PHYTOTHERAPY RESEARCH
Phytother. Res. (2015)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5405

Consumption of Argan Oil Improves Anti-Oxidant and Lipid Status in Hemodialysis Patients

Rachid Eljaoudi,[1]*† Driss Elkabbaj,[2†] Abdelali Bahadi,[2] Azeddine Ibrahimi,[3] Mohammed Benyahia[2] and Mourad Errasfa[4]

[1]Pharmacology and Toxicology Department, Faculty of Medicine and Pharmacy, University Mohammed V, 10000 Rabat, Morocco
[2]Nephrology Department, Military Hospital Mohammed V, 10000 Rabat, Morocco
[3]Medical Biotechnologie lab (MedBiotech), Faculty of Medicine and Pharmacy, University Mohammed V, 10000 Rabat, Morocco
[4]Pharmacology Department, Faculty of Medicine and Pharmacy, University Sidi Mohamed Ben Abdellah, 30000 Fes, Morocco

Objective: Virgin Argan oil (VAO) is of interest in oxidative stress and lipid profile because of its fat composition and antioxidant compounds. We investigated the effect of VAO consumption on lipid profile and antioxidant status in hemodialysis patients after a 4-week period of consumption. Methods: In a crossover, controlled trial, 37 patients (18 men, 19 women) with end-stage renal disease on maintenance hemodialysis, were randomly assigned to a 4-week VAO diet. Fasting plasma lipids, vitamin E and oxidized LDL (ox-LDL) were analyzed. Malondialdehyde (MDA) was determined before and after hemodialysis session. Results: There was no significant change in serum total cholesterol and ox-LDL. However, VAO consumption decreased the levels of triglyceride ($p = 0.03$), total cholesterol ($p = 0.02$) and low-density lipoprotein ($p = 0.03$) and increased the levels of high-density lipoprotein ($p = 0.01$). Plasma vitamin E contents significantly increased from baseline only in VAO-group ($p < 0.001$). Hemodialysis session increased MDA levels, but the increase in VAO group was less than in control group. Conclusion: VAO consumption improved lipid profile and oxidative stress status in hemodialysis patients. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: Argan oil; chronic renal failure; oxidative stress; lipid profile.

INTRODUCTION

Chronic renal failure (CRF), a serious health problem all over the world, is characterized by a progressive decline of kidney function. Patients with CRF have three to five times higher risk of having cardiovascular event compared to the general population. This risk increases up to 20 times in hemodialysis (HD) patients. This great increase in cardiovascular risk cannot be explained entirely by traditional cardiovascular risk factors. Many studies have demonstrated that CRF is associated with oxidative stress which is proposed as a non-traditional cardiovascular risk factor in these patients (Danielski *et al.,* 2003; Ahmadpoor *et al.,* 2009; Andreucci *et al.,* 2004).

Oxidative stress (OS) refers to excessive production of reactive oxygen species (ROS) and inadequate antioxidant protection. In CRF patients under HD treatment, the formation of ROS is amplified (Mimic-Oka *et al.,* 1999; Ceballos-Picot *et al.,* 1996) damaging intracellular macromolecules, oxidizing lipids, carbohydrates, DNA or proteins (Köken *et al.,* 2004). Numerous biomarkers of OS were studied in hemodialyzed patients such as antioxidant defense systems (erythrocyte superoxide dismutase and glutathione peroxidase activities), free radical scavengers (vitamin E and β-carotene) and malondialdehyde (MDA) which is generated *in vivo* via peroxidation of polyunsaturated fatty acids. In hemodialyzed patients, most of the studies reported decreased levels of antioxidant defense systems and free radical scavengers with a rise in plasma MDA (Bonnefont-Rousselot *et al.,* 1997). HD session may also increase the level of MDA in serum (Elkabbaj *et al.,* 2012).

Virgin Argan oil (VAO) is produced from the fruits of *Argania spinosa*, which is an endemic tree of south-western Morocco. In some regions of Morocco, this noble oil is intended for everyday therapeutic use both for internal and external treatment. It has been shown that VAO has a powerful antioxidant effect because of its particular phyto-chemical composition (Drissi *et al.,* 2004; Guillaume and Charrouf, 2011; Monfalouti *et al.,* 2010). VAO is composed of 99% acylglycerides but it is rich in unsaturated fatty acids (80%) principally oleic and linoleic acids. The oleic/linoleic ratio is generally about 1.25. Interestingly, the unsaponifiable fraction (1% of the oil constituents) of VAO is mainly rich in antioxidant compounds such as tocopherols, which is present in a higher proportion (Khallouki *et al.,* 2003). Moreover, this non-glyceric fraction is rich in phenolic compounds, principally ferulic and syringic acids and some sterols such as schottenol (Cherki *et al.,* 2005).

We hypothesize that the consumption of VAO could improve OS biomarkers and lipid profile in CRF patients, which in turn may lead to protection against the development CRF complications.

The aim of this study was to evaluate the impact of VAO on OS and lipid profile in HD patients after a period of 4 weeks on VAO diet.

* Correspondence to: Rachid Eljaoudi, Pharmacology and Toxicology Department, Faculty of Medicine and Pharmacy, University Mohammed V, 10000 Rabat, Morocco.
E-mail: eljaoudi_rachid@yahoo.fr
†These authors contributed equally to this work.

Received 17 October 2014
Revised 18 May 2015
Accepted 01 June 2015

MATERIALS AND METHODS

Argan oil. VAO was purchased from a local women-cooperative production in the southwest of Morocco. The composition of the oil as shown on the bottle label was: oleic acid (47%), linoleic acid (32%) and tocopherols (44 mg/100 g).

Study design. This prospective unicenter study was conducted according to the principles of the declaration of Helsinki and was approved by the local Ethical Committee from the Faculty of Medicine and Pharmacy in Rabat, Morocco, with assigned number of 479/2012. The protocol and objectives of the study were explained to the participants, and a written informed consent was signed by all of them. All patients were with end-stage renal disease on maintenance HD. Inclusion criteria were patients on HD for at least 12 months. None of the patients received lipid-lowering agents, allopurinol, vitamin E, vitamin C or any antioxidant drugs. The patients were instructed to maintain their usual patterns of dietary intake during the study. HD process was performed with online-produced ultrapure dialysis fluid (Diasafe and heat disinfection with hot feed Fresenius Medical Care, with reverse osmosis, deionization and carbon filtration). All patients received single-use biocompatible synthetic low-flux membranes (Polyamide, Polyflux Renal Products Gambro). Blood flow rates were chosen between 300 and 350 mL/min, and ultra filtration rates were set according to individual needs. Dialysate flow rate was fixed at 500 mL/min.

Using a crossover, controlled trial, 37 patients were randomized to either group A or group B using computer-generated random numbers. In the first period, group A consumed 30 mL/day of VAO for 4 weeks taken in a single dose with bread at breakfast while group B was the control group. After 8 weeks as a washout period, the groups were reversed (Fig. 1). No placebo was used during the study because of particular smell and taste of VAO.

We determined sample size on available results from previous study (Sour *et al.,* 2012). Using G*Power software, a final sample size of 36 was needed to provide 90% power at $\alpha = 0.05$ to detect a 30% increase of serum α-tocopherol by the VAO diet.

Study parameters

Blood sample. Venous blood was collected after 12 h fast. Routine blood chemistry and levels of lipids: total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were analyzed using fresh blood samples. LDL levels were calculated using Fridewald's formula. For MDA, vitamin E and oxidized LDL analysis, samples were collected in EDTA-containing tubes. Blood venous samples (10 mL) were centrifuged at 1500 ×*g* for 10 min just after being collected. The resulting plasma samples were frozen at −80 °C until analysis.

MDA. MDA was determined by Thiobarbituric Acid Reactive Substances (TBARS) method as previously described with a slight modification (Yagi, 1976). All chemicals and reagents used (from Merck) were of analytical grade, and Milli-Q water was used for each dilution. Plasma (100 µL) was mixed to 300 µL of a 42 mM thiobarbituric acid solution and 700 µL of a phosphoric acid solution (1%). The whole volume was incubated in a water bath at 95 °C for 45 min. The reaction was then stopped at ice-cold temperature, and an equal volume of n-butanol was added to the reaction mixture. Samples were then centrifuged, and an aliquot of the supernatant was read at 532 nm. During the measurement, each sample was analyzed in duplicate. MDA was determined before and after dialysis session for each patient.

Oxidized LDL. Plasma level of oxidized LDL (ox-LDL) was measured in duplicate by a commercially available sandwich ELISA (Mercodia, Uppsala, Sweden). This assay is based on a murine monoclonal anti-body (mAb-4E6) directed against a conformational epitope in the apoB-100 moiety of LDL, which is generated as a consequence of reaction of lysine residues with aldehydes.

Vitamin E. Serum α-tocopherol was determined on all patients by high-performance liquid chromatography as described (Milne and Botnen, 1986) with some modifications. Tocopherol acetate was used as an internal standard and methanol as a mobile phase. Calibrators and controls purchased from Recipe (Munich, Germany) were used during the analysis. After sample preparation, 30 µL was injected into an ACQUITY UPLC® system coupled to an ACQUITY UPLC® Photodiode Array (Waters). The column was ACQUITY UPLC® BEH C18 1.7 µm 2.1 × 50 mm. The whole HPLC system was controlled by MassLynx Software (version 4.1). The internal standard and vitamin E peaks were detected at 295 nm.

Statistical analysis. All analyses were performed using the SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL,

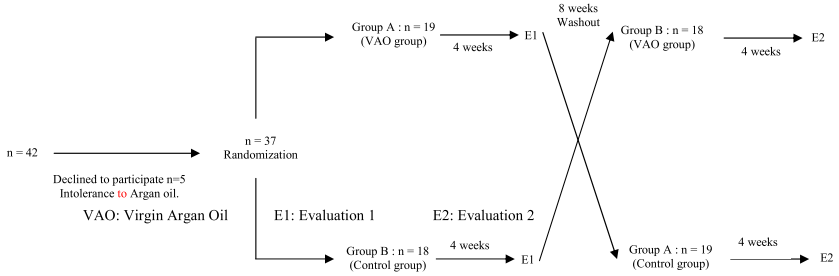

Figure 1. Study design diagram.

USA). Depending on their normal or skewed distribution, data are reported as mean ± standard deviation (SD) or median (full range). Comparison between variables was performed using the t-test, Wilcoxon's or chi square test. The analyze crossover experiment (analyses of variance for a 2 × 2 crossover study) was used to evaluate the effect of VAO diet and performed by Stata SE v.11 software. Value of $p < 0.05$ was considered statistically significant.

RESULTS

The characteristics of the patients, including sex, age, duration in HD and other baseline levels are detailed in Table 1.

Table 2 shows that the group and period had no effect on studied parameters. However, VAO consumption decreased the levels of TG ($p = 0.03$), TC ($p = 0.02$) and LDL ($p = 0.03$) and increased the levels of HDL ($p = 0.01$).

MDA, Ox-LDL and vitamin E contents, used as OS markers, were determined for each subject both at baseline and after 4 weeks either in VAO and control groups. MDA increased in blood's patient following HD session ($p < 0.05$ for all the groups and phases) but the increase in control group was more than that of VAO group ($p = 0.002$). Plasma vitamin E contents significantly increased from baseline only in VAO-group ($p < 0.001$). However, there was no effect of VAO consumption on the ox-LDL concentrations.

Table 1. Baseline characteristics of the hemodialysis patients expressed as the mean ± standard deviation or median (full range)

	Value		
Variable	Group A	Group B	*p*
Sex M/W	8/11	10/8	0.35
Age (years)	50.7 ± 16.5	47.9 ± 21.5	0.21
Duration in hemodialysis (months)	36 (6–132)	39 (13–115)	0.29
BMI (kg/m^2)	23.1 ± 2.9	23.3 ± 3.4	0.81
TG (g/L)	1.27 ± 0.46	1.35 ± 0.55	0.80
TC (g/L)	1.60 ± 0.43	1.63 ± 0.34	0.81
LDL (g/L)	1.00 ± 0.36	1.02 ± 0.35	0.63
HDL (g/L)	0.35 ± 0.08	0.34 ± 0.05	0.35
OxLDL (U/L)	33.29 (21.23–89.31)	35.15 (20.16–95.41)	0.38
Vitamin E (mg/l)	7.89 ± 2.37	8.17 ± 2.41	0.72
ΔMDA (μMol/L)	2.50 ± 0.89	2.76 ± 0.53	0.49

M/W: men/women BMI: body mass index. TG: triglyceride. TC: total cholesterol. LDL: low-density lipoprotein. HDL: high-density lipoprotein. OxLDL: oxidized Low-density lipoprotein. ΔMDA: difference between malondialdehyde level before and after hemodialysis session.
Data are reported as mean ± standard deviation or median (full range).

DISCUSSION

The aim of our study was to evaluate the impact of VAO consumption on OS and lipid profile in HD patients. We hypothesized that this diet may be beneficial to the status of OS and lipid parameters in a HD population.

After VAO diet, we noticed an increase of plasma α-tocopherol concentration accompanied by a downward trend in the levels of MDA. This result suggests an antioxidant effect of this oil and supported by previous studies (Cherki *et al.,* 2005; Drissi *et al.,* 2004). It should be noted that the main tocopherol that we can find in VAO is the γ-tocopherol. The increase of α-tocopherol in plasma could be a consequence of the eventual conversion from γ to α-tocopherol because of the close similarity between the chemical structures of both molecules (Elmadfa *et al.,* 1989). Tocopherols are molecules with strong antioxidant and free radical scavenging properties. They also act synergistically with other molecules found in VAO such as polyphenols and sterols. Polyphenol compounds might exert their antioxidant effect by acting as a ROS scavenger. This antioxidant activity is principally defined by the presence of orthodihydroxy substituent's, which stabilize radicals and chelate metals. The antioxidant effect of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule. VAO contains important phenolic compound such as ferulic acid and syringic acid, which can be more effective than ascorbic acid and tocopherols (Berrougui *et al.,* 2006).

Table 2. Effect of group, period and consumption of VAO on different studied parameters (analyses of variance for a 2 × 2 crossover study) expressed as the mean ± standard deviation or median (full range)

	Control n = 37	VAO diet n = 37	Group effect (p value)	Period effect (p value)	Traitement effect (p value)
BMI (kg/m^2)	23.2 ± 3.1	23.3 ± 3.4	0.81	0.78	0.65
TG (g/L)	1.36 ± 0.51	1.18 ± 0.52	0.44	0.15	**0.03***
TC (g/L)	1.64 ± 0.35	1.55 ± 0.33	0.69	0.50	**0.02***
LDL (g/L)	1.02 ± 0.33	0.91 ± 0.30	0.80	0.66	**0.03***
HDL (g/L)	0.34 ± 0.05	0.40 ± 0.09	0.74	0.56	**0.01***
OxLDL (U/L)	33.58(20.67–93.28)	34.29(22.43–93.08)	0.98	0.58	0.25
Vitamin E (mg/L)	8.48 ± 1.97	11.67 ± 3.05	0.37	0.06	<**0.001***
ΔMDA (μMol/L)	2.54 ± 1.07	1.76 ± 1.01	0.98	0.12	**0.002***

M/W: men/women BMI: body mass index. TG: triglyceride. TC: total cholesterol. LDL: low-density lipoprotein. HDL: high-density lipoprotein. OxLDL: oxidized low-density lipoprotein. VAO (Virgin Argan Oil). ΔMDA: difference between malondialdehyde level before and after hemodialysis session.
Data are reported as mean ± standard deviation or median (full range).
*Statistically significant.
The bold data indicates that the difference is statistically significant ($p < 0.05$).

As proposed by previous studies (Pryor and Stanley, 1975; Franckel and Neff, 1983), oxidized lipids are able to produce MDA as a decomposition product from polyunsaturated fatty acids with two or more double bonds. MDA has been found elevated in HD patients compared to normal controls because of the state of OS that accompanies this disease. Blood MDA concentration is generally increased following the dialysis session as we found it in a previous study (Elkabbaj *et al.,* 2012). This is probably because of blood cell activation by dialysis membrane during the process of HD. However, MDA increase was reduced following VAO consumption compared to control group. Several studies have shown that consumption of VAO reduces lipid peroxidation and therefore contribute to the reduction of the concentration of MDA (Cherki *et al.,* 2005; Drissi *et al.,* 2004; Berrougui *et al.,* 2006). Incubation of LDL with tocopherol, sterol and phenolic extracts of Argan oil significantly prolonged the initial lag-phase of LDL peroxidation. Also, the phenolic extract slowered the rate of lipid peroxidation and reduced the disappearance of Vitamin E in a concentration-dependent manner (Berrougui *et al.,* 2006; Drissi *et al.,* 2004).

VAO consumption was associated, in addition, with a low levels of TC ($p = 0.02$), LDL ($p = 0.03$) and a high level of HDL ($p = 0.01$). The same results were found by previous studies (Cherki *et al.,* 2005; Drissi *et al.,* 2004; Sour *et al.,* 2012). The decrease of cholesterol level may be because of not only unsaturated fatty acid contained in Argan oil, but also to minor compounds such as sterols. Indeed, sterols have molecular structure very similar to that of human cholesterol, so they reduce cholesterol absorption by mixing with the micelles and blocking cholesterol from doing so (Drissi *et al.,* 2004). Our results showed that HDL levels are significantly higher after VAO diet. HDL is antiatherogenic lipoproteins that are implicated in the protection of LDL against oxidation (Mackness *et al.,* 1993). Paroxonase 1 (PON1) has been targeted as the principal enzyme contained within HDL and responsible for their antioxidant effect (Blatter *et al.,* 1993). It has been shown that, VAO consumption increases the PON1 activity and decreases the susceptibility of LDL to lipid peroxidation (Cherki *et al.,* 2005). However, in our study, although vitamin E levels were found to be increased, we found no difference between the levels of ox-LDL before and after VAO consumption. This result could be because of the fact that VAO consumption may not affect *in vivo* LDL oxidation and/or that vitamin E was not incorporated enough in LDL to influence their level of oxidation. Interestingly, a clinical trial (Drissi *et al.,* 2004) has shown that in healthy volunteers, consumption of Argan oil has induced an increase of blood vitamin E levels and a decrease of LDL levels, though no difference of LDL oxidation *in vitro* was found when compared to LDL from control volunteers. Instead, another clinical study have shown that vitamin E supplementation in HD patients (Islam *et al.,* 2000) proved to be effective in protecting *in vitro* oxidation of LDL. The possible anti-oxidant effect of VAO *in vivo* may not be the same on the different molecular species, as we have found an effect on lipid peroxidation (MDA levels), and no effect was found on ox-LDL levels upon VAO consumption.

One of the limitations of our study was sample size that could have limited the ability to find additional or stronger association between VAO diet and studied parameters. However, this pilot study showed for the first time the beneficial effect of VAO consumption in HD patients by improving the status of vitamin E and lipid profile on one hand. On the other hand, our data show that dialysis membrane-related MDA production during HD process is reduced following VAO consumption.

This study should be complemented by large-scale trials but, based on these preliminary results, we can recommend the consumption of this oil to this category of patients as a natural antioxidant.

Acknowledgements

This work was supported by a grant from the University of Sidi Mohamed Ben Abdellah, Faculty of Medicine and Pharmacy, Fez, Morocco.

Conflict of Interest

None declared

REFERENCES

Ahmadpoor P, Eftekhar E, Nourooz-Zadeh J, Servat H, Makhdoomi K, Ghafari A. 2009. Glutathione, glutathione-related enzymes, and total antioxidant capacity in patients on maintenance dialysis. *Iran J Kidney Dis* **3**: 22–27.

Andreucci VE, Fissell RB, Bragg-Gresham JL, *et al*. 2004. Dialysis Outcomes and Practice Patterns Study (DOPPS) data on medications in hemodialysis patients. *Am J Kidney Dis* **44**: 61–67.

Berrougui H, Cloutier M, Isabelle M, Khalil A. 2006. Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. *Atherosclerosis* **184**: 389–396.

Blatter MC, James RW, Messmer S, Barja F, Pometta D. 1993. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur J Biochem* **211**: 871–879.

Bonnefont-Rousselot D, Jaudon MC, Issad B, *et al*. 1997. Antioxidant status of elderly chronic renal patients treated by continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant* **12**:1399–1405.

Ceballos-Picot I, Witko-Sarsat V, Merad-Boudia Nguyen AT, *et al*. 1996. Glutathione antioxidant system as a marker of oxidative stress in chronic renal failure. *Free Radic Biol Med* **6**: 845–853.

Cherki M, Derouiche A, Drissi A, *et al*. 2005. Consumption of argan oil may have an antiatherogenic effect by improving paraoxonase activities and antioxidant status: intervention study in healthy men. *Nutr Metab Cardiovasc Dis* **15**: 352–360.

Danielski M, Ikizler TA, McMonagle E, *et al*. 2003. Linkage of hypoalbuminemia, inflammation, and oxidative stress in patients receiving maintenance hemodialysis therapy. *Am J Kidney Dis* **42**: 286–294.

Drissi A, Girona J, Cherki M, *et al*. 2004. Evidence of hypolipemiant and antioxidant properties of argan oil derived from the argan tree (*Argania spinosa*). *Clin Nutr* **23**: 1159–1166

Elkabbaj D, Bahadi A, Cherrah Y, Errasfa M, Eljaoudi R. 2012. Impact of improving quality of dialysis fluid on oxidative stress and lipid profile in hemodialysis patients. *ISRN Nephrol* **19**: 717849.

Elmadfa I, Kim SW, Reutlinger M, Siewert R. 1989. The transformation of gamma-tocopherol to alpha-tocopherol in the animal organism: a generational study in rats. *Z Ernahrungswiss* **28**: 36–48.

Franckel EN, Neff WE. 1983. Formation of malonaldehyde from lipid peroxidation products. *Biochim Biophys Acta* **754**: 264–270

Guillaume D, Charrouf Z. 2011. Argan oil. Monograph. *Altern Med Rev* **16**: 275–279.

Islam KN, O'Byrne D, Devaraj S, Palmer B, Grundy SM, Jialal I. 2000. Alpha-tocopherol supplementation decreases the oxidative susceptibility of LDL in renal failure patients on dialysis therapy. *Atherosclerosis* **150**: 217–224.

Khallouki F, Younos C, Soulimani R, *et al*. 2003. Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemo preventive effects. *Eur J Cancer Prev* **12**: 67–75.

Köken T, Serteser M, Kahraman A, Gökçe C, and Demir S. 2004. Changes in serum markers of oxidative stress with varying periods of hemodialysis. *Nephrology* **2**: 77–82.

Mackness MI, Abbott CA, Arrol S. 1993. The role of high-density lipoprotein and lipid soluble antioxidant vitamins in inhibiting low density lipoprotein oxidation. *Biochem J* **294**: 829–834.

Milne DB, Botnen J. 1986. Retinol, α-tocopherol, lycopene, and α- and β-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clin Chem* **32**: 874–876.

Mimic-Oka J, Simic T, Djukanovic L, Reljic Z, Davicevic Z. 1999. Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. *Clin Nephrol* **4**: 233–241.

Monfalouti HE, Guillaume D, Denhez C, Charrouf Z. 2010. Therapeutic potential of argan oil: a review. *J Pharm Pharmacol* **62**: 1669–1675.

Pryor WA, Stanley JP. 1975. Letter: A suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Non enzymatic production of prostaglandin endoperoxides during autoxidation. *J Org Chem* **40**: 3615–3617.

Sour S, Belarbi M, Khaldi D, *et al*. 2012. Argan oil improves surrogate markers of CVD in humans. *Br J Nutr* **107**:1800–1805.

Yagi K 1976. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* **15**: 212–216.