

ORIGINAL ARTICLE

# Tocopherols and Saponins Derived from *Argania spinosa* Exert, an Antiproliferative Effect on Human Prostate Cancer

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## ABSTRACT

The aim of our study is to evaluate the antiproliferative effect of tocopherols obtained from alimentary virgin argan oil extracted from the endemic argan tree of Morocco and of saponins extracted from argan press cake on three human prostatic cell lines (DU145, LNCaP, and PC3). The results were compared to 2-methoxyestradiol as antiproliferative drug candidates. Cytotoxicity and antiproliferative effects were investigated after cells' treatment with tocopherols and saponins compared to 2-Methoxyestradiol as the positive control. Tocopherols and saponins extracted from argan tree and 2-methoxyestradiol exhibit a dose-response cytotoxic effect and an antiproliferative action on the tested cell lines. The best antiproliferative effect of tocopherols is obtained with DU145 and LNCaP cell lines (28  $\mu\text{g/ml}$  and 32  $\mu\text{g/ml}$ , respectively, as  $\text{GI}_{50}$ ). The saponins fraction displayed the best antiproliferative effect on the PC3 cell line with 18  $\mu\text{g/ml}$  as  $\text{GI}_{50}$ . Our results confirm the antiproliferative effect of 2-methoxyestradiol and show for the first time the antiproliferative effect of tocopherols and saponins extracted from the argan tree on hormone-dependent and hormone-independent prostate cancer cell lines. These data suggest that argan oil is of potential interest in developing new strategies for prostate cancer prevention.

## INTRODUCTION

Prostate cancer (PC) is among the most frequent metastatic (1) malignancies contributing to cancer deaths in males (1, 2). The exact causes of PC are not known. In addition, the tumor

development of the human prostate gland is critically dependent on the deregulated balance between various different growth factor regulators (3, 4). It strongly is suggested, however, that differences in the incidences of clinical prostate cancer and the passage from an indolent tumor to a clinically detected tumor are not due to genetic variations but to environmental and dietary factors. For example, although the incidence of PC is similar in diverse geographical areas (5), PC mortality rates are 4–5 times higher in Northern European countries and the United States than in Japan. Compared with the rates observed in Japan, the incidence of PC in Japanese men who migrated to the United States increased 4–9 times within one generation (6).

Dietary modifications for PC prevention continue to gain attention following demonstrations that various dietary nutrients/supplements such as fatty acids (7, 8) and antioxidants (9–12) are related to a reduced risk of developing PC. In this respect, several antioxidant plant compounds such as green tea, olive oil, and wine have been well individualized. Of all these nutrients, those having the most beneficial influence on PC prevention are lycopene, polyphenols, vitamin E and selenium (13–16).

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**Keywords:** Argan tree, Virgin argan oil, Tocopherols, Saponins, 2-Methoxyestradiol, Prostate cancer, Antiproliferative effect.

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Virgin argan oil is extracted from the pit of argan tree, *Argania spinosa*, which is endemic to southwestern Morocco. We evaluated the effect of tocopherols obtained from virgin argan oil and saponins extracted from the argan press cake, on proliferation in three human prostatic cell lines (PC3, DU145, and LNCaP) as a possible means of diet-associated prostate cancer prevention. This oil is rich in unsaturated fatty acids (45 percent oleic and 37 percent linoleic) and minor compounds (tocopherols, polyphenols, sterols, carotenoids, xanthophylls, and squalene). The unsaponifiable fraction is very abundant in these compounds, which have powerful antioxidant effects as proved on isolated LDL from human plasma (17). These interesting findings, with regard to the prevention of LDL oxidation due to the rich minor compounds, provoked our interest in investigating virgin argan oil's antiproliferative effect on PC. The results are compared to 2-methoxyestradiol (2ME<sub>2</sub>) as antiproliferative drug candidate (18).

## MATERIALS AND METHODS

### Cell lines

Three human prostatic cell lines (PC3, DU145, and LNCaP) had grown as monolayers in RPMI 1640 medium (Invitrogen, Cergy Pontoise, France) supplemented with 5 percent fetal bovine serum (FBS, Invitrogen) and 2 mM L-glutamine (Invitrogen), at 37°C with 5 percent CO<sub>2</sub> and 95 percent humidity. The human prostate cancer cell lines LNCaP, DU145, and PC3 were purchased from the American Type Culture Collection.

### Argan compounds extraction

The virgin argan oil used in this study was extracted by a cold pressing process (19); its chemical composition is listed in Table 1 (20). The fruit provided is from the city of Essaouira in southwestern Morocco. The argan oil used was in its rough state,

without any preliminary processing. In order to investigate the antiproliferative effect of this oil, we analyzed the unsaponifiable fraction that represents one percent of the total mass of the oil, from which we extracted the tocopherols. The tocopherols were separately obtained after extraction of the unsaponifiable fraction of argan oil, which was obtained using the hexane extraction method. Using the HPLC technique, the purified fraction of tocopherols was reconstituted in chloroform (20). While the saponins were isolated from the MeOH extract of the argan press cake (21).

### Steroid

2-Methoxyestradiol (2ME<sub>2</sub>) was provided by Steraloids Laboratory (New Hampshire, USA). A stock solution was prepared in DMSO (Sigma, Saint-Quentin Fallavier, France) and stored at -20°C. 2ME<sub>2</sub> is synthesized in vivo by hydroxylation at the 2-position of estradiol and subsequent O-methylation by catechol-O-methyltransferase (COMT) (22, 23).

### Methyl Thiazolyl Tetrazolium (MTT) assay

PC3, DU145, and LNCaP cell lines were seeded on 96-well plates at density of 10,000 cells/well in a total volume of 100  $\mu$ l. Cells were incubated for 24 hours prior to addition of saponins, tocopherols and 2ME<sub>2</sub>. The different media were changed 24 hours after. Saponins, tocopherols, and 2ME<sub>2</sub> were added at 7 different concentrations between 1 and 100  $\mu$ g/ml. The amounts of DMSO used to dissolve tocopherols and saponins were the same as in the control plate. The maximum final concentration of DMSO was 1  $\mu$ l per ml of culture medium (0.1% v/v).

Living cells were counted at 48 hours using the MTT method (24). Cells were incubated for 2 hours at 37°C with 1 mg/ml MTT (Sigma). MTT was then discarded and replaced by isopropanol. Optical density (OD) was measured on an EL800 universal microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 550 nm. IC<sub>50</sub> was defined as the concentration entailing a 50 percent reduction of OD in comparison with the controls.

### Tritiated thymidine incorporation (3HdThd)

PC3, DU145, and LNCaP cell lines were cultured on 24-well plates at a density of 20,000 cells/well in a total volume of 1 ml. After plating in 24-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) and 24-hour incubation, cells were treated with saponins, tocopherols, and 2ME<sub>2</sub> for 24 hours at different concentrations between 1 and 100  $\mu$ g/ml. A 2 hour pulse of 2  $\mu$ Ci/ml <sup>3</sup>[H] thymidine was then applied to cells. Thereafter, cells were incubated for 10 minutes at 4°C in 10 percent trichloroacetic acid (TCA), washed 3 times with phosphate buffered saline (PBS) pH 7.4 and lysed in 200  $\mu$ l of 0.2 M NaOH/1% SDS solution. Radioactivity incorporated in the cells was measured by scintillation counting for one minute on Beckman LS 6000SC scintillation counter (Beckman Coulter, Roissy, France). The GI<sub>50</sub> was defined as the concentration inhibiting growth by 50 percent compared to the controls.

**Table 1.** Composition of virgin argan oil (20)

Fatty acid	%
C16:0	13.4
C18:0	5.1
C18:1 n-9	44.8
C18:2 n-6	35.7
C18:3 n-3	0.1
Sterols	mg/100 g oil
Schottenol	142
Spinasterol	115
Stigmasta-8,22-dien-3 $\beta$ -ol	9
Others	29
Tocopherols	mg/kg oil
$\alpha$	35
$\beta$	122
$\gamma$	480
Phenolic compounds	$\mu$ g/kg oil
Vanillic acid	67
Syringic acid	37
Ferulic acid	3147
Tyrosol	12

**Table 2.** Relative cytotoxicity of compounds extracted from argan and 2-methoxyestradiol, (2ME<sub>2</sub> used as the control), in 3 human cancer cell lines (measured after 48 hours)

Cell lines	IC <sub>50</sub> , $\mu\text{g/ml}$		
	Tocopherols	Saponins	2ME <sub>2</sub>
DU145	24.89 $\pm$ 3.05	35.08 $\pm$ 2.39	20.11 $\pm$ 1.84
LNCaP	20.04 $\pm$ 1.32	50.34 $\pm$ 4.19	15.63 $\pm$ .32
PC3	59.54 $\pm$ 4.12	24.24 $\pm$ 2.84	6.16 $\pm$ 1.17

IC<sub>50</sub>, the concentration that inhibits growth of cell by 50 percent versus that of control cells. Data are presented as the mean  $\pm$  SD of 3 separated experiments on each cell line.

### Statistics

Statistical analysis was performed using stat view software (SAS Institute, Inc., Cary, NC, USA). The results are reported as mean  $\pm$  SD.

## RESULTS

### Relative cytotoxicity of saponins, tocopherols, and 2ME<sub>2</sub>

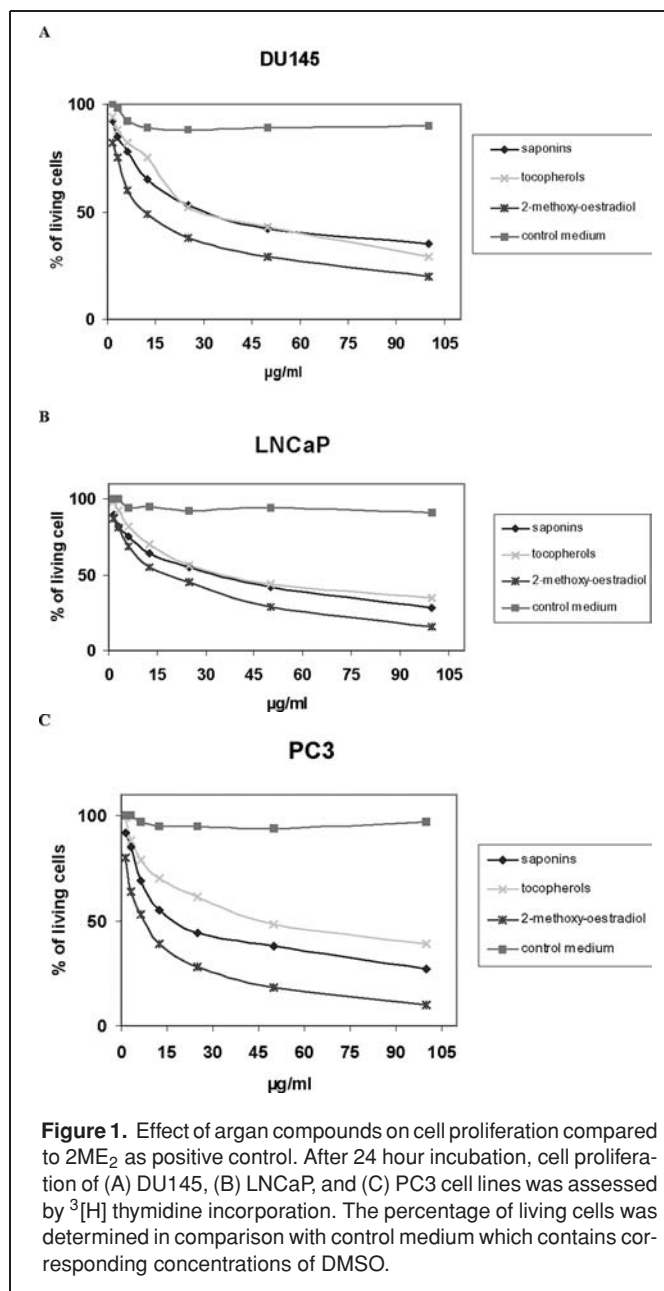
The relative cytotoxic activity of saponins, tocopherols, and 2ME<sub>2</sub> on human tumor cells was measured by means of a MTT assay in human cancer cells including the DU145, LNCaP, and PC3 lines. Table 2 summarizes the mean IC<sub>50</sub> values for saponins, tocopherols, and 2ME<sub>2</sub>. The data indicated that tocopherols, saponins, and 2ME<sub>2</sub> all effectively inhibited the growth of the 3 human tumor cells after 48 hours. For the DU145 and LNCaP cell lines, the most cytotoxic effect was obtained with the tocopherol fraction at 24.89  $\mu\text{g/ml}$  and 20.04  $\mu\text{g/ml}$ , respectively, as IC<sub>50</sub>. The saponins fraction showed the most cytotoxic effect on the PC3 cell line with 24.2  $\mu\text{g/ml}$  as IC<sub>50</sub>.

### Saponins, tocopherols, and 2ME<sub>2</sub> inhibit cell proliferation

Saponins, tocopherols, and 2ME<sub>2</sub> showed a dose-dependant antiproliferative effect on DU145, LNCaP, and PC3 cell lines (Figure 1). The calculated GI<sub>50</sub> at 24 hours on DU145, LNCaP, and PC3 cell lines were, respectively, 28, 32, 48  $\mu\text{g/ml}$  for tocopherols, 26, 30, 18  $\mu\text{g/ml}$  for saponins, and 12, 11, 6  $\mu\text{g/ml}$  for 2ME<sub>2</sub>. Proliferation in the 3 cell lines was more inhibited by 2ME<sub>2</sub> than tocopherols and saponins.

## DISCUSSION

Several studies have focused on the antioxidant effects of various dietary substances on the prevention of prostate cancer. Natural antioxidants in vegetable foods such as tea, olive oil, and wine are believed to be responsible for the reduced risk of PC observed in association with these foods (9, 11, 15). Evidence supporting this hypothesis is based on epidemiological observations, animal case-studies, and cell culture experiments.



**Figure 1.** Effect of argan compounds on cell proliferation compared to 2ME<sub>2</sub> as positive control. After 24 hour incubation, cell proliferation of (A) DU145, (B) LNCaP, and (C) PC3 cell lines was assessed by <sup>3</sup>[H] thymidine incorporation. The percentage of living cells was determined in comparison with control medium which contains corresponding concentrations of DMSO.

Argan tree compounds also can be an important dietary source of antioxidants (20). Argan oil—a product from the fruit of the argan tree produced and consumed largely in southwestern Morocco—is known for its cosmetic, pharmaceutical, and nutritional virtues. It has been demonstrated that compounds such as tocopherols, sterols, and polyphenols from argan oil have an antioxidative effect (17, 25), while saponins from argan cake press have antiinflammatory properties (26), suggesting a possible role in inhibiting carcinogenesis. To our knowledge, no study of the effects of argan-derived saponins or tocopherols on prostate cancer has been reported yet.

In the present study, we investigated the antiproliferative effect of tocopherols obtained from virgin argan oil and saponins extracted from argan press cake compared with 2ME<sub>2</sub> as an

antiproliferative drug candidate. We have studied the compounds' viability and proliferation in two hormone-independent (DU145 and PC3) and one hormone-dependent (LNCaP) human prostate cell lines. Our results indicate that tocopherols and saponins display cytotoxic activity and exert an inhibitory effect on the proliferation of the three prostatic cell lines. The effect is not the same for the three cell lines; LNCaP was clearly the most sensitive of the three. These results are significant because tocopherols' role as a candidate molecule for the prevention of prostate cancer have been well documented (27, 28).

Venkateswaran et al. (28) demonstrated that physiological concentrations of vitamin E induced cell cycle arrest mediated by up-regulation of the P27 cell cycle regulatory protein. This observation provides a theoretical basis for the putative chemopreventive effect of vitamin E. Also, such clinical trials as the SELECT study (29, 30), the second large-scale study of chemoprevention for prostate cancer which included more than 32,000 men, has suggested that both selenium and vitamin E have potential efficiency in prostate cancer prevention. Considering  $\gamma$ -tocopherols in comparison with all vegetable oils, argan oil seems to be a unique  $\gamma$ -tocopherols-rich oil. Thus, it would be possible that  $\gamma$ -tocopherols extracted from argan oil could have more benefit in chemopreventive action against cancer of the prostate. Taken together, our data are promising for the future use of argan oil in patients developing prostate cancer. The use of  $\gamma$ -tocopherols should be considered for study in future prevention trials.

Only a few studies have showed an antiproliferative effect of saponins on human prostate cancer, notably from ginseng (31, 32). As reported in the literature, 7 major saponins have been identified in argan press cake, which is rare in vegetable oils (33). Saponins isolated from argan press cake are specific and chemically different from those extracted from ginseng and soy. In our study, the saponins isolated from the press cake exhibited cytotoxic activity and inhibited proliferation of LNCaP, DU145, and PC3 cell lines. Our observations that saponins' antiproliferative effect is dose dependant and that PC3 was the most sensitive cell line are similar to those of Kim et al. (31) and Liu et al. (32), who reported the antiproliferative activity of ginseng saponins in the LNCaP and PC3 cell lines. They suggested that ginseng saponins activate the expression of P21 and P27 as cyclin kinase inhibitors. Also, these saponins blocked LNCaP cells at G1 phase and subsequently inhibited cell growth. Since the chemical structure of argan saponins is different from that of other saponins, it is expected that argan saponins may act with a different mechanism. Thus, the argan saponins could offer a new opportunities for cancer prevention. We believe it would be of use to investigate all argan saponins separately in order to elucidate the role of each in prostate cancer prevention.

Before drawing final conclusions on the mechanisms of the anticancer effects of tocopherols and saponins derived from the argan tree, further investigations concerning the cell proliferation and apoptosis would be necessary and of interest.

Our study suggests for the first time that argan tree compounds could play a role in developing new strategies for the

prevention and treatment of prostate cancer. Argan oil and argan press cake constitute additional products that reduce prostate cancer risk factors and delay the onset of prostate carcinogenesis.

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