
Myrtus Communis Aqueous Extract is an Effective Inhibitor of in Vitro Oxidation of Human Serum LDL/VLDL

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Abstract

Background: Myrtus communis L. (Fam. Myrtaceae) is distributed all over Iraq. It is a medicinal plant with a wide pharmacological actions. A solubilizing effect upon cholesterol gallbladder stones has already been reported.

Objective: To investigate the effect of an aqueous extract of Myrtus communis L. on the oxidation of human serum low density lipoprotein (LDL)/ very low density lipoprotein (VLDL).

Setting: Department of Pharmacology and Department of Biochemistry, College of Medicine, Al-Mustansiriya University, Baghdad-Iraq.

Design: *in vitro* pharmacognoso-biochemical study.

Methods: Human serum LDL/VLDL is treated, *in vitro*, with aqueous extract of Myrtus communis L., related to two morphological varieties. In another series of experiments, human serum LDL/VLDL is oxidized, *in vitro*, with 5 μ mol CuSO₄ for three hours in presence or absence of an aqueous extract of Myrtus communis L.

Results: Aqueous extract of Myrtus communis L. of both varieties showed a significant reduction in serum native oxidized - LDL/VLDL (ox-LDL/VLDL) level. Aqueous extracts of Myrtus communis L. inhibited CuSO₄-induced oxidation of LDL/VLDL by 15.8% and 22.6% in respect to its morphological variety.

Conclusion: Aqueous extract of Myrtus communis, of whatever variety, has inhibitory effects on oxidation of serum LDL/VLDL, whether it is native or induced by copper. These effects are most probably related to its content of polyphenolic compounds and Myrtus communis may be a useful medicinal plant in the prevention of development of atherosclerosis.

Key words: Myrtus communis, Serum LDL, Oxidation, Polyphenolic compounds

Introduction

Myrtus communis L. (Fam. Myrtaceae) is distributed all over Iraq, especially cultivated in gardens and parks as a hedge plant^[1]. Its medicinal parts are the leaves and the branches. Volatile oils are extracted by a percentage that ranges from 0.1-0.5% and are composed mainly from polyphenolic compounds^[2].

The Assyrians and the Egyptians were aware of the analgesic effects of a decoction of myrtle leaves for joint pains^[3]. Myrto-commulone A which was isolated from myrtus communis L. showed a significant antibacterial activity against multidrug-resistant clinically - relevant bacteria^[4]. Hayder *et al* 2003 demonstrated, by using Ames mutagenicity test, the antimutagenic activity of aqueous extract and essential oils isolated from Myrtus communis^[5].

From the toxicological point of view, essential oils extracted from leaves and flowers of Myrtus communis L. were found to be the most toxic substances and showed insecticidal effects against fourth-Instar larvae of the mosquito Culex pipiens molestus Forskal with a lethal concentration (LC₅₀) equivalent to 16 mg/L^[6]. In human, overdoses of myrtle oil (more than 10 gm) can lead to life-threatening poisonings, due to the high cineol content^[2].

A mixture of the essential oil related to Myrtus communis (Myrtol) was used to solubilize cholesterol gallbladder stones^[7]. Lyophilized glutaraldehyde-treated erythrocytes saturated with

phytohaemoagglutinins obtained from Myrtus communis L. seeds decreased the content of triglycerides in serum samples by more than 70%^[8].

Low density lipoprotein oxidation is the primary event in atherosclerotic plaque formation^[9].

Antioxidants such as polyphenols were shown to inhibit LDL oxidation and atherosclerosis development^[10]. Therefore, in this study we investigated the antioxidant properties of Myrtus communis L., popularly known as Yas or As. Although it is rich in polyphenolic compounds, the ethnopharmacological actions of this plant did not previously include the inhibition of lipid oxidation or scavenging oxidized lipids.

Materials & Methods

This study was conducted at Departments of Pharmacology and Biochemistry, College of Medicine, Al-Mustansiriya University, Baghdad – Iraq in June 2004.

Plant collection

Two varieties of Myrtus communis L. Leaves (Fig. 1) were collected as the newly formed upper buds from Al-Jadiriya area in Baghdad. Leaves were dried at room temperature (ranged 25-32°C) in a dry atmosphere, away from sunlight, after dryness these parts were packed in plastic bags and used within few days.

Preparation of aqueous extract

Dried leaves of *Myrtus communis* (of each variety) were crushed and ground by using an electric grinder into a fine powder. A known weight of this powder is mixed with distilled water in a ratio of 1 gm of powder to 10 mL of distilled water (i.e 10%). This mixture is left in volumetric flask

at room temperature with dim light for 12 hours. The mixture is separated into three layers, the lower layer composed of heavy particles residue, the middle layer of clear aqueous greenish colour and the upper layer is composed of floating light particles of myrtle powder (Fig.2).



Variety [A]



Variety [B]

Fig.1. The morphology of *Myrtus communis* L leaves (Variety A & B).

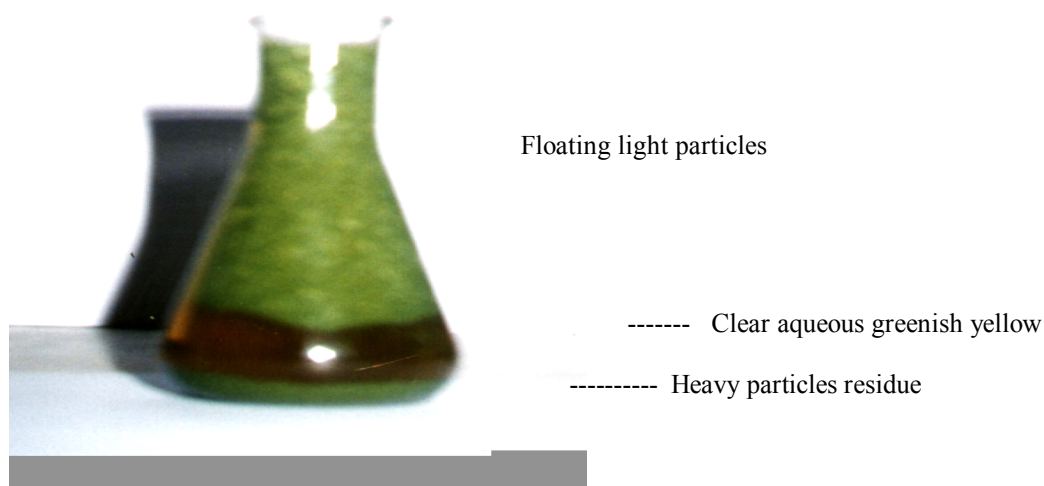


Fig.2. The separated three layers of extracted *Myrtus communis* L milled leaves. The aqueous extract is aspirated from the middle layer and processed as mentioned in methods.

The middle aqueous layer is aspirated by Pasteur pipette, and then filtered through a filter paper to get rid of the fine particles. Thereafter is filtered by filter paper (0.2 μ m). The resultant aqueous solution is immediately used in this study. Isolation of low and very low density lipoproteins (LDL/VLDL) from human sera

Venous blood samples (10 mL) were obtained from twenty subjects after an overnight fasting from the brachial vein in the antecubital fossa under strict aseptic conditions. Whole blood was collected into test tubes and underwent centrifugation for ten minutes at 3000 rounds per minute (rpm), to obtain the serum. Fractionation of serum lipoproteins was achieved by mixing 0.1 mL of working reagent (composed from 14 mmol/L phosphotungstic acid and 2 mmol/L magnesium chloride) with one milliliter of serum. Then the mixture was allowed to stand at room temperature for ten minutes and centrifuged at 4000 rpm for 20 minutes. The pellet (i.e. LDL/VLDL precipitate) was obtained by decanting the supernatant (HDL) and resuspended in one milliliter of isotonic saline.

Determination of oxidized low/very low density lipoproteins (ox-LDL/VLDL) in human sera. Natio *et al* (1993) reported a simple method for assessment of iperoxides in serum, plasma or tissue samples ^[11]. In brief, the steps of **determination the ox-LDL/VLDL are:**

- 1-Pipette 3mL of 0.05N HCl into a glass-stoppered centrifuge test tube.
- 2-Add 0.3 mL serum, and mix well.
- 3-Add 1 mL 0.67% (v/v) thiobarbituric acid solution, freshly prepared and mix well.
- 4-Heat the mixture at 95°C for exactly 30 minutes.
- 5-Cool it immediately with tap water.
- 6-Add 4 mL of methanol/n-butanol (3:17) mixture.
- 7-Spin at 2500 rpm for 20 minutes.
- 8-Measure the absorbance of ox-LDL/VLDL of the upper (butanol) layer at 535nm.
- 9-The yield of ox-LDL/VLDL was calculated from $\varepsilon = 1.56^5 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

Experimental design of the effect of Myrtus communis L. aqueous extract on ox-LDL/VLDL of human sera

I. Effect of aqueous extract of Myrtus communis on native ox-LDL/VLDL

Three equal volumes of each sample of reconstituted pellet of LDL/VLDL with isotonic saline were subgrouped into:

Group A (n=20): the level of native ox-LDL/VLDL was assayed in reconstituted pellet of LDL/VLDL with isotonic saline.

Group B (n=20): the samples were incubated with 25 μ L of Myrtus communis (variety A) aqueous extract for ten minutes prior to the determination of ox-LDL/VLDL.

Group C (n=20): the samples were incubated with 25 μ L of Myrtus communis (variety B) aqueous

extract for ten minutes prior to the determination of ox-LDL/VLDL.

II. Effect of aqueous extract of Myrtus communis on CuSO₄-induced oxidation of LDL/VLDL

Three equal volumes of each sample of reconstituted pellet of LDL/VLDL with isotonic saline were subgrouped into: Group D (n=20): each sample was incubated with 5 μ mol CuSO₄ for three hours, then the level of ox-LDL/VLDL was measured.

Group E (n=20): each sample was mixed with 25 μ L of Myrtus communis (variety A) aqueous extract for ten minutes prior to the incubation with 5 μ mol CuSO₄ for three hours, then the level of ox-LDL/VLDL was measured.

Group F (n=20): each sample was mixed with 25 μ L of Myrtus communis (variety B) aqueous extract for ten minutes prior to the incubation with 5 μ mol CuSO₄ for three hours, then the level of ox-LDL/VLDL was measured.

Statistical analysis

The results are presented as mean $\pm$ SEM of number of observations. The data are analysed by using paired, two tailed Student's test taking $p < 0.05$ as the lowest limit of significance.

Results

I. Effect of aqueous extract of Myrtus communis on native ox-LDL/VLDL

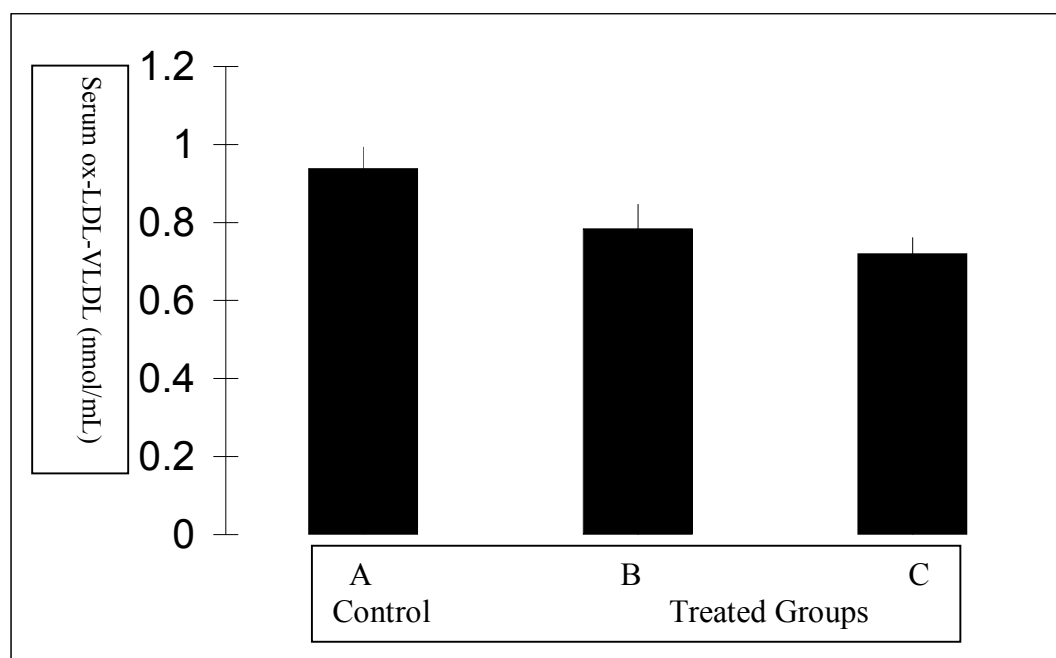
Aqueous extract of Myrtus communis L. (of both varieties A or B) showed a significant reduction in serum ox-LDL/VLDL level (Fig.3). The human serum level of native ox-LDL/VLDL was 0.939 ± 0.055 nmol/mL while that treated with 25 μ L of Myrtus communis (variety A) reduced to 0.784 ± 0.063 nmol/mL (the mean $\pm$ SEM difference was -0.151 ± 0.047 nmol/mL, $t = 3.212$, $p < 0.01$) and that treated with 25 μ L of Myrtus communis (variety B) reduced to 0.720 ± 0.042 nmol/mL (the mean $\pm$ SEM difference was -0.219 ± 0.029 nmol/mL, $t = 7.448$, $p < 0.0005$).

The percent of native oxidized LDL/VLDL to LDL was 0.121 % (the mean levels of LDL and VLDL in the study samples were 0.7772 mmol / L and 0.466 mmol / L respectively. The calculation of percentage was related to LDL because LDL is the major constituent of LDL/VLDL pellet in precipitation method). This percent was reduced to 0.1 % and 0.092 % in samples treated with aqueous solutions of Myrtus communis varieties A and B respectively.

Although aqueous extract of Myrtus communis (variety B) showed a higher level of reduction in native serum ox-LDL/VLDL than that of variety A, but such a difference did not reach a significant level (the mean $\pm$ SEM difference was -0.064 ± 0.0571 nmol/mL, $t = 1.12$, $p > 0.05$). Further analysis revealed that aqueous extracts of Myrtus

communis variety A and B reduced the serum level of native ox-LDL/VLDL by 16.1% and 23.3 %

respectively.



(A) Control (untreated group).

(B) Treated group with Myrtus communis L. (variety A). P value < 0.01 in comparison with untreated group (A).

(C) Treated group with Myrtus communis L. (variety B). P value < 0.00 in comparison with untreated group (A).

Fig. 3. Shows the significant inhibitory effect of aqueous extract of Myrtus Communis L. on human serum native ox-LDL/VLDL.

II. Effect of aqueous extract of Myrtus communis on CuSO_4 -induced oxidation of LDL/VLDL

Samples of reconstituted pellet of LDL/VLDL with isotonic saline showed a significant higher level of ox-LDL/VLDL when treated with oxidizing agent of $5 \mu\text{mol}$ CuSO_4 solution at room temperature for three hours. It amounted the level of 2.526 ± 0.137 nmol/mL in comparison with untreated level of 0.939 ± 0.055 nmol/mL (the mean $\pm$ SEM difference was 1.586 ± 0.097 , $t = 16.286$, $p < 0.00001$).

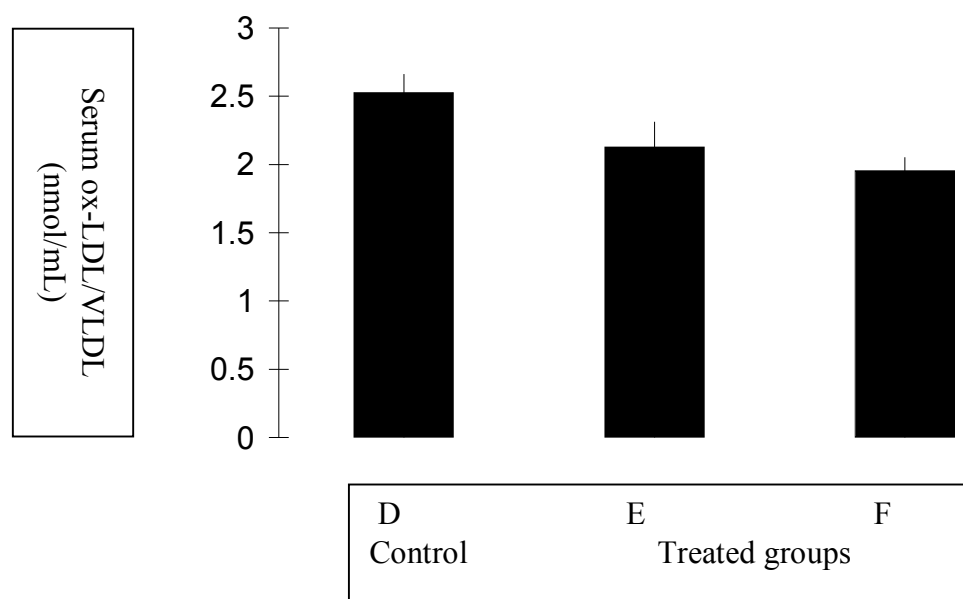
Once again, aqueous extracts of Myrtus communis L. related to both varieties showed a significant inhibition of LDL/VLDL oxidation induced by $5 \mu\text{mol}$ CuSO_4 solution (Fig.4). The level of ox-LDL/VLDL of human sera treated with aqueous extract related to variety A of Myrtus communis L. was 2.127 ± 0.187 nmol/mL in comparison with 2.526 ± 0.137 nmol/mL of untreated group (the mean $\pm$ SEM difference was

-0.399 ± 0.132 , $t = 3.022$, $p < 0.01$). The aqueous extract related to variety B of Myrtus communis L. showed a more pronounced inhibitory effect on CuSO_4 -induced LDL/VLDL oxidation. The human sera level of ox-LDL/VLDL was 1.955 ± 0.099 nmol/mL. This level is highly significant than that of untreated group (the mean $\pm$ SEM difference was -0.572 ± 0.079 , $t = 7.240$, $p < 0.0005$).

The percent of CuSO_4 - induced oxidized LDL/VLDL to LDL was 0.325 %. This percent was reduced to 0.273% and 0.251% in samples treated with aqueous solutions of Myrtus communis varieties A and B respectively.

Aqueous extracts of Myrtus communis L. related to varieties $\pm$ A and B inhibited CuSO_4 -induced oxidation of LDL/VLDL by 15.8% and 22.6% respectively. Although a higher percentage of inhibition has been observed with aqueous extract related to variety A than B of Myrtus communis L. but it did not reach the level of significance (the mean $\pm$ SEM difference was -0.2209 ± 0.1759 , t

= 1.255, $p > 0.05$).



(D) Control (untreated group).

(E) Treated group with Myrtus communis L. (variety A). P value < 0.01 in comparison with untreated group (D).

(F) Treated group with Myrtus communis L. (variety B). P value < 0.00 in comparison with untreated group (D).

Fig. 4. Shows the significant inhibitory effect of aqueous extract of Myrtus Communis L. on oxidation of human serum ox-LDL/VLDL induced by CuSO_4 .

Discussion

The results reported *herein* demonstrated that aqueous extracts of Myrtus communis L. are effective inhibitor of *in vitro* serum LDL/VLDL oxidation. Aqueous extracts of Myrtus communis L. like many herbs and natural plants seem to be able to scavenge oxidized lipids and peroxides in human sera [12-14]. This effect may be related to the polyphenolic compounds which are available in them [15]. As mentioned in several articles there was no specific phenolic compound which owned the property of scavenging the oxidized lipids i.e. phenolic compounds, of whatever nature or source have the ability of scavenging the lipid peroxides [16-18]. Moreover, the difference in the morphology of myrtus leaves was reflected on the efficacy of their extracts in scavenging the oxidized lipids. This observation could be related to the difference in concentrations of available polyphenolic compounds in both tested varieties which differed in morphology of their leaves i.e. differed in genus of Myrtus communis.

Aqueous extracts of Myrtus communis did not only scavenge the oxidized lipids in *in vitro* human

sera but also inhibited the oxidation of these lipids. The extent of this effect is more or less similar to the property of scavenging ox-LDL/VLDL. This observation was reported with a limited number of herbs or natural plants [15,16,19-22]. Several possibilities can explain this effect. One of these is the ability of aqueous extract to chelate the copper ions and prevent or retard the oxidation process [18]. The possibility of up-regulation of LDL-receptors in experimental cell lines should also be taken into consideration [17]. The other possibility is that aqueous extracts of Myrtus communis may contain specific inhibitors of LDL oxidation that is induced by CuSO_4 solution. Most of published literature concerned with the inhibition of lipid oxidation did not search for the exact mechanism of inhibition of that process.

We concluded that the aqueous extract of Myrtus communis, of whatever variety has inhibitory effects on oxidation of LDL/VLDL, whether it is native or induced by copper, that are related to its contents of polyphenolic compounds and it may be a useful

medicinal plant in the prevention of development of atherosclerosis.

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