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**Journal of Chemical and Pharmaceutical Research, 2015, 7(2):42-46**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Inhibitory effects of myrtle (*Myrtus communis* L.) leaves hydroalcoholic extract on LDL oxidation *in vitro*

**Bahador Fadaei[1], Hassan Ahmadvand[2,3*], Maryam Ghasemi Dehnoo[2] and Sako Mirzaei[1,4]**

[1]*Department of Biology, Kurdistan Science and Research Branch, Islamic Azad University, Sanandaj, Iran*
[2]*Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*
[3]*Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*
[4]*Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*

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**ABSTRACT**

*Oxidation of low-density lipoprotein (LDL) has been strongly implicated in the pathogenesis of atherosclerosis. The use of some compound in dietary food stuff include Myrtle (Myrtus communis L.) leaves hydroalcoholic extract (ME) may lead to the inhibition of production of oxidized LDL and may decrease both the development and the progression of atherosclerosis. The present work investigated the effects of ME on LDL oxidation induced-CuSO4 quantitatively in vitro. Fasting blood samples from normal people after an overnight fasting were collected and then LDL was isolated. LDL was incubated without CuSO4 as control and incubated with CuSO4 and several concentration of ME (0.2, 2 and 20 µg/ml); and measured the formation of conjugated dienes, lag time and thiobarbituric acid reactive substances (TBARS). Inhibition of this Cu-induced oxidation was studied in the presence of several concentration of ME (0.2, 2 and 20 µg/ml). It was demonstrated that ME is able to inhibit CuSO4-induced LDL oxidation and decrease the resistance of LDL against oxidation in vitro. Myrtle (Myrtus communis L.) leaves extract showed an increase rate of 5%, 42% and 50% at concentrations ranging from 0.2 to 20 µg /ml, respectively. The inhibitory effects of the ME on LDL oxidation were dose-dependent at concentrations ranging from 0.2 to 20 µg /ml. This study showed that ME prevented the oxidation of LDL in vitro and it may suggest that they have the similar effect in vivo.*

**Keywords**: Myrtle (*Myrtus communis* L.) leaves extract, Low-density lipoprotein (LDL) oxidation

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## INTRODUCTION

LDL oxidation plays an important role in the genesis and development of atherosclerosis [1, 2]. The consumption of oxidant substances in foods causes LDL to oxidize and this can result in initiation and progression of atherosclerosis [2-4]. When LDL is oxidized, its tendency to its receptor is reduced [2]. The accumulation of oxidized LDL in macrophages leads to the formation of foamy cells and also the formation of atherosclerosis [5, 6]. Using antioxidants such as vitamin E and plants such as myrtle leaves, which are rich in antioxidants, we can prevent the oxidation of LDL, and thus prevent the development of atherosclerosis [5, 6]. The pathogeneses of atherosclerosis is a complex process, but there is some evidence to suggest that lipids and LDL oxidation are among of the major events that are involved in the formation of atherosclerotic plaques [7-10]. On the other hand, atherosclerosis is also known as an inflammatory disease, and it is said that oxidative and inflammatory factors in the form of a defective cycle are involved in the initiation and development of atherosclerosis [7-10].

The scientific name of a plant used in this study is "*Myrtus communis L.*" which is known as "myrtle" by local name [11]. Myrtle is an evergreen plant in the form of shrub and belongs to Myrtaceae family which exists in Europe, Asia and America [11]. The leaves of this plant is strongly aromatic and major chemical constituents of its extracts include 1, 8-cineole (18.3 %), Linalool (16.3%) , Myrtinil acetate (14.5%) , These compounds have

antioxidant and bacterial properties [9]. Other studies showed that the leaves of myrtle have been used as bitter herbs in traditional medicine [12]. Myrtle leaves also have antiseptic, antibacterial, hypoglycemic effects which can cause the elimination of active oxygen's [12, 13]. In one study it is shown that myrtle oil essence can meaningfully reduce the blood glucose levels in diabetic animals , and was traditionally used as a treatment for lowering the blood sugar , also this plant can lower the level of triglycerides and lipid peroxidation [14, 15]. According to the theoretical evidences, oxidative stress is the first main cause of atherosclerosis. As a result, we can use these compounds which are useful in inhibition of oxidative stress. Perhaps this kind of treatment with extracts and other antioxidants can be effective in inhibition of oxidative stress and other similar harmful mechanisms. In spite of important ingredients of myrtle and different benefits of this plant, there have not been any complete investigation so far to explore the relationship between myrtle and the oxidation of LDL, so, in this study we investigate the antioxidant effects of ethanol extract of myrtle leaf on inhibition of LDL oxidation induced by copper sulfate.

## EXPERIMENTAL SECTION

**Materials:** Disodium Ethylene Diamine Tetra Acetate (Na2EDTA), Potassium Bromide, Sodium Chloride, Disodium Hydrogen Phosphate (Na2HPO4) all purchased from Sigma Company.

**Preparation of hydroalcoholic extract of myrtle leaves:** Hydroalcoholic extract of myrtle leaves were prepared from Medical plants research center of Lorestan University Medical Sciences. For preparation of hydroalcoholic extract of myrtle leaves first, the green leaves were dried in shadow away from direct sunlight and then they were powdered , 100g of obtained powder poured in 50% alcohol for a period of 9 hours at 50°C , using Suxhlet apparatus .After drying, the obtained extract was stored in 4°C .

**Blood Sampling**: The blood samples were taken from healthy individuals and the serums were separated using centrifugation (300 rmp for 10 min), and Sodium Azide were added to the final concentration (0.06%) to prevent the sample from oxidation.

**LDL Isolation:** Serum LDL was separated in cardiovascular Research Center of Isfahan University of Medical Sciences, using discontinues gradient ultracentrifugation method. Adding Potassium Bromide, the density reached from 0.365g/ml to 1.21g/ml/3.5ml chloride sodium (0.145mol/lit) and 1.5ml from concentrated serum were added to centrifugal tubes, and ultracentrifuge, Beckman Model L7-55 with 40000 rpm during 2.5 hours at 10°C was centrifuged. The isolated LDL was dialyzed for 48h at 4 °C against three changes of deoxygenated-PBS (0.01 mol/L Na2HPO4, 0.16 mol/L NaCl, pH 7.4) [6].

**LDL Oxidation:** After isolation of total LDL, the protein content of LDL was measured. LDL was adjusted to 150 µg/mL of LDL protein with 10 mM PBS, pH7.4 and then aliquots of ESK were added to the solution [14]. Then the sample containing LDL and copper sulfate without extract and samples containing 10 micromolar copper sulfate and hydroalcoholic extract of myrtle leaf with 0.2, 2, 20 micrograms per milliliter were prepared and solvent equivalent to extract volume was added to control sample and copper, also oxidative modification of LDL by measuring UV absorption at 234 nanometer once every ten minutes for a period of 5 hours were done[17]. To determine the LDL oxidation kinetics absorption curve , obtained absorptions were plotted based on sample times, then the time lag and final density of conjugated diene were obtained after 5 hours , using plotted curve (by means of molar extinction coefficient) (29500liter/mol/cm).

**Measurement of Malondialdehyde (MDA) formation:** Lipid peroxidation end products were determined as MDA according to modified method of Buege and Aust. After initiating the oxidation process with CuSO4, the sample mixtures were incubated at 37 °C for 5 h in a water bath and the reaction was terminated by adding EDTA (2 mmol/L). MDA formation was measured in a spectrophotometer at 532 nm. The results were recorded as MDA equivalent content (nmol/mg LDL protein) [18, 19].

**Statistical analysis:** The data were presented as mean ±SD of three experiments performed in duplicate. These parameters were obtained using the y man whitny test (using SPSS 13.0 statistical software) for independent data and the differences were considered significant when $p < 0.05$.

## RESULTS

After adding the desired concentrations of copper and hydroalcoholic extract of myrtle leaf to samples and reading the absorption samples once every ten minutes , LDL oxidation kinetics curves plotted and the amount of conjugated diene, and formed MDA were measured. The results showed that hydroalcoholic extract of myrtle leaf would meaningfully reduce LDL oxidation in vitro ($p<0.05$). The effect of hydroalcoholic extracts of myrtle leaf on

inhibition of LDL oxidation is proportional to the consecration of hydroalcoholic extract of myrtle leaves (this dependence has linear density). To study the kinetics of LDL oxidation , the obtained absorptions in 234 nanometer with notice to time plotted , which is shown is figure 1 .In this diagram there are three distinct parts , the lag phase and propagation phase in which the rate of LDL oxidation is increased, and decomposition phase that is the end of LDL oxidation. The oxidative modification of LDL was obtained by measuring the UV absorbance at 234 nanometer after 5 hours, and accordingly, the late conjugated diene concentration was obtained. (using molar extinction coefficient 2950 liter/mol.cm). The obtained results are shown in figure number 2, with regard to this results the effect of hydroalciholic extract of myrtle leaf is significant and also using kinetic curve, and the lag time was obtained. The results are shown in figure 3. For studying the formed MDA in accordance with the method described before, the absorption of samples measured at 532 nm, and the amount of absorptions were obtained using a molar coefficient as MDA formation in nm/mg-LDL protein .The obtained results are shown in figure 4. Based on the results , the effects of different concentrations of hydroalcoholic extract of myrtle leaf in reduction of MDA comparing to samples having copper sulfate lacking hydroalcoholic extract of myrtle leaf is significant.

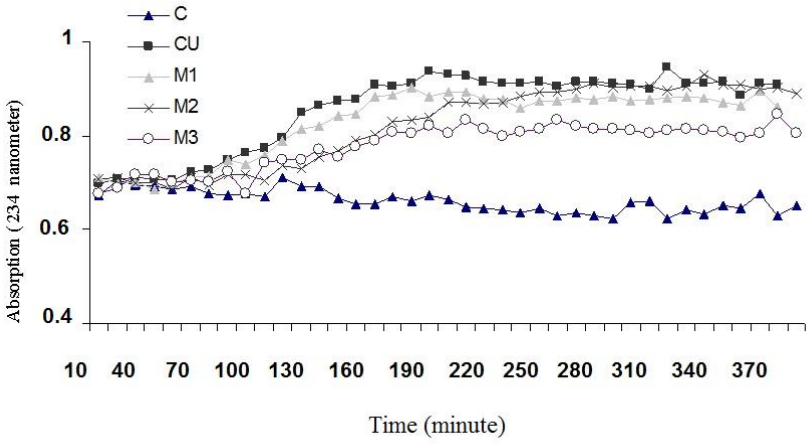


**Figure 1. The effects of ME on LDL oxidation in 10 mmol/L PBS, pH 7.4 at 37 °C for 5 h. C: C: n-LDL; Cu: n-LDL+copper; M1: n-LDL +copper +ME (0.2 µg/mL); M2: n-LDL+ copper+ME (2 µg/mL); M3: n-LDL+ copper+ME (20 µg/mL)**
*Each point represents the mean of five experiments*

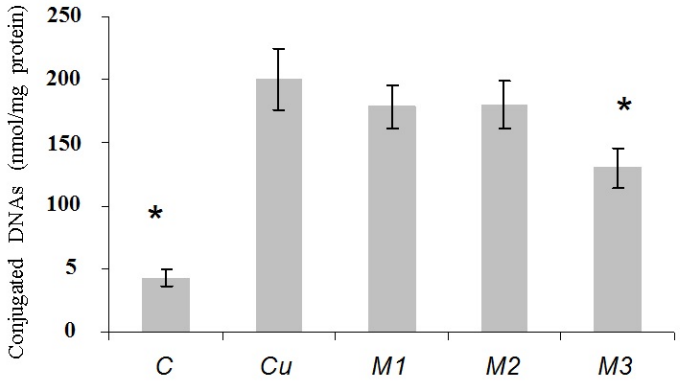


**Figure 2. The effects of ME on the formation of conjugated dienes of LDL oxidation. C: n-LDL; Cu: n-LDL+copper; M1: n-LDL +copper +ME (0.2 µg/mL); M2: n-LDL+ copper+ME (2 µg/mL); M3: n-LDL+ copper+ME (20 µg/mL)**
*Each point represents the mean of five experiments. *significant compared to Cu by man whitny test (P<0.01).*

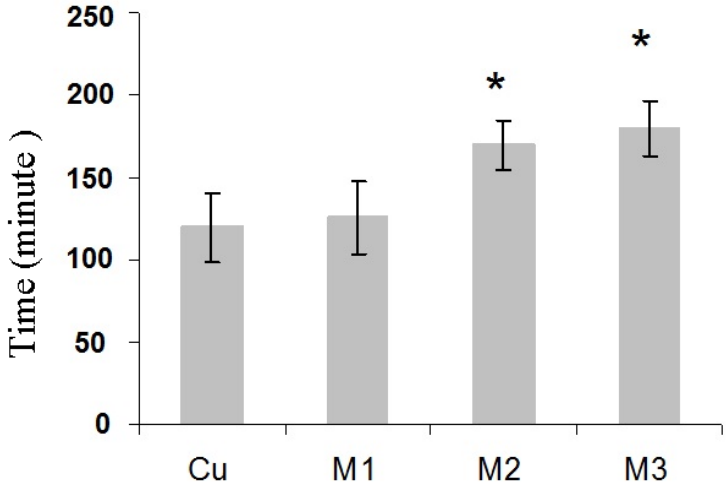


**Figure 3. The effects of ME on lag time of CuSO4-induced LDL oxidation. C: n-LDL; Cu: n-LDL+copper; M1: n-LDL +copper +ME (0.2 µg/mL); M2: n-LDL+ copper+ME (2 µg/mL); M3: n-LDL+ copper+ME (20 µg/mL)**
*Each point represents the mean of five experiments. *significant compared to Cu by man whitny test (P<0.01).*

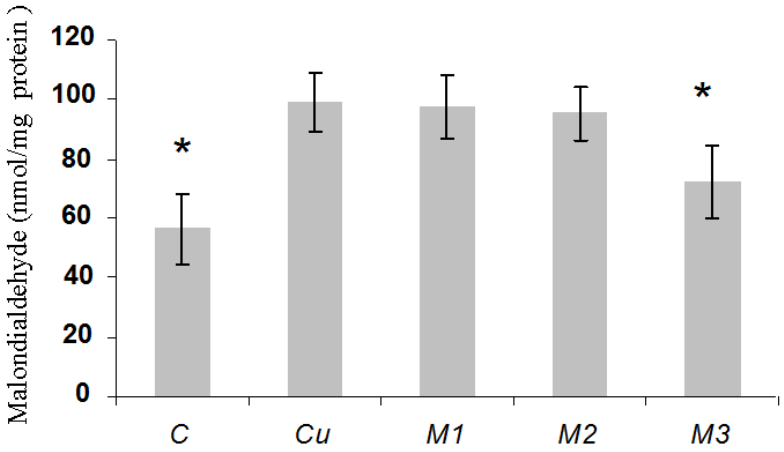


**Figure 4. The effects of ME on the formation of MDA. C: n-LDL; Cu: n-LDL+copper; M1: n-LDL +copper +ME (0.2 µg/mL); M2: n-LDL+ copper+ME (2 µg/mL); M3: n-LDL+ copper+ME (20 µg/mL)**
*Each point represents the mean of five experiments. *significant compared to Cu by man whitny test (P<0.01).*

## DISCUSSION

Oxidation of LDL in the arterial walls is known as the main factor in genesis and development of atherosclerosis [2]. According to the studies conducted in patients with atherosclerosis, there is a lower level of antioxidants (comparing healthy people) [20]. Copper causes the free radicals to release through Fenton & Haber-Weiss reaction, and probably in this way the LDL oxidation is happen, following that, the amount of lipid peroxidation and antioxidants existing in LDL structures such as vitamin E is reduced by copper due to interaction with free radicals [4, 21, 22]. Based on the results obtained in this study, the ME can meaningfully reduce conjugate diene and MDA, and also the lag time is increased induced by ethanol ME. According to this results ME leaves has an antioxidant role in this process. Although little research has been done regarding antioxidant role of ME so far , but studies indicate that the most important compound in the myrtle leaves are anthocyanins and flavonoids which are both well known antioxidants [23, 24]. As results the presence of antioxidant compounds such as anthocyanins and flavonoids in myrtle leaf correspond to the results obtained. Researchers have reported that the extracts of myrtle fruit have potent

radical scavengers as also electron donors [25]. Also, researchers have reported that the aqueous extracts myrtle fruit considerable antioxidant activity, as measured by their capacity to scavenge the stable-free radical DPPH [24, 25]. Results of our study are in accordance with others researchers' study that showed ME have potent antioxidant activity. ME as a source of potent natural antioxidants, with multi beneficial properties can be introduced as supplement antioxidant could prevent or be helpful in reducing the oxidative stress in chronic diseases such as coronary heart diseases and diabetes.

The studies showed that ME acts as an oxygen radical scavenger whenever oxygen free radicals are generated, decreases lipid peroxidation in vivo. Also another researcher suggested possible explanations the mechanisms of some natural antioxidant: "1. It may act independently as chain breaking antioxidants, providing hydrogen atoms to reduce peroxyl and/or alkoxyl radicals. 2. A redox interaction may exist between antioxidant and another lipid soluble antioxidant such as vitamin E, in its one-electron oxidized form, vitamin E phenoxyl radical" [3-7]. The results showed that ME can effectively inhibit the oxidation of LDL so it can be used as a dietary supplement, or even with a more serenity we can use them as a medicine for reducing cardiovascular diseases.

## CONCLUSION

As a conclusion from these results, it can be suggested that the consumption of myrtle can probably offer some dietary benefits, as they contain antioxidant constituents, which can protect against lipid peroxidation and can scavenge free radicals. Based on the results obtained in this study, similar studies can be conducted in vivo, and other can be done for evaluating of its effects on cardiovascular and diabetic patients, and it can be used to treat cardiovascular patients easier or improve the life style of cardiovascular and diabetic patients.

**Acknowledgment**
The authors wish to thank Deputy of Research of Lorestan Medical University, Lorestan. Iran.

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