Antiatherogenic activity of extracts of *Argania spinosa* L. pericarp: beneficial effects on lipid peroxidation and cholesterol homeostasis[1]

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Abstract: Prevention of lipoprotein oxidation by natural compounds may prevent atherosclerosis via reducing early atherogenesis. In this study, we investigated for the first time the beneficial properties of methanolic extract of argania pericarp (MEAP) towards atherogenesis by protecting human low-density lipoprotein (LDL) against oxidation while promoting high-density lipoprotein (HDL)-mediated cholesterol efflux. By measuring the formation of malondialdehyde (MDA) and conjugated diene as well as the lag phase and the progression rate of lipid peroxidation, the MEAP was found to possess an inhibitory effect. In addition, MEAP reduced the rate of disappearance of α-tocopherol as well as the apoB electrophoretic mobility in a dose-dependent manner. These effects are related to the free radical scavenging and copper-chelating effects of MEAP. In terms of cell viability, MEAP has shown a cytotoxic effect (0–40 μg/mL). Incubation of ^{3}H-cholesterol-loaded J774 macrophages with HDL in the presence of increasing concentrations of MEAP enhanced HDL-mediated cholesterol efflux independently of ABCA1 receptor pathways. Our findings suggest that argania seed pericarp provides a source of natural antioxidants that inhibit LDL oxidation and enhance cholesterol efflux and thus can prevent development of cardiovascular diseases.

Key words: methanolic extract of argania pericarp (MEAP), LDL, HDL, lipid peroxidation, cholesterol efflux.

Résumé : La prévention de l'oxydation des lipoprotéines par les produits naturels pourrait prévenir l'athérosclérose en réduisant le développement précoce de l'athérogenèse. Dans la présente étude, nous avons examiné pour la première fois les propriétés antiathérogènes d'un extrait méthanolique du péricarpe de l'arganier (EMPA) via la protection des LDL contre l'oxydation et l'augmentation de l'efflux de cholestérol véhiculé par les LDL. La mesure de la formation de malondialdéhyde (MDA) et de diènes conjugués, du temps de latence ainsi que de la vitesse de progression de la peroxydation lipidique a permis d'observer l'effet inhibiteur de l'EMPA. De plus, l'EMPA réduit la vitesse de disparition de l'α-tocophérol et la mobilité électrophorétique de l'apoB d'une manière dose-dépendante. Ces effets sont associés à la capacité de piégeage des radicaux libres et de chélation du cuivre de l'EMPA. En matière de viabilité des cellules, l'EMPA a montré un effet cytotoxique (0–40 μg/mL). L'incubation de macrophages J774 chargés en ^{3}H-cholestérol avec les HDL en présence d'une concentration élevée d'EMPA stimule l'efflux de cholestérol véhiculé par les HDL indépendamment des voies des récepteurs de ABCA1. Nos résultats portent à penser que le péricarpe des graines de l'arganier constitue une source d'antioxydants naturels capables de prévenir le développement des maladies cardiovasculaires en inhibant l'oxydation des LDL et en stimulant l'efflux de cholestérol.

Mots-clés : extrait méthanolique du péricarpe de l'arganier (EMPA), LDL, HDL, peroxydation lipidique, efflux de cholestérol.

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Introduction

Cardiovascular diseases are considered to be the principal cause of morbidity and mortality in the western world (de Lorgeril and Salen 2000). Low-density lipoprotein (LDL) peroxidation constitutes the first step in a complex process leading to atherosclerosis and to its clinical manifestations. Oxidized LDLs are not recognized by the normal LDL-receptor apolipoprotein (apo) B/E and are taken up by macrophages in a nonregulated manner through the "scavenger-receptor." This leads to cellular cholesterol accumulation and foam cell formation, the hallmarks of early atherosclerotic lesions (Witztum and Steinberg 1991) and the underlying cause of coronary heart disease (Stocker 1999).

On the other hand, high-density lipoproteins (HDLs) are considered to be antiatherogenic lipoproteins due to their effect in protecting LDL against oxidation and in promoting cholesterol efflux from macrophages (Kritharides et al. 1998). These beneficial effects are principally attributed to apoA-I and paraoxonase 1. ApoA-I is implicated in HDL-mediated cholesterol efflux especially by interacting with ABCA1 transporters, whereas paraoxonase 1 enhances this process by increasing the HDL and ABCA1 binding (Rosenblat et al. 2005). Moreover, paraoxonase 1 has been shown to produce an important antioxidant activity, which contributes significantly to the capacity of HDL to protect LDL against oxidation (Jaouad et al. 2006).

Several studies have shown that antioxidant nutrients and (or) natural medicine positively modulate the susceptibility of LDL to oxidation and enhance the antiatherogenic properties of HDL, thus playing an important role in the prevention of cardiovascular diseases (Gugliucci and Menini 2002; Hertog et al. 1993).

Argan oil, from the seeds of *Argania spinosa* L. (an endemic oleaginous plant of the southwest area of Morocco), is eaten raw and is also used in traditional medicine. Chemical analysis of this oil highlighted a glyceride fraction (99%) that is rich in polyunsaturated fatty acids like oleic (44.8%) and linoleic acid (33.7%) (Khallouki et al. 2003). Studies with the unsaponifiable fraction revealed that argan oil is rich in tocopherols (637 mg/kg, which is represented by 75% of its γ-isoform). This fraction also contains other important compounds such as squalene, sterols (schottenol and spinasterol), and phenols (ferulic, syringic, and vanillic acid) (Khallouki et al. 2003). These compounds make argan oil an important source of antioxidants, which might play an important role in the prevention of atherosclerosis.

Indeed, we have previously reported an antihypercholesterolemic and antihypertensive effect of argan oil (Berrougui et al. 2003, 2004). Treatment of hypertensive animals by argan oil prevents an increase in blood pressure and improves endothelial function by promoting the nitric oxide and cyclooxygenase pathways. Moreover, argan oil presents a high antioxidant activity associated with its content in phenolic compounds that protect LDL and HDL against lipid peroxidation. These compounds also promote the HDL capacity to mediate cholesterol efflux from macrophages (Berrougui et al. 2006).

The seed or almond of *A. spinosa* L. is enclosed in the pericarp. Chemical analysis of the pericarp has revealed it is rich in polysaccharides, mainly the xylans (Habibi and Vignon 2005). Published data indicate that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants (Ng et al. 2004). Therefore, the aim of this study was to investigate the antioxidant activity of methanolic extract of argan pericarp (MEAP) and its capacity to modulate cholesterol efflux mediated by HDL.

Materials and methods

Plant material

Fruits of *A. spinosa* were collected during summer 2005 from the southwest region of Morocco. The plant was identified by the botanical section of the poly-disciplinary Faculty of Beni Mellal in Morocco, where a specimen was preserved (number AF-0001). The extract was also preserved in our laboratory at the Research Centre on Aging, Faculty of Medicine, University of Sherbrooke, in Canada.

After removing the seeds from the fruits (endosperm), the pericarp was ground in a mixer–grinder and sieved to give a homogeneous powder. This powder was subjected to water–methanol (20:80, *v*/*v*) maceration for 6 h at 4 °C and then dried by lyophilisation. The yield of this MEAP was 2% (*m*/*m*).

Phytochemical study

Phytochemical analyses of free quinones, anthraquinones, alkaloids, terpenoids, saponosides, coumarins, flavonoids, and tannins were performed as previously described by Douhou et al. (2003).

Chemicals

Acetic acid, sulfuric acid, sodium phosphate, thiobarbituric acid, *n*-butanol, methanol, ethanol, *n*-isopropanol, and hexane were purchased from Fisher (Montreal, Que.). 1,1,3,3-tetraethoxypropane, D-α-tocopherol, DL-α-tocopherol, butylated hydroxytoluene (BHT), cupric sulfate ($CuSO_4$), ethylenediaminetetraacetic acid (EDTA), lithium perchlorate, DPH (1,6-diphenyl-1,3,5-hexatriene), 2-β-mercaptoethanol, [1α,2α(n)-^{3}H] cholesterol, [methyl-^{3}H] thymidine, DPPH (1,1-diphenyl-2-picryl-hydrazyl), and methylthiazoletetrazolium (MTT) were obtained from Sigma (St. Louis, Mo.). Dialysis bags were purchased from Spectrum Medical Industries (Houston, Tex.). J774 cells were purchased from American type Culture Collection (ATCC) (Manassas, Va.).

Biochemical study

Lipoprotein preparation

Human plasma was collected from 12 healthy volunteers (ages 20–25 years) with normal ranges of blood pressure and normal glycemic and lipid profiles. The ethics committee of the University of Sherbrooke Geriatric Institute approved the study, and all subjects gave written informed consent.

HDL and LDL isolations were performed according to the method of Sattler et al. (1994). Briefly, human plasma was collected in heparin, LDL (density range 1.019–1.063 g/mL) and HDL (density range 1.063–1.19 g/mL) were separated by 2 h of ultracentrifugation (543 200*g*) at 15 °C in a TLA

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Table 1. Phytochemical analysis of methanolic extract of argan pericarp.

Compound family	Presence
Quinones	++
Anthraquinones	++
Alkaloïds	Minor
Terpenoids	–
Saponosides	++
Coumarins	–
Flavonoids	Minor
Tannins	
Catechic	+
Gallic	–

Note: ++, present in high quantity; +, present; minor, minimal level of detection; –, not present.

100.4 rotor. Isolated lipoproteins were dialysed overnight at 4 °C with 10 mmol/L sodium phosphate buffer (pH 7). Protein concentrations were measured by the Bradford method according to the recommended procedure (Biorad, Mississauga, Ont.).

Copper-mediated LDL oxidation

Induction of LDL peroxidation was carried out as previously described by using transition metal ions as oxidizing agents (Khalil et al. 2000). Briefly, lipoproteins (LDL 100 μg/mL) were suspended in 10 mmol/L sodium phosphate buffer (pH 7) and incubated with or without MEAP (0–20 μg/mL) at different incubation times (0–8 h) at 37 °C in the presence of 10 μmol/L cupric sulphate. Oxidation reactions were stopped by cooling in an ice bath after EDTA addition and the resulting lipid peroxides were measured immediately.

Biochemical markers of lipid peroxidation

Conjugated diene formation

LDL oxidized alone or in the presence of increasing concentrations of MEAP (0 to 20 μg/mL) were continuously monitored at 234 nm to detect the formation of conjugated dienes (CDs) as previously described (Berrougui et al. 2006).

Kinetic profile parameters of LDL oxidation

The kinetic profile of lipid peroxidation curves is characterized by 3 mathematical parameters: the lag preceding rapid oxidation (lag phase), the maximal rate of oxidation (V_{max}), and the maximal accumulation of oxidation products (OD_{max}). These 3 parameters were determined as previously described by Pinchuk and Lichtenberg (2002).

α-Tocopherol measurement

LDL endogenous content of vitamin E was assayed by means of the α-tocopherol content at different oxidation times by using reverse-phase HPLC with electrochemical and UV detection (at approximately 292 nm) as previously described (Khalil et al. 2000). α-Tocopherol was assayed on a Sephasil peptide column (C_{18} 5 μm ST 4.6/250) (Pharmacia Biotech, Piscataway, N.J.). The mobile phase was made up with methanol–ethanol–isopropanol (88:24:10 by volume) containing lithium perchlorate (20 μmol/L) at a flow rate of 1 mL/min.

Relative electrophoretic mobility of LDL

The relative electrophoretic mobility of LDL (native and oxidized LDL) was used as an indicator of oxidation of the protein moiety and was measured by using agarose gel (Titan gel lipoprotein electrophoretic system). Electrophoresis was performed on 2 μL (LDL 100 μg/mL) samples in barbital buffer at pH 8.6 on 0.6% agarose gels (Helena Lab., Montreal, Que.) at a constant voltage (80 V) for 45 min, then oven dried at 75 °C. The gels were stained with 0.1% (*m*/*v*) Fat Red 7B in 95% methanol.

Free radical scavenging activity

Determination of the free radical scavenging activity of MEAP was conducted using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test as previously described (Mensor et al. 2001). Briefly, DPPH solution dissolved in ethanol was added to aqueous solution of MEAP at increasing concentrations (0 to 40 μg/mL) and allowed to react at room temperature. After 30 min, the absorbance was measured at 517 nm. DPPH containing ethanol was used as a negative control. Antioxidant activity was calculated by using the following formula:

$$\%\quad AA = 100 - \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}$$

where AA is the antioxidant activity and Abs the absorbance.

Copper-chelating capacity of MEAP

In this assay, LDLs (100 μg/mL) were oxidized during 4 h at 37 °C with increasing concentrations of copper (0, 5, 10, 15, 20, 25, and 30 μmol/L) in the presence or absence of increasing concentrations of MEAP (0 to 20 μg/mL). Oxidation level was evaluated by measuring conjugated diene formation as previously described (Berrougui et al. 2006).

Cell study

Cell culture and [^{3}H]-free cholesterol efflux measurements

J774 macrophages were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C, 5% CO_2. J774 macrophages were plated at 0.5 10^6 cells/mL. Macrophages were loaded with [^{3}H] cholesterol (2 μCi/mL) for 24 h in DMEM containing 1% FBS. The cells were then washed and incubated with 0.2% bovine serum albumin (BSA) in DMEM medium with or without 0.3 mmol/L AMPc for 12 h. To measure the effect of MEAP (0 to 20 μg/mL) on cholesterol efflux, cells were incubated for 24 h with 50 μg/mL of native-HDL in the presence or absence of MEAP. At the end of the time course, cells were centrifuged (54 320*g*, 4 °C, 20 min) to remove the medium and then lysed. Aliquots (100 μL) from the medium and cells were counted separately by using liquid scintillation counting.

Cholesterol efflux was determined by liquid scintillation counting, and the percentage of radiolabelled cholesterol re-

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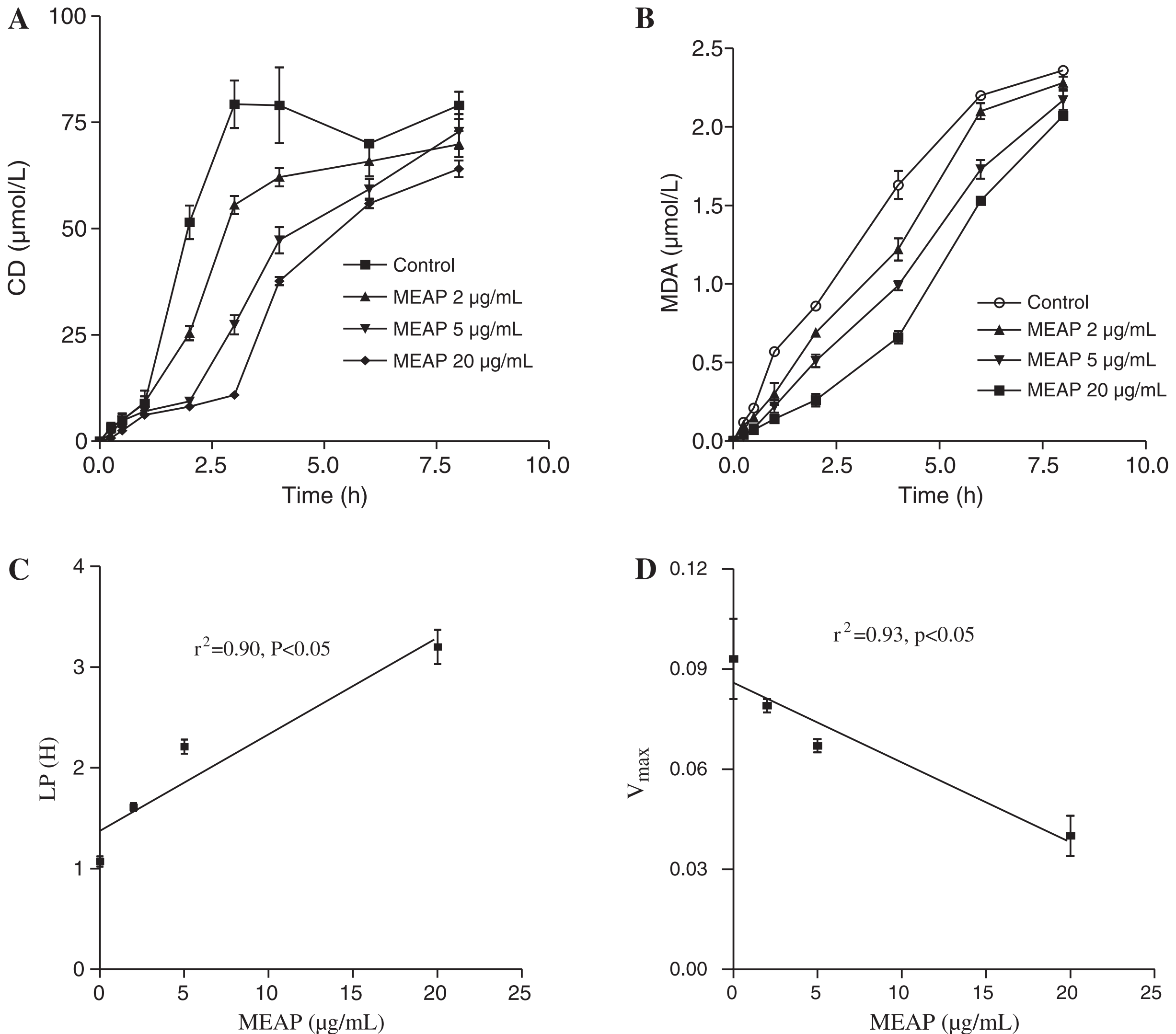
Fig. 1. Kinetics of (A) conjugated diene, (B) malondialdehyde (MDA) formation, (C) lag phase, and (D) maximal rate of LDL oxidation (V_{max}) on incubation of human LDL (100 μg/mL) with 10 μmol/L $CuSO_4$ in the presence of increasing concentrations (0–20 μg/mL) of methanolic extract of argan pericarp (MEAP). Results are means ± SE of a minimum of 3 independent experiments.



leased (% cholesterol efflux) was calculated as (cpm in medium/(cpm in the cell + medium)) × 100 (Berrougui et al. 2006).

Western blotting

Twenty-five micrograms of cell lysate proteins were loaded and electrophoresed on 7.5% SDS–PAGE. After transfer, membranes were incubated with specific antibodies against ABCA1 (Santa Cruz Biotechnology, Calif.). Anti-mouse IgG-HRP antibody (Sigma) was directed against the primary antibody and protein was subsequently revealed with enhanced chemiluminescence (ECL) reagents.

Assessment of cell viability

Cell viability was measured by quantitative colorometric assay using the tetrazolium salts MTT (Denizot and Lang 1986). After treatment of cells with increased concentrations of MEAP (0 to 40 μg/mL) for 24 h, the medium was removed and fresh medium containing MTT (0.5 mg/mL) was added to each well. Cells were then incubated for 3 h at 37 °C. After centrifugation, cells in the bottom were lysed with DMSO and followed by reading absorbance at 595 nm.

Statistical analysis

Values are expressed as the means ± SE. One-way analysis of variance (ANOVA) was used for multiple comparisons. Linear regression analysis was used to assess the association between 2 continuous variables. Statistical analyses were performed with Prism 4.0 version software.

Results

Phytochemical composition of MEAP illustrated in the Table 1 show that this extract is rich in quinons, saponosids, and tannins.

LDL oxidation was monitored by the formation of conjugated diene (CD) and thiobarbituric acid-reactive substances (TBARS) as malondialdehyde (MDA) equivalent. Oxidation of LDL results in a high production of CD and MDA ($p < 0.001$). However, this oxidation was inhibited in a

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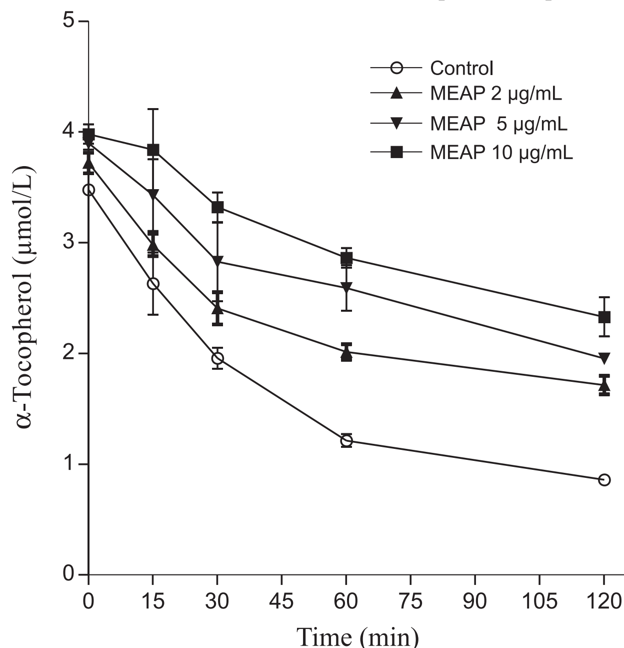
Fig. 2. Effect of increasing concentrations of methanolic extract of argan pericarp (MEAP) (0–20 μg/mL) on endogenous α-tocopherol disappearance during low-density lipoprotein (LDL) oxidation. Results are means ± SE of a minimum of 3 independent experiments.



dose-dependent manner in the presence of increasing concentrations of MEAP, as reported in Figs. 1A and 1B. Indeed, oxidation of LDL in the presence of MEAP induced a significant increase in a dose-dependent manner of lag phase ($r^2 = 0.90$, $p < 0.05$; linear regression) and a significant decrease of V_{max} ($r^2 = 0.93$, $p < 0.05$; linear regression). Figure 1D shows CD formation, whereas no significant differences were observed with respect to OD_{max}. After 3 h of LDL oxidation, CD formation was inhibited by 30%, 65%, and 86% in the presence of 2, 5, and 20 μg/mL of MEAP, respectively. The disappearance rate of LDL endogenous α-tocopherol during oxidation was also reduced in the presence of increasing concentrations of MEAP (Fig. 2).

We further investigated the effect of MEAP on the LDL electronegative charge as an indicative of the oxidative modification of the LDL-protein moiety (apoB100). Figure 3 shows that oxidation of LDL alone induces a great increase in the LDL electrophoretic mobility, whereas the presence of MEAP (20 μg/mL) during oxidation of LDL significantly reducesthis increase (Fig. 3A, 3B).

To gain more insight into the mechanism behind the antioxidant capacity of the MEAP, we investigated both the free radical-scavenging activity using DPPH test and the copper ions-chelating effect of MEAP. Free radicals scavenging activity of MEAP was determined by monitoring the decrease in DPPH absorbance at 517 nm (Fig. 4). MEAP at concentrations from 10 to 40 μg/mL reduced the DPPH absorbance by 64.315, 86.295, 90.305, and 90.61%, respectively, when compared with the control (in absence of MEAP). Moreover, MEAP shown also a dose-dependant inhibitory effect on LDL oxidation in the presence of increased concentrations of copper, which suggests that MEAP might present a chelating activity towards copper ions (Fig. 5).

Before this investigation into the effect of MEAP on the cholesterol homeostasis in J774 macrophages, we studied its effect on cells viability. The colorimetric MTT reduction assay was used to evaluate the J774 macrophages viability in the presence of MEAP at concentrations comprised between 0 and 40 μg/mL. Treatment of J774 with increased concentrations of MEAP (0 to 40 μg/mL) does not significantly affect their viability (data not shown). The maximum inhibition of cells viability was noted with MEAP at 40 μg/mL and was lower than 20%.

In light of these results, we have investigated the effect of MEAP on cholesterol homoeostasis and especially on the cholesterol efflux process. Our results show that the HDL capacity to mediate cholesterol efflux in the presence of [^{3}H]-cholesterol-loaded J774 macrophages is increased significantly in the presence of MEAP (Fig. 6A). Stimulation of ABCA1 expression in J774 results in a nonsignificant increase of cholesterol efflux indicating that MEAP promotes cholesterol efflux independently to the ABCA1 pathway. This hypothesis was confirmed by Western blot analysis in which it is shown that incubation of cells with MEAP do not affect the level of expression of ABCA1 (Fig. 6B). Moreover, incubation of HDL in the presence of the extract has no effect on the phospholipidic bilayer fluidity of HDL (results not shown).

Discussion

Oxidative modification of LDL plays a crucial role in the pathogenesis of athrosclerosis and high levels of oxidized LDL are found in various acute coronary syndromes, indicating that oxidized LDL might be a marker for atherosclerosis (Marrugat et al. 2004). LDL peroxidation is characterized by 3 principal phases: lag, propagation (V_{max}), and decomposition phases (OD_{max}). The lag phase is the initial interval between the additions of $CuSO_4$ and the beginning of the extensive lipid peroxidation process. During this process, the rate of oxidation is dependent on endogenous antioxidants in LDL (principally vitamin E). This phase is followed by the propagation phase, which is characterized by auto-acceleration and formation of oxidation products. The last phase corresponds with a complete oxidation of cholesterol and the accumulation of oxysterols (Pinchuk et al. 1998; Scheffer et al. 2000). LDL oxidation may require the generation of hydroxyl radicals produced by the Fenton reaction (Zhang et al. 1999). Copper-induced oxidation of polyunsaturated fatty acid of LDL, which results in an elevation of lipid peroxides and depletion of endogenous natural antioxidants.

Over the last decade, dietary antioxidants have attracted considerable attention as preventive and therapeutic agents towards development of atherosclerosis and cancer. Derived product from argan tree like argan oil and oil cake have been shown to be rich in antioxidant compounds (vitamin E squalene, saponin, furilic acid, sterols, etc.) (Khallouki et al. 2003) and to modulate cardiovascular disease risk factors (LDL oxidation, diabetes, hyperlipemia, and hypertension) (Berrougui et al. 2003, 2004, 2006; Samane et al. 2006). However, the composition and the antioxidant properties of argan seed pericarps have not yet been investigated.

The present study aimed to investigate chemical composi-

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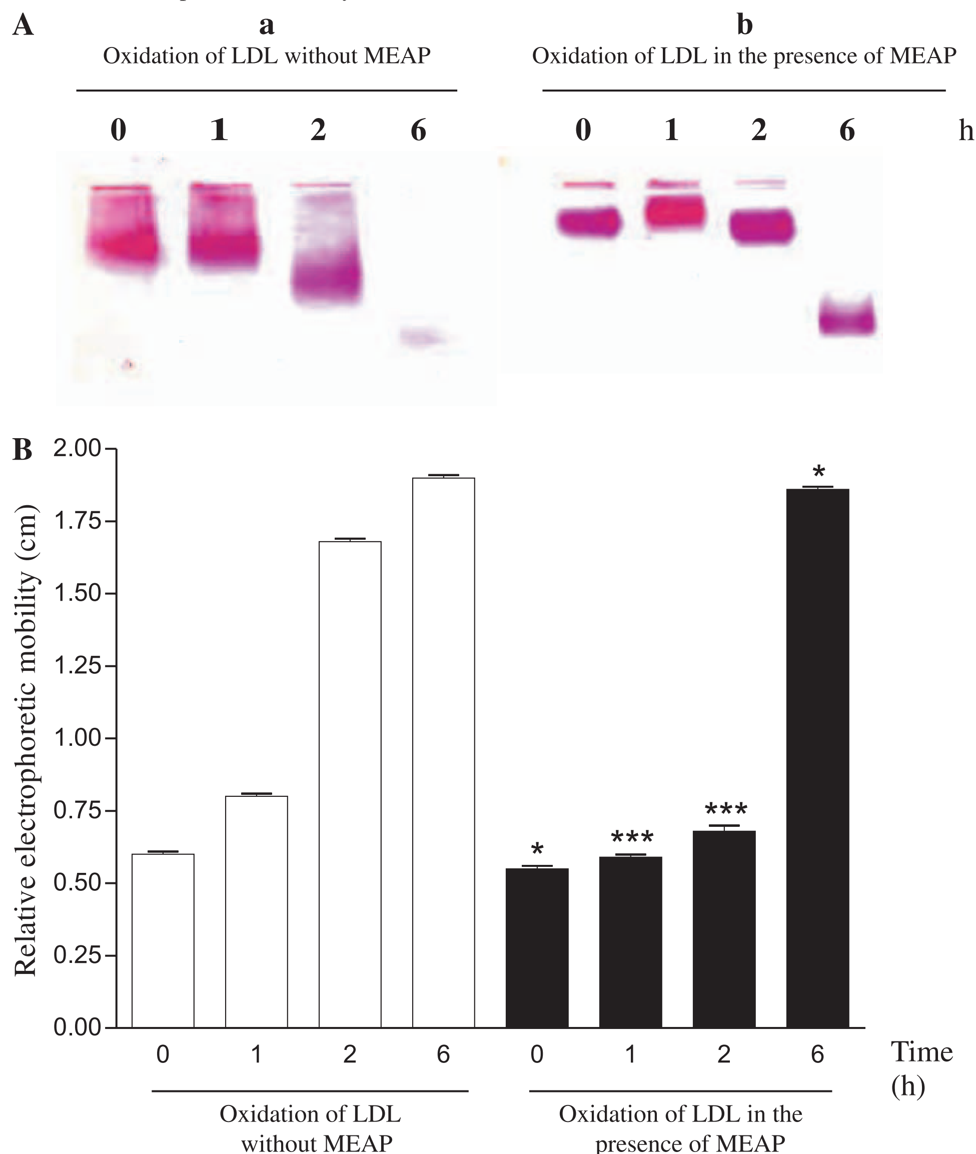
Fig. 3. (A) Electrophoretic mobility of low-density lipoprotein (LDL) on agarose gel. Samples were electrophoresed for 45 min at 80 V and then stained with Titan gel lipoprotein stain (Fat red 7B). Lanes are identified as control LDL incubated with copper (10 μmol/L) and methanolic extract of argan pericarp (MEAP)-treated LDL incubated with copper (10 μmol/L) for 0, 1, 2, and 6 h in the presence of 20 μg/mL of MEAP. (B) Data are expressed as relative electrophoretic mobility of each band at increasing oxidation time in the absence or presence of MEAP (20 μg/mL). Experiments were repeated 3 times and the gel shown is typical of the results obtained. Statistical significance at $*p < 0.05$ and $***p < 0.001$. Statistical comparison was analysed for each band (oxidated LDL vs. oxidated LDL + MEAP).



tion of MEAP and to evaluate its ability to inhibit LDL oxidation and to promote HDL-mediated cholesterol efflux capacity.

Our results showed an antioxidant activity of the extract at concentrations between 1 and 20 μmol/L. This was supported by the reduction of CD and MDA formation, extent of lag phase, and attenuation the oxidation rate formation, as well as by a decrease in the rate of vitamin E disappearance. The beneficial effect of MEAP could be attributed to its copper-chelating property leading to the inhibition of redox-inactive complexes and thus reducing metal-catalyzed oxidation. This funding is supported by the fact that copper chelators prolong the lag phase and reduce the rate of oxidation (Pinchuk and Lichtenberg 2002). In addition, this protective effect also occurs by the free radical scavenging activity of the extract as demonstrated in this work. In this case, MEAP can interact with free radicals to form a relatively stable agents and nonradical products.

It is well known that a naturally antioxidant product acts by scavenging free radicals, thus prolonging the lag phase in a dose-dependent manner without affecting the maximal rate of oxidation and the maximal accumulation of oxidized products (Pinchuk and Lichtenberg 2002). However, in our case, MEAP, in addition of its extended action on the lag phase, also reduces the rate of oxidation, which suggests that MEAP can also react as an inhibitor of apolipoprotein copper binding and subsequently preventing the modification of amino acids of apoB protein residue. This is in accordance with our results showing that MEAP abolishes the copper-induced electrophoretic shift in LDL.

These beneficial effects might be explained by the rich-

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Fig. 4. Free radical scavenging activity of methanolic extract of argan pericarp (MEAP) measured by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. MEAP was used at concentrations of 0 to 40 µg/mL and absorbance was read at 517 nm. Results are means ± SE of a minimum of 3 independent experiments. Statistical significance at $^{**}p < 0.01$ vs. control.

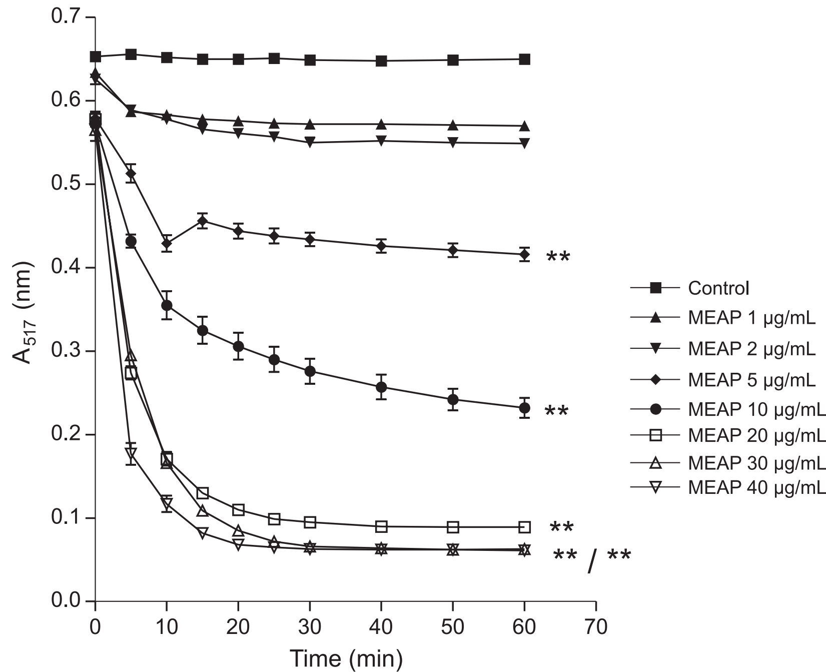
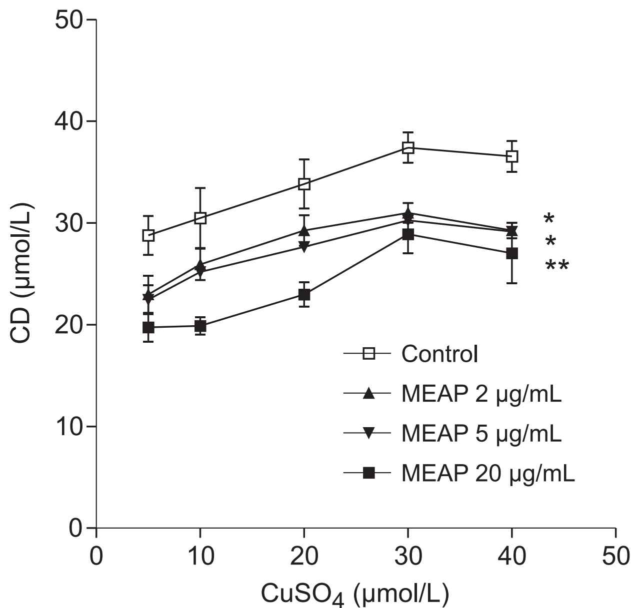


Fig. 5. Copper ion chelating effect of methanolic extract of argan pericarp (MEAP). MEAP was used at concentrations 0 to 20 µg/mL and low-density lipoprotein (LDL) oxidation was initiated at increasing concentration of $CuSO_4$ (0 to 40 µmol/L). Results are means ± SE of a minimum of 3 independent experiments. Statistical significance at $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control.



ness of MEAP in quinones, anthraquinones, and saponosides. Indeed, the free radical scavenging activity of quinones has been demonstrated (Nathan and Chaudhuri 1998). In addition, it was reported that anthraquinones also protect LDL against copper oxidation by a free radical scavenging mechanism (Zhang et al. 2005; Iizuka et al. 2004), whereas saponosides were shown to inhibit LDL peroxidation by both scavenging free radicals and chelating metal ions (Gulcin et al. 2004). Several natural antioxidants are reducing agents that interact with free radicals or reduce transition metals. In this case, MEAP components can interact with a free radical to form a relatively stable free radical and nonradical product. It may also reduce Cu^{2+} to Cu^{+}, resulting in the formation of a nonradical product and stable free radical that, under conditions of high oxidative stress, quenches another free radical. As reviewed in the literature (Pinchuk and Lichtenberg 2002), natural antioxidants acting by this mechanism are likely to inhibit both copper-induced and free radical-induced peroxidation, prolonging the lag phase in a dose-dependent manner, but without effect on the maximal rate and the maximal accumulation of oxidation products. However, in addition of its extended action on the lag phase, MEAP also reduces the V_{max}. This antioxidant profile suggests that MEAP might also act as an inhibitor of copper binding via interaction with the apolipoprotein. Such binding can inhibit peroxidation either by blocking the copper binding site or by binding to another (allosteric) site and by that reduce the binding of copper.

Unlike LDL, HDLs are considered as antiatherogenic lipoproteins. One of the more important properties of HDLs is their capacity to mediated cholesterol efflux (Kritharides et al. 1998). This process is in part dependent on the physical and chemical properties of HDL. As demonstrated by our laboratory (Berrougui et al. 2006), HDL oxidation significantly affects their capacity to promote cholesterol efflux from macrophages. This oxidation results in a reduction of HDL phospholipidic layer fluidity (Davidson et al. 1995), and alteration of paraoxonase1 activity and apoA-1 structure, leading to inhibition of the capacity of HDL to remove free cholesterol excess. In the present work, we demonstrate

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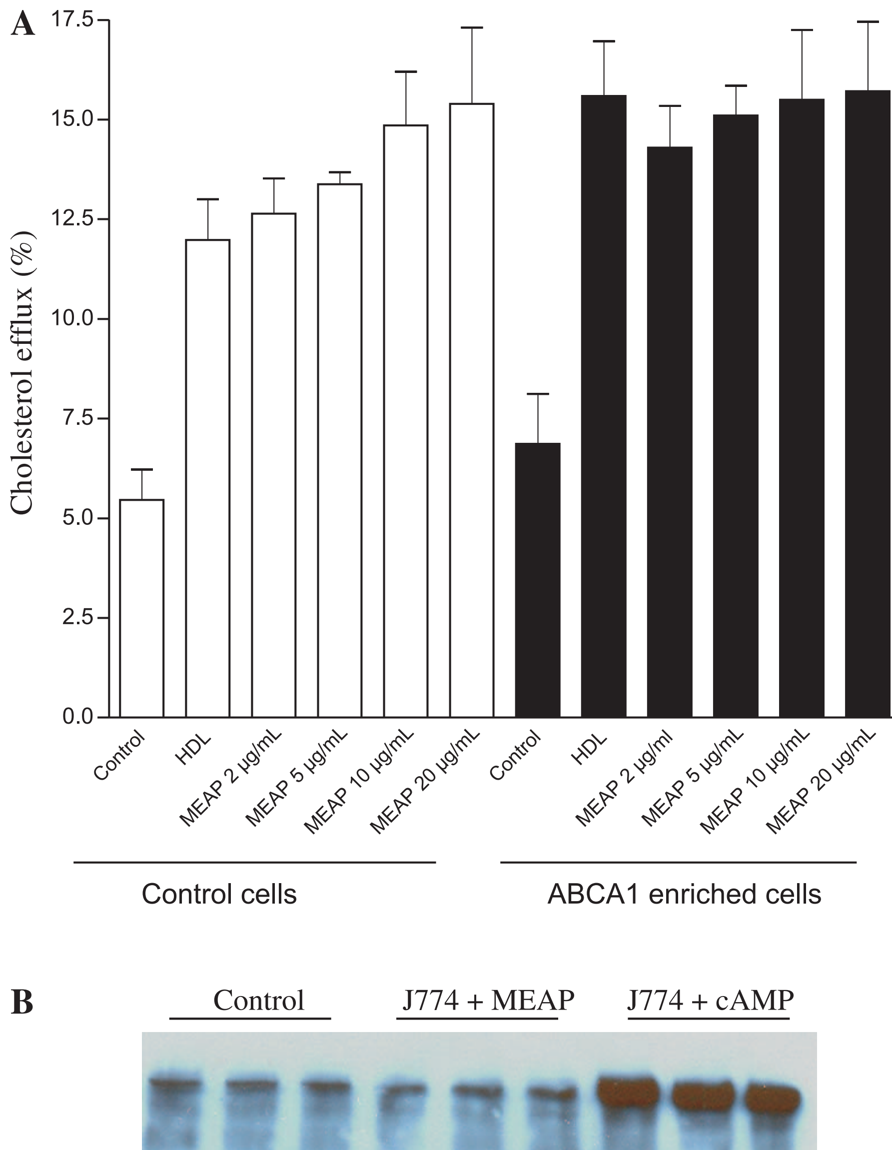
Fig. 6. Effect of methanolic extract of argan pericarp (MEAP) on HDL-mediated cholesterol efflux capacity. (A) Measure of HDL-mediated cholesterol efflux in the absence or presence of MEAP. J774 macrophages were labelled with 2 µCi/mL [^{3}H]-cholesterol for 24 h. Cells were then incubated for 12 h with FBS-free DMEM containing 0.1% BSA in the absence (control) or presence of 0.3 mmol/L cAMP (ABCA1-enriched cells). Preparations were then incubated for 24 h with HDL in the presence or absence of MEAP (0–20 µg/mL). Results are means ± SE of a minimum of 3 independent experiments. (B) ABCA1 expression was measured in J774 macrophages incubated alone (control) or in the presence of MEAP or AMPc. 20 µg of protein of lysed cells were loaded in SDS–PAGE (7.5%) and immunoblotted with ABCA1 monoclonal antibody.



that MEAP promotes HDL-mediated cholesterol efflux. This effect was independent of ABCA1 pathway because stimulation of J774-ABCA1 expression by adding AMPc-analogue did not affect the MEAP ability to promote cholesterol efflux, nor did incubation of J774 with MEAP affect ABCA1 expression. These results lead us to suggest that the components of this extract may interact in a nonselective manner with scavenger receptors like SR-BI. The most plausible hypothesis is that the extract protects HDLs against macrophage oxidation by preserving their physical and chemical properties and consequently promotes HDL-mediated cholesterol efflux. In fact, Rifici and others (Rifici and Khachadurian 2002; Rifici et al. 2002) have demonstrated that in the absence of antioxidant agents such as polyphenols, incubation of HDL with J774 macrophages increases their oxidative state and affects their capacity to remove cholesterol excess.

In conclusion, we suggest that MEAP enhances HDL-mediated cholesterol efflux and protects lipoproteins from oxidation by scavenging free radicals and chelating metals inducing oxidation. Additional studies are needed to investigate the pharmacological properties of this extract.

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