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Original Research

Protective Effects of *Myrtus communis* Linn Fruit and Leaf Extracts on Isoproterenol-Induced Heart Failure in Rat

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Abstract

Myrtus communis Linn. (MC) is a cardiotonic plant in traditional Persian medicine. This study was conducted to evaluate the protective effect of MC on isoproterenol-induced heart failure (HF) in rats. Isoproterenol was injected subcutaneously in all groups except the control group for 4 consecutive days to induce myocardial injury in male Wistar rats. In the case of treatment groups, the animals were treated with different doses of the hydro-alcoholic extract of MC fruit or leaves (150, 300, and 700 mg/kg), and were compared with healthy and HF rats. In order to evaluate cardiac function, echocardiography was performed on day 28 after treatment. MC fruit and leaf extracts were administered to all groups except the healthy control group for 28 days by gavage. At the end of the experimental period, the animals were sacrificed and the left ventricle regions of tissue hearts were collected to measure the levels of oxidative stress factors (MDA, SOD, GSH) using ELISA methods. Cardiac fibrosis was evaluated by Mason's trichrome staining. Hematoxylin-eosin staining was used to assess histopathological changes in cardiac structure. Our results showed that administration of MC fruit and leaf extracts significantly reduced the MDA level and increased SOD and GSH levels in treated HF rats compared to the HF group ($P<0.05$). In addition, MC mitigated fibrosis and improved cardiac histological changes compared to the HF group. Collectively, our findings show that MC can be considered as a good candidate to provide cardioprotective effects in HF rats through reduction of oxidative stress and myocardial fibrosis.

Keywords: *Myrtus communis*; Heart failure; Herbal medicine; Traditional medicine; Oxidative stress; Isoproterenol

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Introduction

Heart failure (HF) is considered a global challenge with a prevalence of 0.4% - 20% in different age groups [1,2], and congestive heart failure (CHF) is estimated to be the cause of about 10-13% of global mortality [3].

The increasing prevalence of HF is mainly due to the improved survival of patients with predisposing conditions that may lead to developing HF, and also the implementation of effective therapies. High morbidity and mortality rates and frequent hospital admissions are still attributed to HF, and it could impose a significant economic burden on patients and healthcare systems [4]. The mechanism of development of HF has been partly specified. Several studies have shown that apoptosis, necrosis, and hypertrophy of cardiomyocytes, interstitial fibrosis, inflammatory response, abnormal angiogenesis, and oxidative stress may be implicated in the pathophysiology of heart failure [5]. Stress factors can lead to changes in heart tissue, which are followed by changes in the heart function [6]. It has been determined that oxidative stress plays a fundamental role in all types of heart diseases such as HF, atherosclerosis, cancer, and the aging process [7-10].

To find new effective and affordable therapeutic or preventive methods for HF, research on conventional or non-conventional methods are conducting.

Due to rapidly growing the use of complementary and alternative medicine (CAM) worldwide (such as about 30 % in patients with chronic HF in 2005 in the USA) [11], conducting animal studies and clinical trials to evaluate the safety and efficacy of CAM therapies in HF is inevitable [12].

Persian medicine (PM) is one of the holistic and active complementary systems of medicine in the world. The method of treatment in PM is taking natural medicine (usually medicinal herbs) along with modifying the lifestyle [13].

Myrtus communis (MC) (Myrtle) is an aromatic evergreen shrub, which is native to west Asia, Southern Europe, and North Africa. In Persian medicine, it is called "*Moord or Us*" and is said to be a cardiotonic herb [14].

It is known that MC leaves have various chemical compounds such as tannins, flavonoids, saponins, vitamin C, and phenolic compounds such as carvacrol, rosmarinicacid and thymol. MC seeds contain essential oil, which its major components were linalool, α-pinene, 1,8-cineole linalyl acetate, limonene and α-terpineol. Also, MC seed extract contains fatty acid methyl esters including linoleic acid methyl ester, oleic acid methyl ester, and palmitic acid methyl ester and tetradecane [15, 16]. MC has hypoglycemic activity [15] protective effects against liver and intestinal ischemic/reperfusion injury [17,18]; can reduce cholesterol, Low-Density Lipoproteins (LDL), and free radicals; and possibly prevent cardiovascular injuries [19,20]. In addition, the antioxidant [19,21] and anti-inflammatory [22,23] activity of MC has been found in some studies.

Since oxidative stress is one of the main mechanisms of cardiac dysfunction, and as the anti-inflammatory and antioxidant activities of MC have been proven, the aim of this study was to

examine the possible protective effects of MC on HF in rats and the role of oxidative stress as one of the possible mechanisms involved in this phenomenon.

Methods

Selection of animals

Male Wistar rats (40 rats, body weight: 200–250 g) were purchased from the animal laboratory of Iran University of Medical Sciences (IUMS) and were randomly divided into HF and non-HF groups at the beginning of the experiment. The animals were housed in an animal room at 22 ± 2 °C with a 12h dark: 12h light cycle and given free access to food and water. The procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the local animal care committee. The project was approved by the ethics committee of Iran University of Medical Sciences (the ethics code: IR.IUMS.REC.1395.95-04-130-29997).

Chemicals

Isoproterenol was purchased from Sigma Company (St. Louis, MO, USA). Assessment of GPX activity (Cayman Chem, Ann Arbor, MI, USA) and measurement of SOD activity were performed (Cayman Chem, Ann Arbor, MI, USA). MDA level was measured using a commercial chemical colorimetrical assay kit pursuant to the manufacturer's instructions (ZellBio GmbH, Ulm, Germany).

Folin–Ciocalteu reagent, Gallic acid monohydrate and sodium carbonate, used for the determination of total phenolic content were purchased from Sigma-Aldrich.

Plant material

MC leaves and fruits were purchased from a local herbal market in Tehran, Iran, whose authenticity was approved by Dr. G. Amin (School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran). The voucher numbers PMP 1602 for MC fruits and PMP 417 for MC leaves were deposited in the herbarium of the pharmacy school. The hydro-ethanolic extract (30:70) of the leaves and fruits were prepared by maceration method (with ethanol 96o) for 72 h (three times) at room temperature and the extracts were dried by a rotary evaporator at 40oC (the extraction yield was 19 %) [24].

Measurement of Total Phenol with Folin-Ciocalteu Reagent (FCR)

The total phenolic content of the extract was determined according to the Folin–Ciocalteu (FCR) method with minor modifications [22]. For preparing the calibration curve, 1 ml aliquots of 75, 100, 150, and 200 µg/ml hydro-ethanolic (50:50) Gallic acid solutions were mixed with 5 ml FCR (diluted tenfold) and 4 ml (75 mg/ml) sodium carbonate. The absorption was read after 30 minutes (min) at 765 nm and the calibration curve was drawn. Further, 1 ml of the plant extract (1 mg/ml) was mixed with the same reagents as described above, and the absorption was measured for determining the plant phenolic. All determinations were performed in triplicate. The total content of pheno-

lic compounds in the extracts was expressed as mean ± standard deviations of µg of Gallic acid equivalents (GAE) /mg extract. The following equation was used to calculate the total amount of phenolic compounds with Gallic acid content in methanol extracts: Absorbance = 0.0012 × Gallic acid (µg) + 0.0033

Induction of HF

HF was induced by a subcutaneous injection of Isoproterenol (170 mg/kg) in four consecutive days. Echocardiography was performed in animals for confirming HF 28 days following the last injection of Isoproterenol.

Experimental design

Rats were treated with oral administration of MC fruit (F) or leaf extracts (L) 150, 300, or 700 mg/kg for 4 weeks. The animals were divided into 8 groups (n = 5), healthy control (C), HF, HF + F150, HF + F300, HF + F700, HF + L150, HF + L300, HF + L700 [24,25].

Tissue Preparation for biochemical analysis

At the end of the experiment, the rats were sacrificed under deep anesthesia by ketamine (60 mg/kg) and xylazine (5 mg/kg) and the heart tissues were stored at -80 °C prior to biochemical assessments.

Measurement of MDA (Malondialdehyde) level

For biochemical evaluation, after washing them with cold 1.15% KCl, the heart samples were dried with blotting paper. Then, the samples were homogenized in KCl at 1600 rpm for 2 min. To determine the Malondialdehyde (MDA) level (one of the end products of lipid peroxidation), Esterbauer and Cheese man method was employed based on Thiobarbituric Acid Reactive Substances (TBARS) formation [26]. Tissue homogenates were mixed with 10% trichloroacetic acid (1 ml) and 10% thiobarbituric acid (2 ml), and were further heated at 100 °C for 1 hour. Once the resulting solution was cooled at room temperature, the precipitate was removed by centrifugation. Measurement of mixture reaction absorbance was performed at 535 nm against a blank containing all components except for the tissue homogenates. The MDA level was defined as µm/l of sample proteins.

Measurement of SOD (Superoxide dismutase) activity

For biochemical evaluation, after washing the heart samples with 1 M cold (+4 °C) PBS, they were dried with blotting paper. Then, the samples were homogenized in phosphate buffer saline (0.15 M) at 1600 round per minute (rpm) for 2 min. After centrifugation of the homogenate (7000 rpm, 30 min at + 4 °C), Superoxide dismutase (SOD) activity was determined via spectrophotometry [27] (Note that SOD activity was defined as U/mL of sample proteins).

Measurement of GSH (Glutathione) content

For biochemical evaluation, once washed with 1 M cold (+4 °C) PBS, the heart samples were dried with blotting paper. Next, the samples were homogenized in metaphosphoric acid (0.5 M) at 1600 rpm for 2 min. After centrifugation of the homogenate (3500 rpm, 10 min at +4 °C) and the reaction of supernatant with 5, 5′-dith-

iobis-(2-nitrobenzoicacid), the absorbance was measured at 412 nm. Glutathione (GSH) content in tissue was presented as mg/ml of sample proteins.

Histological Examination

The hearts were dehydrated and embedded in paraffin and then cut into 7 µm thick sections. In order to evaluate the level of collagen deposition in the heart tissue, we used Masons' trichrom staining. To assess the histological changes and structure of the myocardial muscle fibers along with the status of cardiomyocytes (in terms of normal or abnormal nuclei) Hematoxylin-Eosin (H&E) staining was applied.

Five slices were selected at 50 µm from the heart of each animal. A high magnification light microscope with OLYSIA Bio Report Soft Imaging System (Muenster, Germany) was used to take pictures with different magnifications in order to assess the collagen deposition in the cardiac tissue and pyknotic cells. Evaluation of the percentage of collagen fibers was carried out using the Image J software (NIH, Bethesda, MD, USA). All these assays were performed in a blinded manner.

Statistical analysis

All the data were expressed as mean ± standard deviation. Data analysis was carried out using Graph Pad Prism-5 statistic software (LaJolla, CA, USA). One-way analysis of variance followed by Tukey test for post hoc analysis was employed to analyze variations among three or more groups. A two-tailed Student's t-test was applied to analyze the difference between the two groups. A value of $P < 0.05$ was considered as statistically significant.

Results

The total phenolic content of leaf and fruit extracts were 214.75 ± 0.01 µg GAE/mg and 92.25 ± 0.01 µg GAE/mg extract, respectively.

Effects of MC extracts on myocardial MDA level

As displayed in figure 1, induction of HF significantly elevated the MDA level as compared to the normal control group ($P < 0.0001$). In addition, the groups receiving MC fruit and leaf extracts revealed significant reductions in the MDA level as compared to HF group ($P < 0.05$ for HF + F150 and HF + L300 groups; $P < 0.01$ for HF + L150 group; $P < 0.0001$ for HF + F300, HF + F700 and HF + L700 groups).

In addition, the MDA level in groups receiving MC fruit and leaf extracts was significantly higher as compared to normal control group ($P < 0.01$ for HF + F700; $P < 0.0001$ for HF + F150, HF + L150 and HF + L300 groups). There were no significant differences at MDA level in HF + F300 and HF + L700 groups when compared to the normal control group.

When comparing the groups receiving MC fruit extract with different doses relative to each other, it was observed that MDA level in HF + F300 group was significantly lower than in HF + F150 group ($P < 0.0001$). There was no significant difference between HF + F700 and HF + F150 groups. On the other hand, HF + F700 group showed a significant increase in MDA level as compared to HF + F300 group ($P < 0.05$).

There were no significant differences in HF +

L300 and HF + L700 groups as compared to HF + L150 group. HF + L700 group revealed a significant fall in MDA level as compared to HF + L300 group ($P < 0.01$).

It was observed that MDA level in HF + L300 group was significantly higher than in HF + F300 group ($P < 0.0001$), but there were no significant differences between HF + F150 and HF + L150; HF + F700 and HF + L700 (Figure 1).

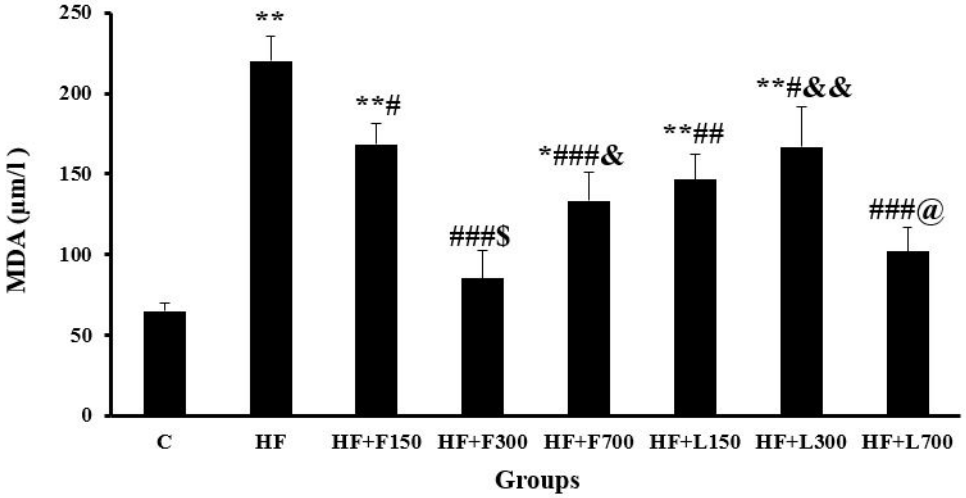

Figure 1. the effects of MC fruit and leaf extracts on myocardial MDA level in normal control and HF groups (n = 3 for each group). The data were expressed as Mean ± standard deviation. (*P < 0.01 as compared to C group, ** P < 0.0001 as compared to C group, ♯ P < 0.05 as compared to HF group, ♯♯ P < 0.01 as compared to HF group, ♯♯♯ P < 0.0001 as compared to HF group, $ P < 0.0001 as compared to HF + F150 group, &P < 0.05 as compared to HF + F300 group, && P < 0.0001 as compared to HF + F300 group, @ P < 0.01 as compared to HF + L300 group. C: Control, HF: HF, HF + F150: HF + 150 mg/kg MC fruit, HF + F300: HF + 300 mg/kg MC fruit, HF + F700: HF + 700 mg/kg MC fruit, HF + L150: HF + 150 mg/kg MC leaf, HF + L300: HF + 300 mg/kg MC leaf, HF + L700: HF + 700 mg/kg MC leaf).

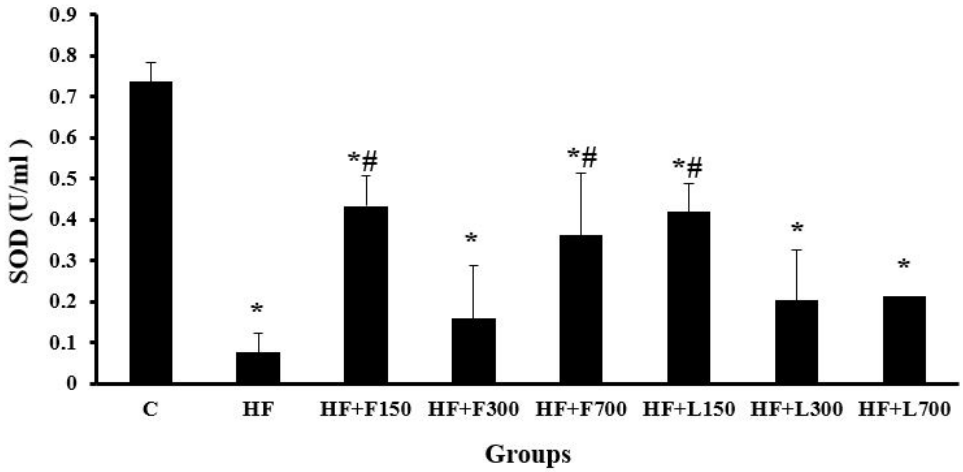

Figure 2. the effects of MC fruit and leaf extracts on myocardial SOD activity in normal control and HF groups (n=3 for each group). The data were expressed as Mean ± standard deviation. (*P < 0.0001 as compared to C group, ♯ P < 0.0001 as compared to HF group. C: Control, HF: HF, HF + F150: HF + 150 mg/kg MC fruit, HF + F300: HF + 300 mg/kg MC fruit, HF + F700: HF + 700 mg/kg MC fruit, HF + L150: HF + 150 mg/kg MC leaf, HF + L300: HF + 300 mg/kg MC leaf, HF + L700: HF + 700 mg/kg MC leaf).

Effects of MC extracts on myocardial SOD activity

As observed in figure 2, induction of HF significantly reduced the SOD activity as compared to the normal control group ($P < 0.0001$). On the other hand, administration of MC significantly enhanced the SOD activity in HF + F150, HF + F700 and HF + L150 groups as compared to HF group ($P < 0.0001$). Further, the SOD activity was significantly lower in groups receiving MC fruit and leaf extracts as compared to the normal control group ($P < 0.0001$).

There were no significant differences in SOD activity between HF + F150, HF + F300 and HF + F700 groups. There were no significant differences between either HF + L150, HF + L300 or HF + L700 groups.

When comparing the groups receiving similar doses of MC fruit and leaf extracts relative to each other, no significant difference was observed between groups with similar doses (Figure 2).

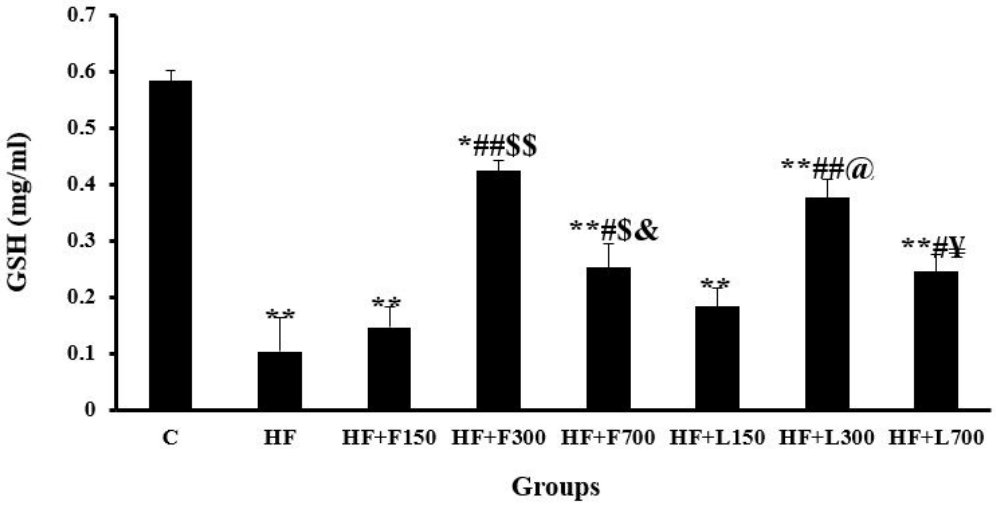

Figure 3. the effects of MC fruit and leaf extracts on myocardial GSH content in normal control and HF groups (n=3 for each group). The data were expressed as Mean ± standard deviation. (*P < 0.01 as compared to C group, ** P < 0.0001 as compared to C group, ♯ P < 0.01 as compared to HF group, ♯♯ P < 0.0001 as compared to HF group, $ P < 0.05 as compared to HF + F150 group, $$ P < 0.0001 as compared to HF + F150 group, &P < 0.01 as compared to HF + F300 group, @ P < 0.0001 as compared to HF + L150 group, ¥ P < 0.01 as compared to HF + L300 group. C: Control, HF: HF, HF+ F150: HF + 150 mg/kg MC fruit, HF + F300: HF + 300 mg/kg MC fruit, HF + F700: HF + 700 mg/kg MC fruit, HF + L150: HF + 150 mg/kg MC leaf, HF + L300: HF + 300 mg/kg MC leaf, HF + L700: HF + 700 mg/kg MC leaf).

Effects of MC extracts on myocardial GSH content

As shown in figure 3, induction of HF significantly lowered the GSH content as compared to the normal control group ($P < 0.0001$). On the other hand, the groups receiving MC fruit and leaf extracts revealed a significant increase in GSH content as compared to HF group ($P < 0.01$ for HF + F700 and HF + L700 groups; $P < 0.0001$ for HF + F300 and HF + L300 groups).

There were no significant differences regarding GSH content in HF + F150 and HF + L150 groups as compared to HF group.

Meanwhile, the GSH content was significantly lower in groups receiving MC fruit and leaf extracts as compared to the normal control group ($P < 0.01$ for HF + F300 group; $P < 0.0001$ for HF + F150, HF + F700, HF + L150, HF + L300 and HF + L700 groups).

It was observed that GSH content in HF + F300

($P < 0.0001$) and HF + F700 ($P < 0.05$) groups was significantly higher than in HF + F150 group. On the other hand, HF + F700 group revealed a significant decrease in GSH content as compared to HF + F300 group ($P < 0.01$).

Comparing different doses of MC leaf extract revealed that GSH content in HF + L300 group was significantly higher than in HF + L150 group ($P < 0.0001$). On the other hand, there was no significant difference in GSH content between HF + L700 and HF + L150 groups. Note that HF + L700 group showed a significant decrease in GSH content as compared to HF + L300 group ($P < 0.01$).

When comparing the groups receiving similar doses of MC fruit and leaf extracts relative to each other, no significant differences were observed between groups with similar doses of MC fruit and leaf extracts (figure 3).

Effects of Myrtus communis Linn fruit and leaf extracts on cardiac fibrosis

The replacement of collagen fibers in the necrotizing area of cardiac tissue occurs following the migration of fibroblasts and formation of collagen fibers. Therefore, Masson's trichrome staining is a good method for evaluating cardiac fibrosis.

As shown in figure 4. A, normal distributed areas of fibrosis deposition are visible in the normal control group. However, the fibrosis of HF group is remarkably greater compared to the normal control group. Surprisingly, administration of different doses of MC fruit and leaf extracts reduced cardiac fibrosis compared to HF group (Figure 4. A).

We also calculated the percentage of interstitial fibrosis compared with the ventricular area (figure 4. B) and found that induction of HF significantly augmented the percentage of interstitial fibrosis as compared to the normal control group ($P < 0.0001$).

In addition, the groups receiving MC fruit and leaf extracts showed a significant decrease in interstitial fibrosis percentage as compared to HF group ($P < 0.0001$).

The percentage of interstitial fibrosis in groups receiving MC fruit and leaf extracts was significantly higher as compared to the normal control group ($P < 0.05$ for HF + F700 group; $P < 0.01$ for HF + F300 group; $P < 0.0001$ for HF + F150 group, HF + L150 and HF + L300 groups). On the other hand, there was no significant difference regarding the interstitial fibrosis percentage in HF + L700 group as compared to the normal control group.

The interstitial fibrosis percentages in HF + F300 ($P < 0.05$) and HF + F700 groups ($P < 0.01$) were significantly lower than in HF + F150 group. However, there was no significant difference in interstitial fibrosis percentage between HF + F700 and HF + F300 groups.

There was no significant difference in interstitial fibrosis percentage between HF + L300 and HF + L150 groups, but the interstitial fibrosis percentage in HF + L700 group was significantly lower than in HF + L150 group ($P < 0.0001$). In addition, HF + L700 group showed a significant decrease in interstitial fibrosis percentage as compared to HF + L300 group ($P < 0.0001$). The interstitial fibrosis percentage in HF + L300 group was significantly higher than in

HF + F300 group (P < 0.0001). However, there were no significant differences between HF + F150 and HF + L150; HF + F700 and HF + L700 (Figure 4b).

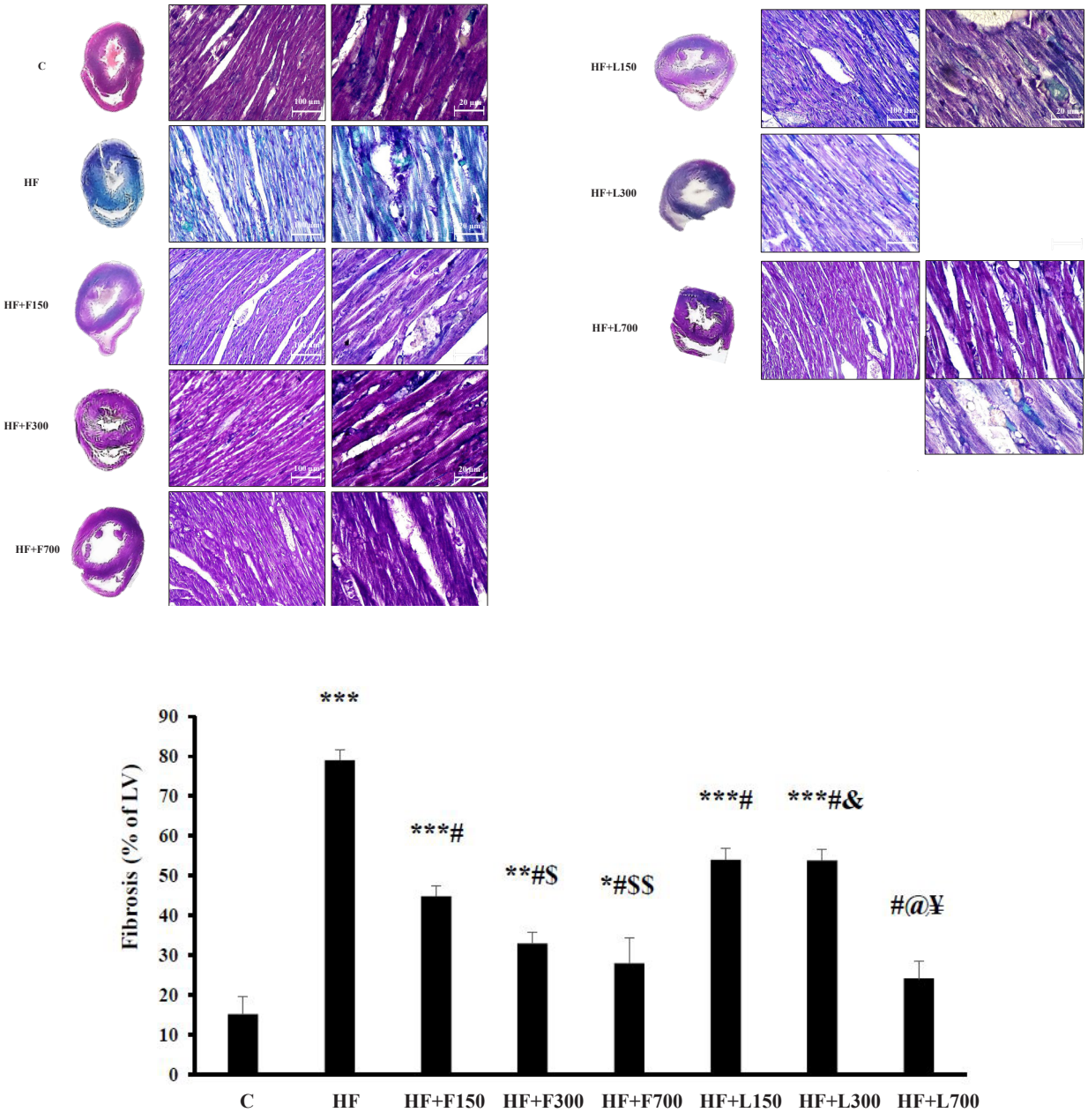

Figure 4. a) Cross sections of the middle part of left ventricle tissue stained with Masson's trichrome in normal control and HF groups and the effect of MC fruit and leaf extracts on it. (Original magnification from left to right 4×, 10× and 40× respectively). Collagen fibers in blue are well detected. **b)** Quantification of interstitial fibrosis (n=3 for each group). The data were expressed as mean ± standard deviation. (*P < 0.05 as compared to C group, ** P < 0.01 as compared to C group, *** P < 0.0001 as compared to C group, ♯ P < 0.0001 as compared to HF group, $ P < 0.05 as compared to HF + F150 group, $$ P < 0.01 as compared to HF + F150 group, & P < 0.0001 as compared to HF + F300 group, @ P < 0.0001 as compared to HF + L150 group, ¥ P < 0.0001 as compared to HF + L300 group. C: Control, HF: HF, HF+ F150: HF + 150 mg/kg MC fruit, HF + F300: HF + 300 mg/kg MC fruit, HF + F700: HF + 700 mg/kg MC fruit, HF + L150: HF + 150 mg/kg MC leaf, HF + L300: HF + 300 mg/kg MC leaf, HF + L700: HF + 700 mg/kg MC leaf).

Effects of Myrtus communis Linn fruit and leaf extracts on cardiac histological changes

H&E staining was employed to evaluate the cardiac histological changes in different groups. As shown in figure 5, cardiomyocytes in normal control group are clear, regular, branched and have one or two large and bright central nuclei (euchromatin) with distinct nuclei.

In HF group, myocardial intracellular spaces have increased and cardiomyocytes have an irregular structure with lateral, darker and pyknotic nuclei, and have a small number of nuclei indicating cardiac distress. Surprisingly, in groups receiving MC fruit and extracts, the intracellular spaces have been reduced and the structure of cardiomyocytes is more regular with clearer and brighter central nuclei as compared to HF group (Figure 5).

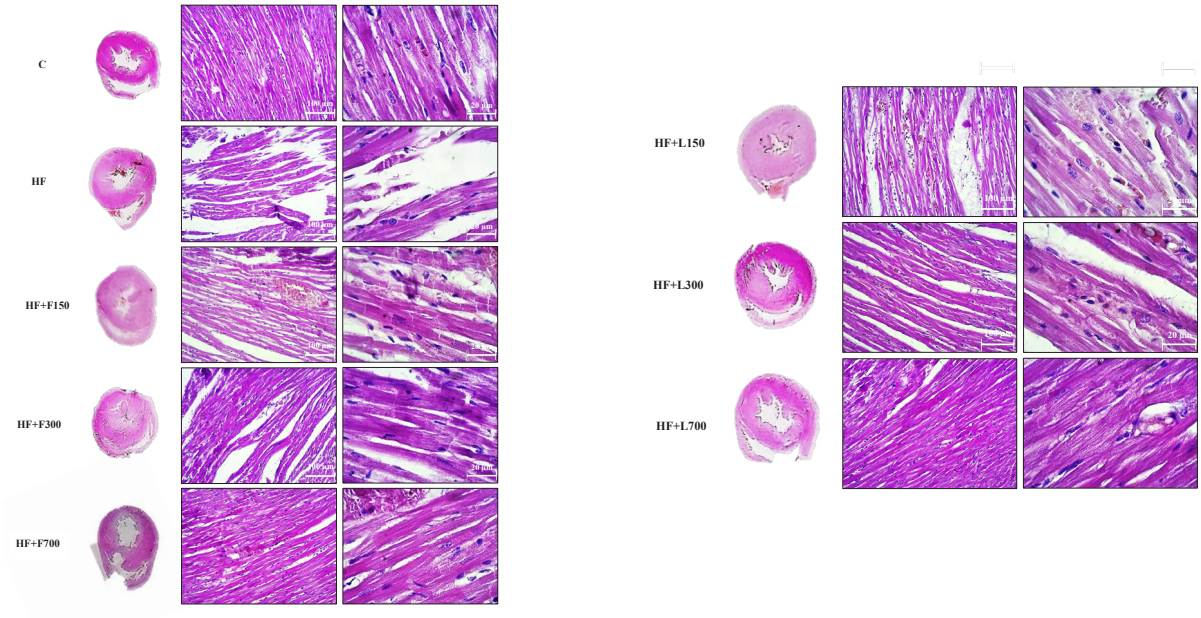

Figure 5. Cross sections of the middle part of left ventricle tissue stained with H&E in normal control and HF groups and the effect of MC fruit and leaf extracts on it. (Original magnification from left to right 4×, 10× and 40× respectively). C: Control, HF: HF, HF+ F150: HF + 150 mg/kg MC fruit, HF + F300: HF + 300 mg/kg MC fruit, HF + F700: HF + 700 mg/kg MC fruit, HF + L150: HF + 150 mg/kg MC leaf, HF + L300: HF + 300 mg/kg MC leaf, HF + L700: HF + 700 mg/kg MC leaf).

Discussion

Many diseases are associated with excessive activity of oxygen free radicals [27]. Reactive oxygen species (ROS) are produced in response to cellular metabolism and oxidative stress occurs when ROS production exceeds the ability of the antioxidant defense system to remove it [6].

Over the past several decades, animal and human models of HF have provided evidence that oxidative stress plays an important role in the progression of HF. Since oxidative stress causes changes in gene expression; also, the death of cardiomyocytes is involved in heart remodeling and failure [6]. Along with excessive production of ROS, myocardial antioxidant defenses are often impaired in CHF experimental models [27].

CHF is characterized by generalized and car-

diac-specific oxidative stress. Chronic oxidant damage also leads to cardiac dysfunction and ultimately the development of HF [28].

Several clinical observations support this hypothesis that ROS plays an important role in human HF. Therefore, antioxidants have attracted a great deal of attention as a potential treatment for many diseases [28].

MC leaves contain different flavonoids such as quercetin, catechin, and myricetin derivatives. Also, MC fruits composed of volatile oils, tannins, carbohydrates, flavonoids, and organic acids. Since MC extracts showed significant levels of phenolic content, so antioxidant activity of MC extracts may due to its phenolic content. Antioxidant components like phenolic compounds can delay or inhibit the oxidation reaction, for example, blocking free radicals, chelating metal ions, and inhibitors of oxidation reaction and therefore these plants can be used in food product because of their antioxidant activity [29].

In this study, we investigated the effects of MC fruit and leaf extracts as an antioxidant on HF in rats by evaluating the changes in oxidative stress indices (MDA, SOD, and GSH), cardiac fibrosis, and histological changes in the heart. According to the results of this study, oral administration of MC fruit and leaf extracts in rats for 4 weeks showed significant protective effects in the HF group. In general, administration of MC fruit and leaf extracts significantly reduced MDA level as compared to the HF group. Specifically, 300 mg/kg MC fruit extract and 700 mg/kg MC leaf extract had a greater effect on the MDA level than the other doses did. On the other hand, administration of MC fruit and leaf extracts significantly enhanced SOD activity and GSH content as compared to the HF group. The results of our study revealed that 150 mg/kg MC fruit and leaf extracts had a greater effect on MDA activity and GSH content compared to other doses. In keeping with our findings, many previous studies have shown that administration of MC fruit and leaf extracts can exert protective effects against different diseases through oxidative stress inhibitory activities of this herbal medicine [20]. For example, Amine Jabri et al. reported that administration of MC extracts resulted in a decrease of lipid peroxidation, an increase of the sulphhydryl groups and glutathione levels, as well as antioxidant enzyme activities [30]. However, Safari et al. showed that MC fruit and leaf extracts are able to increase antioxidant related genes such as SOD and catalase in zebrafish [31].

The mechanisms through which MC inhibits oxidative stress have not been investigated in this study. However, the results of this study open up potential avenues for future studies to examine the protective effects of this extract and its related signaling pathways in the context of cardioprotection under CHF conditions.

Oxidative stress plays an important role in the activation of apoptosis, which seems to be an important contributor to the progression of CHF, especially in the advanced stages of the disease [32]. In other words, apoptosis of cardiomyocytes occurs in hypertrophied, ischemic, and failure hearts and may develop cardiac dysfunction plus HF [33].

Research has shown that oxidative stress stim-

ulates the activation of mitochondrial pathway of apoptosis in the myocardium, which results in the release of cytochrome-c from mitochondria and their activation. Therefore, apoptosis of cardiomyocytes leads to decreased myocardial contractility and ultimately HF [6]. Thus, apoptosis of the cardiomyocytes may play an important] role in the transition of cardiac compensatory function to HF, though it is still controversial [34].

Induction of apoptosis by ROS through up-regulation of Fas-FasL system results in the activation of caspase-8 and downstream caspases. Cytochrome c which is released from mitochondria can interact with Apaf1 which is a major step in the formation of apoptosomes (a complex which consists of Apaf1, cytochrome c, and caspase 9) and initiates mitochondrial apoptosis. Therefore, it can be concluded that mitochondrion is a target and a source of ROS [3].

ROS activates many extracellular factors directly or indirectly, which can induce hypertrophy of cardiomyocytes. Many downstream signaling pathways (PKC, MAPKs, p38 MAPKs, JNK, ASK-1, ERK 1/2; PI3K; Akt and several tyrosine kinases such as NF-κB, FAK, src and calcineurin) which mediate hypertrophic growth in response to these factors can be activated by ROS [3,35,36].

We did not investigate the mechanisms of MC–induced reduction of oxidative stress, but it can be assumed that MC may inhibit apoptosis-signaling pathways as previously reported [6].

In addition to the apoptosis-dependent mechanism, other possible candidates may also trigger the inhibition of apoptosis by MC, which warrants further investigation. In addition, the evidence is not robust so far for concluding that oxidative stress signaling is the only main molecular mechanism for the cardioprotection of MC, since other possible actions of MC in CHF protection have not been studied yet.

Excessive interstitial fibrosis is a serious adverse aspect of chronic HF. Oxidative stress has been proven profibrotic in most organs, and it seems to be the same in the heart. NOX2 oxidase-derived ROS is important for the development of interstitial cardiac fibrosis. NOX2 enhances the expression of profibrotic genes and growth factors, activation of NF-κB and MMPs, and finally infiltration of inflammatory cells [32].

In this study, we investigated the effects of MC fruit and leaf extracts of on myocardial fibrosis in CHF. We also observed that myocardial fibrosis (assessed by Masons' trichrom staining and measuring the percentage of fibrosis) increased in CHF as compared to its level in healthy controls. Notably, MC fruit and leaf extracts administration reduced fibrosis in the HF group. In agreement with our findings, Samareh Fekri *et al.* reported that MC fruit and leaf extracts provide protective effects against experimentally bleomycin-induced pulmonary fibrosis through the reduction of tissue inflammation and inhibition of oxidative stress [37].

Further, having evaluated the cardiac histological changes, we also found that in MC fruit and leaf extracts groups, the intracellular spaces were reduced and the structure of cardiomyocytes was more regular than in the HF group. It seems that MC may mitigate oxidative stress through inhibiting apoptosis signaling path-

ways as its cardioprotective effect (which needs more clarification). Exploring the role of the other important pathways might help us to gain a precise understanding of MC effects on heart protection.

Therefore, the beneficial effects of MC in CHF are also attributable to its multi-mechanistic actions that are directly or indirectly related to its antioxidant activities. Considering the potentials of MC in cardiovascular medicine, it can be said that MC has beneficial effects on CHF conditions due to its effect on oxidative stress.

Conclusions

Our study revealed for the first time that MC fruit and leaf extracts have cardioprotective effects in tissue damage of HF by targeting oxidative stress. Surprisingly, it can be concluded that one of the main mechanisms is inhibition of oxidative stress by reducing the MDA level and enhancing the SOD activity as well as GSH content. It can be concluded that MC can be proposed as a candidate for reduction of severity of HF in the future, but further research is definitely required.

Conflict of Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors' contributions

F.HD. conceptualized the study. N.A. designed the method section and prepared the setting of the animal study. A.Sh. prepared the plant extracts. N.A. and A.E. conducted the animal studies. F.HD. and NA drafted the manuscript; all authors revised and finalized it. F.HD. submitted the paper and tracked the publication process.

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