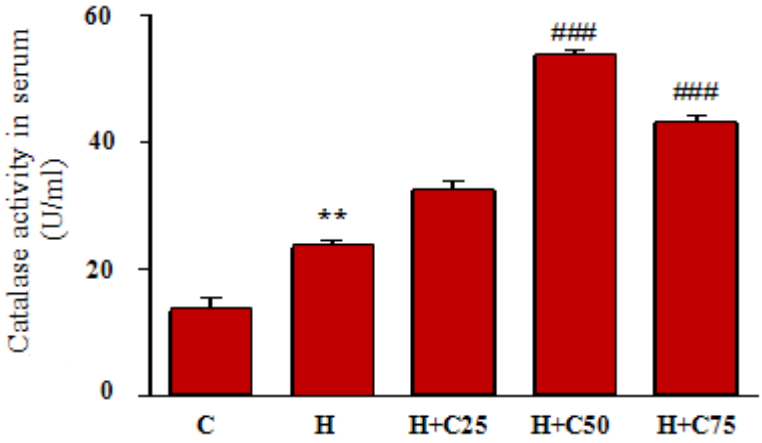


Fig. 2. Catalase activities of serum in the control group (C), hypertrophy without treatment group (H) and animals that received Carvacrol at doses 25, 50 and 75 mg/kg/day(H+C).
Data are presented as Mean ± SEM (n=7 in each group), ###p<0.001 compared to group H
**p< 0.01 indicate the significant difference as compared to group C.

In this section of study, catalase mRNA level was evaluated in the left ventricular tissue in the experimental groups. As shown in Fig. 3, in the H+C50 experimental group, mRNA expression levels of catalase were significantly elevated in comparison to group C ($p<0.01$) and group H ($p<0.05$). In other experimental groups, the mRNA changes were not statistically significant.

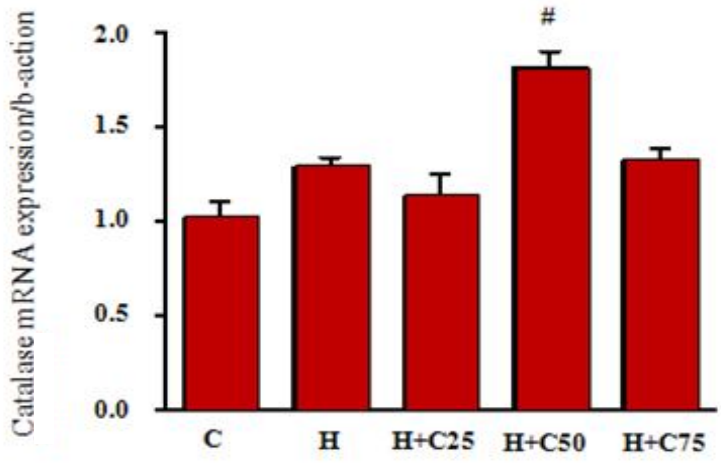


Fig. 3. Catalase mRNA expression of left ventricular tissue in the control group (C) hypertrophy without treatment (H) group and rats that received Carvacrol at doses 25, 50 and 75 mg/kg/day (H+C).
Data are presented as Mean±SEM (n=7 in each group), #p<0.05 indicate the significant difference as compared to group H.

Discussion

As the study findings revealed, blood pressure and heart weight to body weight ratio were increased due to the abdominal aorta banding. Besides, Danijel Juric et al. reported that heart

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weight to body weight ratio and blood pressure increase in hypertrophied rats which is consistent with results of the current study [22]. Results of the authors' previous study indicated that Carvacrol administration at 50 and 75 doses decreases hypertrophy indexes including heart weight to body weight ratio and blood pressure in cardiomyocytes (The data have not been shown). In Aydin et al.'s study, Carvacrol at a dose of 100 mg/kg (i.p.) was demonstrated to reduce blood pressure and heart rate, and prevent the hypertension induced by L-NAME,N(omega)–nitro-L-arginine methyl ester in normotensive rats [18]. Moreover, results of this study demonstrated that serum enzymatic activity of catalase increased in response to myocardial hypertrophy but did not reveal any significant changes in the cardiac tissue indicating dynamic changes of catalase activity in response to hypertrophy and on the other side complexity of catalase activity regulation in the cardiovascular system. An increase in serum activity level of catalase can act as a compensatory response. Dieterich et al. have proposed that increased oxidative stress in last stage of heart failure may lead to high expression of catalase gene, which this can act as a compensatory mechanism in response to heart failure [23]. A study carried out by Satoh et al. *Rac1* gene (regulator of NADPH oxidase activity) was concluded to cause oxidative stress and hypertrophy response in adult heart [24].

In the second section, the study results revealed that Carvacrol administration increases catalase mRNA as well as enzymatic activity in the hypertrophied hearts, though catalase mRNA expression was increased only at the dose of 50 mg/kg/day. In the present study, cardiac catalase mRNA level did not change significantly during thehypertrophy. Previous studies have revealed the antioxidant effects of Carvacrol on liver [25], breast [26] and lung cancer [27]. According to the best of our knowledge, the effect of Carvacrol on catalase activity and mRNA level was evaluated in the current study for the first time. In a study carried out by Peixoto-Neves et al., Carvacrol was proved to induce an endothelium-independent relaxation, possibly including avoidance of Ca^{2+} influx through the membrane. Hence, Ca^{2+} influx inhibition could be the mechanism by which Carvacrol applies its pharmacological effect. Furthermore, calcium channel is a significant factor in the vasorelaxant response to Carvacrol in the rat aorta [28]. Earley et al. have recently examined the effects of Carvacrol on the cerebral artery of rats, that caused potent vasodilation likewise this monoterpene to have significant effects on the human cardiovascular system. Studies by Earley et al. were carried out to investigate the effects of Carvacrol on the TRPV3 channel. Carvacrol caused an influx of Ca^{2+} in endothelial cells by rising intracellular Ca^{2+}, which leads to activation of K^+ channels sensitive to Ca^{2+} medium and low conductance. This monoterpene hyperpolarizes the plasma membrane of endothelial cells, as well as smooth vascular muscles, and thus leads to vasodilatation [29]. On the other hand, Dantas et al. have found that Carvacrol might activate

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K $^{+}$channels in smooth muscle cells [30]. These channels are expressed as main determinants of blood pressure, and vessels tone in the blood vascular walls [31]. The current study results confirm findings of several other studies mentioned earlier on catalase and Carvacrol's essential roles in reduction of blood pressure and as a result prevention of cardiac hypertrophy.

A study conducted by Magyar et al. using the technique of patch clamp in the whole-cell experiments, reported that Carvacrol was likewise able to prevent L-type Ca^{2+}flow in cardiomyocytes from canine and human ventricles [29]. What is the mechanism responsible for Carvacrol in response to oxidative stress suppress in rats involved by aortic banding? There is a strong consensus that exposure to antioxidant activity can be attributed to the existence of hydroxyl group connected to benzene ring. The mild acid character of Carvacrol simplifies its reaction with free radicals, via donating hydrogen atoms to an unpaired electron and generating other radicals stabilized by electron scatter, which is produced at a resonance molecule structure [32]. Considering these findings, a protective effect against oxidative damages of heart tissue is used as a potential mechanism for supplying affects in LVH diseases; nevertheless, it needs to be more explored in the future.

Conclusion

The results of the present study revealed that carvacrol increases catalase activity and mRNA level in the left ventricles of hypertrophied rats. Augmentation of catalase expression and activity can be taken as a novel mechanism into consideration in order to protect the heart against hypertrophy. Assessing the effects of this monoterpene on different components of antioxidant system in the heart can lead us toward using it in treatment of cardiovascular diseases such as left ventricular hypertrophy.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

There is no acknowledgment to declare.

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