Prevention of Urinary Tract Infection with Oximacro®, A Cranberry Extract with a High Content of A-Type Proanthocyanidins: A Pre-Clinical Double-Blind Controlled Study

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Purpose: Urinary tract infections (UTIs) are widespread and affect a large portion of the human population. Cranberry juices and extracts have been used for UTI prevention due to their content of bioactive proanthocyanidins (PACs), particularly of the A type (PAC-A). Controversial clinical results obtained with cranberry are often due to a lack of precise determination and authentication of the PAC-A content. This study used Oximacro® (Biosfered S.r.l., Turin, Italy), a cranberry extract with a high content of PAC-A, to prevent UTIs in female and male volunteers.

Materials and Methods: The Oximacro® PACs content was assayed using the Brunswick Laboratories 4-dimethylaminocinnamaldehyde (BL-DMAC) method, and the dimer and trimer PACs-A and PACs-B percentages were determined via high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS). A balanced group of female (ranging from 19 to over 51 years) and male volunteers (over 51 years) was divided into two groups. The experimental group received 1 capsule containing Oximacro® (36 mg PACs-A) twice per day (morning and evening) for 7 days, and the placebo group was given the same number of capsules with no PACs.

Results: Analysis of Oximacro® revealed a high total PAC content (372.34 mg/g ± 2.3) and a high percentage of PAC-A dimers and trimers (86.72% ± 1.65). After 7 days of Oximacro® administration, a significant difference was found between the placebo and Oximacro® groups for both females (Mann-Whitney *U*-test = 875; $P < .001$; n = 60) and males (Mann-Whitney *U*-test = 24; $P = .016$; n = 10). When the female and male age ranges were analysed separately, the female age range 31-35 showed only slightly significant differences between the placebo and Oximacro® groups (Mann-Whitney *U*-test = 20.5; $P = .095$; n = 10), whereas all other female age ranges showed highly significant differences between the placebo and Oximacro® groups (Mann-Whitney *U*-test = 25; $P = .008$; n = 10). Furthermore, colony forming unit/mL counts from the urine cultures showed a significant difference ($P < .001$) between the experimental and the placebo groups (SD difference = 51688; df = 34, t = -10.27; Dunn-Sidak Adjusted $P < .001$, Bonferroni Adjusted $P < .001$).

Conclusion: Careful determination of the total PAC content using the BL-DMAC method and the authentication of PACs-A with mass spectrometry in cranberry extracts are necessary to prepare effective doses for UTI prevention. A dose of 112 mg Oximacro® containing 36 mg PACs-A was found to be effective in preventing UTIs when used twice per day for 7 days.

Keywords: urinary tract infections; prevention & control; plant extracts; pharmacology; humans; urinalysis; therapeutic use; *Vaccinium macrocarpon*.

INTRODUCTION

Urinary tract infections (UTIs) are widespread and affect a large portion of the human population. Approximately 13 million women in the United States and approximately 150 million people worldwide develop UTIs each year, with societal costs of approximately 3.5 billion USD per year in the USA alone.[1] An estimated 40% of women develop at least one UTI during their lifetimes.[2] UTIs refer to the presence of a certain threshold number of bacteria in the urine (usually > 10^5/mL) and consist of cystitis (or lower UTIs, with bacteria in the bladder), urethral syndrome and pyelonephritis (or upper UTIs, with infection of the kidneys).[3] Bacterial cystitis (also called acute cystitis) can occur in women and men, and the signs and symptoms include dysuria (pain on passing urine), frequency, cloudy urine, and occasionally haematuria (blood in the urine); bacterial cystitis is also often associated with pyuria (urine white cell count > 10^4/mL). Some people also develop recurrent UTIs with an average of two to three episodes/year.[4]

The berries of cranberry (*Vaccinium macrocarpon* Ai-

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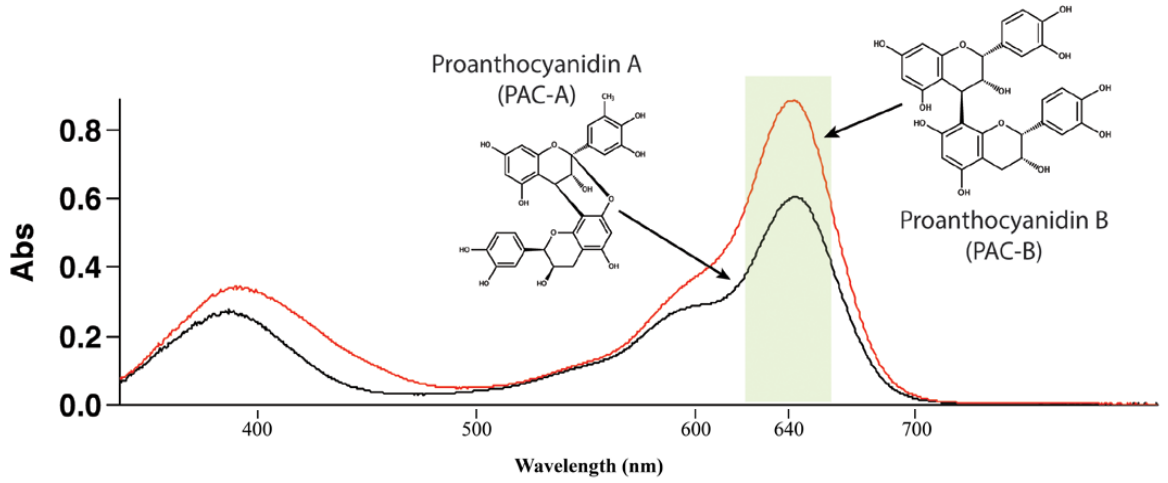

Figure 1. Spectral analysis of the BL-DMAC reaction of PAC-A (black line) and PAC-B (red line). When tested at the same concentration (20 µg mL^{-1}), PAC-B showed a higher absorbance (at 640 nm) than PAC-A.
Abbreviations: PAC, proanthocyanidins; BL-DAMC, Brunswick Laboratories 4-dimethylaminocinnamaldehyde.

ton) have been used for hundreds of years as a remedy for diseases of the urinary tract and have attracted attention due to their potential health benefits.[5,6] The beneficial mechanism of cranberry was historically thought to be due to the fruit's acids causing a bacteriostatic effect in the urine. However, recently, a group of proanthocyanidins (PACs) with A-type linkages (PAC-A) was isolated from cranberries and shown to exhibit bacterial antiadhesion activity against both antibiotic-susceptible and -resistant strains of uropathogenic P-fimbriated *Escherichia coli (E. coli)* bacteria, including multidrug-resistant *E. coli*.[7-10] Central in the efficacy of cranberry extract/juice is the determination of the optimum dose of PAC-A, which is an essential requirement in establishing botanical supplements as viable supports to conventional therapies.[11] Recent studies have revealed that cranberry extract regimens containing 72 mg PACs produce significant bacterial antiadhesion activity in human urine.[2,10] Clearly, the dose of bioavailable PAC-A is central to the issue of cranberry efficacy. Currently, four methods are used to evaluate the content of cranberry PACs; two methods are based on the depolymerisation of PACs (e.g., the hydrochloric acid butanol method known as Bates-Smith and the European Pharmacopoeia method), and two are colorimetric methods (a ultraviolet-visible [UV-VIS] spectrophotometric method based on Prussian-blue or Folin-Ciocalteu reagents and the Brunswick Laboratories 4-dimethylaminocinnamaldehyde [BL-DMAC] method). The BL-DMAC colorimetric method (an aldehyde condensation of 4-dimethylaminocinnamaldehyde) appears to be more accurate than the other methods and has been successfully used to quantify cranberry PACs.[12] In particular, the BL-DMAC method is less likely to be subject to interference from cranberry components, such as anthocyanins, because the reaction is read at 640 nm. However, the BL-DMAC method, although specific for PAC quantification, is not able to distinguish between A- and B-type PACs;[13] therefore, analytical methods, such as high-performance liquid chromatography (HPLC) coupled to mass spectrometry or fluorescence detectors, are necessary for PACs-A authentication.[14] Oximacro® is a cranberry extract with the highest content of PACs (according to the BL-DMAC method) and the highest percentages of PAC-A dimers and trimers (based on Liquid chromatography [LC]/mass spectrometry [MS] identification) available on the market. Here, we report on the chemical analysis of the PAC content of Oximacro® and its action in preventing UTIs based on a pre-clinical double-blind controlled study on male and female volunteers.

MATERIALS AND METHODS

Reagents

Oximacro®, a cranberry (*Vaccinium macrocarpon* Aiton) extract, was provided by Biosfered S.r.l. (Turin, Italy) and produced from cranberries as a reddish powder with a total PAC content > 360 mg/g (Lot # CR0104-PD01). The CoA of the product is available at the company web site (http://www.biosfered.com). Extrasynthese (Lyon, France) provided pure standards of PAC-A and PAC-B. The pure chemicals were dissolved in 96% v/v ethanol (Sigma-Aldrich, Carlsbad, USA) at a final concentration of 100 µg/mL. Aliquots of stock solutions were stored in 1.5-mL HPLC vials at -80°C

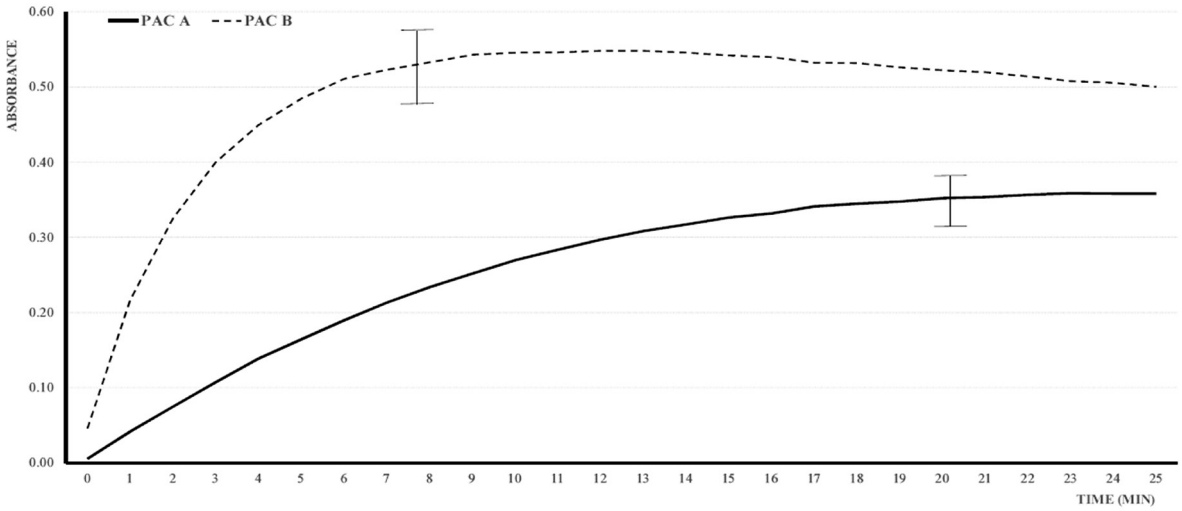

Figure 2. Time-course of the BL-DMAC reaction of 20 µg mL^{-1} PAC-A and PAC-B. When used at the same concentration, PAC-B (dotted line) reacts more rapidly and with a higher absorbance with respect to PAC-A (solid line). Metric bars represent standard deviation.
Abbreviations: PAC, proanthocyanidins; BL-DAMC, Brunswick Laboratories 4-dimethylaminocinnamaldehyde.

until use. The chemical purity and integrity of standard compounds was assessed (see below) prior to use.

Determination of the Total PAC Content with the BL-DMAC Method

The BL-DMAC assay was performed according to the method of Prior and colleagues[12] with minor modifications. Extraction buffer was composed of acidified 75% v/v acetone (VWR International, Milan) with 0.5% v/v acetic acid (Sigma-Aldrich, Carlsbad, USA). Acidified ethanol was composed of 72% v/v ethanol and hydrochloric acid (Sigma-Aldrich, Carlsbad, USA) at a final concentration of 1.52 M. DMAC solution was composed of 0.1% w/v 4-(dimethylamino)-cinnamaldehyde (DMAC) (Sigma-Aldrich, USA) in acidified ethanol; this solution was freshly prepared prior to the assay. Briefly, Oximacro® (20-30 mg) was dissolved in 5 mL of extraction buffer. The powder was extracted in an ultrasonic bath at room temperature for 20 min and then shaken with an orbital shaker for 1 h. Samples were centrifuged at 5,000 g for 10 min and then diluted in the extraction buffer prior to spectrophotometric assay. The colorimetric reaction was performed by mixing 0.84 mL DMAC solution and 0.28 mL of a diluted sample in a 1.5-mL plastic cuvette. The total PACs were quantified via an external calibration curve made with a pure PAC-A standard. The reaction kinetics of both PAC standards (PAC-A and PAC-B) were determined using a time-course BL-DMAC assay. A concentration of 20 µg/mL was tested for both standards. The reaction was incubated in the dark from 1 to 25 min to assess the dynamics of the DMAC reaction, and the absorbance was read at 640 nm (Cary60, Agilent-Technologies, California, USA) against a blank composed of acidified ethanol and DMAC solution. The quantification was performed in triplicate within the linear range of calibration curves (5-30 µg/mL). Oximacro® was then assayed exactly at 20 min, which corresponds to the maximum absorbance value for PAC-A. To test the reactivity of PAC-A and PAC-B to DMAC, 20 µg/mL solutions of PAC-A and PAC-B were tested with increasing percentages of PAC-B (0, 25, 50, 75 and 100%). The final concentration of the tested mixtures was always 20 µg/mL. The absorption spectra were recorded between 350 and 800 nm, exactly 20 min after the beginning of the DMAC colorimetric assay.

Authentication of the PAC-A Content in Oximacro®

PAC-A and PAC-B authentication of Oximacro® were obtained via liquid chromatography (1200 HPLC, Agilent Technologies, California, USA) equipped with a reverse phase (RP) C18 Kinetex (2.6 µm, 100 × 3.0 mm, Phenomenex, California, USA) column. The binary solvent system was A) MilliQ H_2O (Millipore, Billerica, Massachusetts, USA) with 0.1% v/v of formic acid and B) acetonitrile (VWR International, USA) with 0.1% v/v of formic acid. Chromatographic separation was carried out at constant flow rate (200 µL/min) using the following conditions: linear gradient from 5% to 30% of B in 10 min and isocratic elution for 5 min and 20 min at 50% of B and 24 min at 90% of B at 24 min. The initial mobile phase was re-established for 10 min prior to the next injection. Tandem mass spectrometry analyses were performed with a 6330 Series Ion Trap LC-MS System (Agilent Technologies, California, USA) equipped with an electrospray ionization

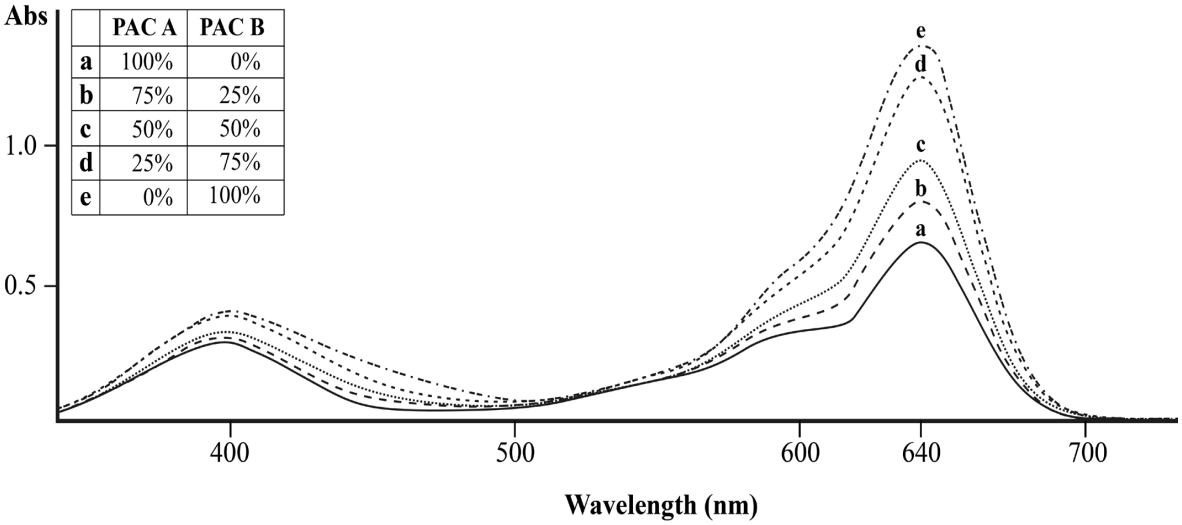

Figure 3. BL-DMAC spectral analysis of a 20 µg mL^{-1} solution of PAC-A and PAC-B used at increasing PAC-B concentrations. Increasing PAC-B causes an absorbance increase at 640 nm.
Abbreviations: PAC, proanthocyanidins; BL-DAMC, Brunswick Laboratories 4-dimethylaminocinnamaldehyde; Abs, absorbency.

source (ESI) operating in negative mode. The identification of PAC-A (dimers and trimers) was performed via multiple reaction monitoring (MRM) by monitoring the following parental ions [M-H]-: 575, 577, 861, 863 and 865 *m/z*.

Study Population and Inclusion and Exclusion Criteria

To assess the effect of Oximacro®, we recruited participants from a population of volunteers (10 male and 60 female) involved in studies performed by the Farmacia Antoniana (San Gillio, Italy) under the supervision of medical doctors. Informed consent was obtained. The inclusion criteria included any woman or man at least 18 years of age to over 51 years of age with at least 2 culture-documented symptomatic UTIs in the calendar year prior to recruitment. The choice of volunteers was completely balanced, and volunteers with known anatomical abnormalities (posterior urethral valves, neurogenic bladder, or any urinary obstruction) were excluded from this study. Urinary infection was defined as a positive culture of a midstream sample with a uropathogenic bacterium at 10^5 colony forming unit (CFU)/mL in symptomatic volunteers with no more than two species of organisms present. We accepted lower counts (10^4 CFU/mL) if the volunteer had typical symptoms of UTI and positive white blood cells and/or nitrites on urine analyses. Specific symptoms and signs included pain before, during, or after micturition; increased frequency of micturition; pain in abdomen; haematuria; foul smell; and signs of common sickness (fever > 37.9°C or 1.5°C above baseline, temperature, chills, nausea, and vomiting). Nonspecific symptoms were considered anorexia, fatigue and reduced mobility, and signs of delirium (e.g., confusion and deterioration in mental or functional status). Asymptomatic bacteriuria was not considered an end point. After explaining the study and obtaining consent, patients were assigned to the placebo group or experimental randomized groups. The randomization was concealed.

Oximacro® Administration and Dosage

Capsules contained 500 mg of the product [112 mg Oximacro® (equivalent to 36% PAC-A), 383 mg mi-

Table 1. Comparative analysis of total PACs content based on a PAC-A calibration curve with an increasing percentage of PAC-B (± standard error; n = 3). In the same column, different letters indicate significant ($P < .05$) differences.

Percentage of a 20 µg mL^{-1} PAC-A Solution	Percentage of a 20 µg mL^{-1} PAC-B Solution	Total PACs, expressed as µg mL^{1} (± standard deviation)
100	0	20.49a (± 0.65)
75	25	22.65b (± 1.48)
50	50	25.49c (± 2.55)
25	75	28.18d (± 2.48)
0	100	31.29e (± 2.19)

Abbreviations: PAC, proanthocyanidins.

Table 2. Volunteers baseline characteristics.

Variables	Experimental Group, Oximacro® Administration, n = 35	Placebo Group, n = 35
Demographics		
Females, no (%)	30 (85.7)	30 (85.7)
Males, no (%)	5 (14.3)	5 (14.3)
Median age (range)	38 (19-61)	38 (19-63)
Age range		
19-24	5 (F)	5 (F)
25-30	5 (F)	5 (F)
31-35	5 (F)	5 (F)
36-40	5 (F)	5 (F)
41-50	5 (F)	5 (F)
> 51	5 (F), 5 (M)	5 (F), 5 (M)
Baseline characteristics		
Acute UTI, no (%)	35 (100)	35 (100)
Bladder and bowel dysfunction, no (%)	35 (100)	35 (100)
Average UTIs in years prior to treatment	2.5	2.6
Number of capsules (days)	2 (7)	2 (7)
Volunteers not completing the study, no (%)	6 (17)	15 (43)
Females, no (%)	6 (17)	13 (43)
Males, no (%)	1 (20)	3 (60)

Abbreviations: M, male; F, female; UTI, urinary tract infections.

crocrystalline cellulose and 5 mg magnesium stearate]. The placebo was indistinguishable in colour, taste, and appearance, consisting of all elements above without Oximacro® and coloured with azorubine. The experimental group (5 males and 30 females) received 1 capsule containing 36% PAC-A twice per day (morning and evening) for 7 days, and the placebo group (5 males and 30 females) was given the same number of capsules with no PACs. A score (from 0, representing no effect, to 10, representing a maximum effect of Oximacro® in preventing UTI) was recorded for all volunteers. To obtain linearity, the logarithm of the scores (Ln scores) was used. The dose was calculated based on previous clinical trials.[10] The administration was performed for 7 days; during this time, the volunteers were followed with alternating visits and telephone calls every 2 days. At the end of the treatment period, a urine sample was sent for urine analysis and urine culture. To avoid contamination, the volunteers were asked to not use antibiotics or any other cranberry products for the duration of the study, with the exception of the placebo group, in which volunteers were asked to immediately report on symptoms. In the latter case, they were asked to use the antibiotic prescribed by the medical doctor and to interrupt the placebo administration. The attending urologists, outcome assessor and statistician were all blinded to the group allocations.

Table 3. Contingency table.

Variables	Placebo	Oximacro®
Not recovered	35	7
Recovered	0	28

Fisher's Exact Test: $P < .001$

Statistical Analysis

We performed Fisher's exact tests on the tabulated frequencies to assess the effect of the treatments. Kolmogorov–Smirnov tests were used to assess the distribution type for the continuous variable, i.e., the average value of the score. The data were log-transformed. Accordingly, a non-parametric analysis of variance was used to assess the differences in the Oximacro® and placebo groups according to the sex and age categories. The median, quartile, maximum and minimum score values are represented in boxplots; outliers are represented by asterisks. A binary logistic regression was performed to test the independent effects of age and sex on Oximacro® outcomes. All statistical analyses were performed using Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 22.0. The inten-

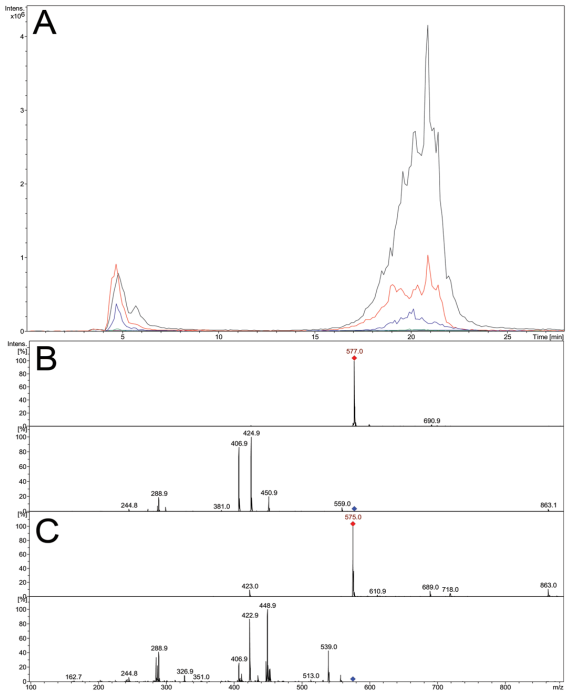

Figure 4. HPLC-ESI-MS/MS analysis of Oximacro®. **A)** HPLC-ESI-MS/MS chromatogram of dimers and trimers of PAC-A and PAC-B. The mass spectrometry analysis performed in MRM mode shows the presence of PAC-A dimers (black line) and trimers (blue line) and PAC-B dimers (red line). **B)** MS^2 spectra in negative mode of a typical PAC-B dimer ([M-H]-1 575 m/z). **C)** MS^2 spectra in negative mode of a typical PAC-A dimer ([M-H]-1 577 *m/z*).
Abbreviations: PAC, proanthocyanidins; HPLC-ESI-MS/MS, high-performance liquid chromatography/electrospray ionization tandem mass spectrometry; MRM, Multiple Reaction Monitoring.

tion-to-treat principle was followed.

RESULTS

One of the key points in UTI prevention is the assessment of the content of bioactive PACs-A, which are contained in the capsule. Total PAC content can be quantitated using various methods, among which the BL-DMAC method is generally recognised as the most accurate.[12,13] However, the BL-DMAC assay is sensitive to the presence of both PACs-A and PACs-B. When we measured the standards of PAC-A and PAC-B using the BL-DMAC method, we found that the same amount of PAC reacted differently with various absorbance spectra (**Figure 1**). In particular, a higher absorbance (at 640 nm) was found for PAC-B than PAC-A (**Figure 1**).

A time-course experiment was then performed by measuring the BL-DMAC reaction of 20 µg/mL PAC-A and PAC-B. PAC-B reached maximum absorbance after 11 min, whereas PAC-A showed a maximum absorbance at 20 min (**Figure 2**). PAC-A and PAC-B purity standards were analysed to assess purity and integrity; the results showed that the purity was identical to that declared by the supplier. To determine whether a mixture of PAC-A and PAC-B with increasing percentages of the two PACs could result in differing PAC quantifications, we assayed the BL-DMAC of the mixtures and found that the presence of PAC-B increased the absorbance values measured at 640 nm (**Figure 3**). Finally, we calculated the total amount of PACs based on a calibration curve prepared with a PAC-A standard using mixtures with increasing percentages of PAC-B and decreasing percentages of PAC-A at a final concentration of 20 µg/mL, as calculated using the gravimetric method. **Table 1** shows that the total amount of PACs increased with increasing PAC-B content despite a constant amount of total PACs used.

These results indicate that the BL-DMAC method is time-sensitive (in our analyses, the best timing for the PAC-A reaction was 20 min) and that shorter reaction times may lead to overestimating the total PAC content. The latter result may occur in cases of high amounts of PAC-B. Furthermore, our results confirm that the BL-DMAC method does not distinguish between PAC-A

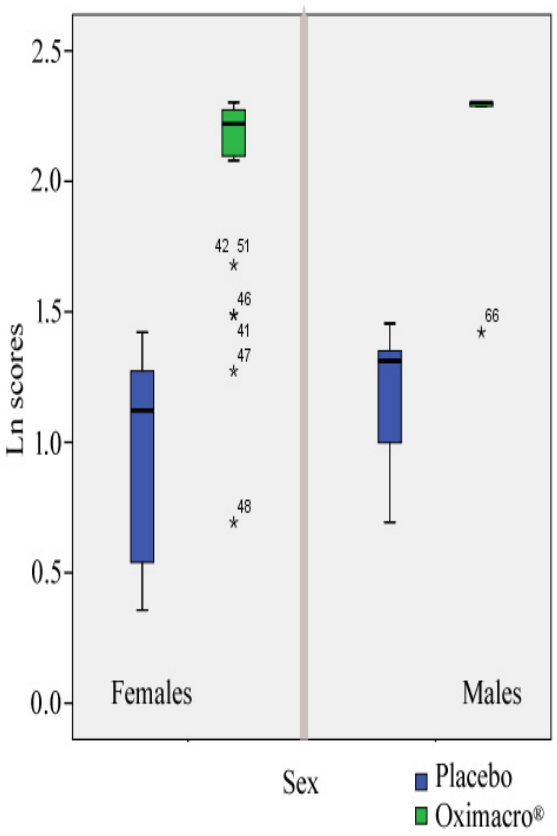

Figure 5. Boxplot representing the logarithm of the scores of the placebo and Oximacro® groups in both females and males volunteers. Females differences between placebo and Oximacro®: Mann-Whitney = 875; $P < .001$; n = 60; males differences between placebo and Oximacro®: Mann-Whitney = 24; $P = .016$; n = 10.

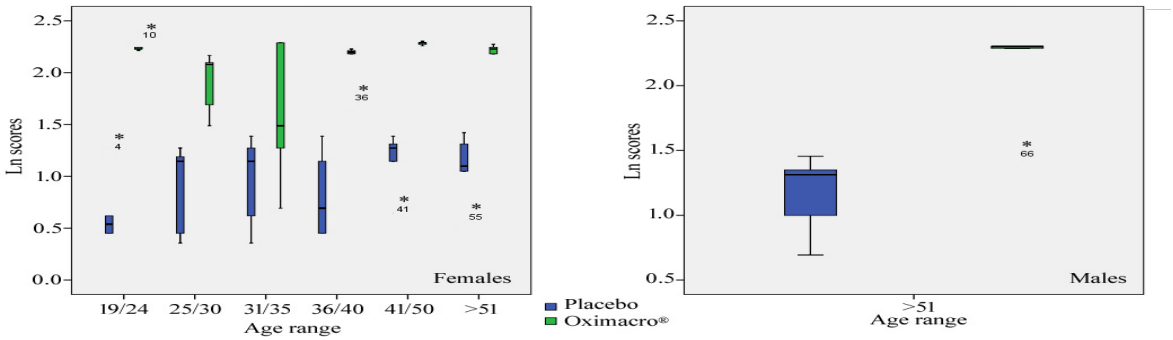

Figure 6. Boxplot representing the logarithm of the scores of the placebo and Oximacro® groups in both females (left panel) and males (right panel) volunteers according to the age range. Left panel, females age range-based (19-24, 25-30, 36-40, 41-50 and > 51 years) differences between placebo and Oximacro®: for each age range, Mann-Whitney = 25; $P = .008$; n = 10; differences between placebo and Oximacro® for the female age range of 31-3 years: Mann-Whitney = 20.5; $P = .095$; n = 10. Right panel, differences between placebo and Oximacro® for the male age range over 51: Mann-Whitney = 24; $P = .016$; n = 10.

and PAC-B and that even when a calibration curve is obtained with PAC-A, an increased amount of PAC-B eventually increases the absorbance at 640 nm, thereby affecting the total PAC quantification. Therefore, a high PAC value obtained with the BL-DMAC method does not necessarily indicate a high amount of PAC-A even though a calibration curve is calculated with a PAC-A standard.

Having assessed the best timing for PAC determination, we next measured the total amount of Oximacro® PACs. The BL-DMAC method showed a total of 372.34 mg/g (± 2.3) PACs, in line with that declared by the producer. To our knowledge, and based on the BL-DMAC method, this is the highest amount of PACs reported for a cranberry extract that is currently on the market.

The bioactivity of cranberry against UTI is dependent on the PAC-A content[9,15,16] (particularly dimers and trimers);[17-19] thus, we analysed Oximacro® via HPLC followed by electrospray ionization (EI) and tandem mass spectrometry (ESI-MS/MS). Oximacro® was primarily composed of PAC-A dimers followed by a lower amount of PAC-A trimers (**Figures 4a and 4c**). The total percentages of PAC-A and PAC-B based on HPLC-ESI-MS/MS were 86.72% (± 1.65) and 13.99% (± 1.03), respectively (**Figures 4a-c**). The percentage of other PAC polymers was below the threshold of detection.

Considering the total PACs (calculated using the BL-DMAC method) and the percentage of PAC-A dimers and trimers, Oximacro® showed a total PAC-A content of 322.89 mg/g (± 1.58). Therefore, 112 mg of Oximacro® contains 360 mg/g PAC-A. Following the assessment and authentication of the PAC-A content of Oximacro®, we prepared capsules containing 112 mg of Oximacro® (which corresponds to 36% PAC-A) and placebo capsules. In all volunteers, the infection prior to recruitment was due to *E. coli* in 85%, other enteric Gram-negative bacilli in 10%, and more than 1 type of bacteria in 5% of the volunteers. **Table 2** shows the volunteers' demographic and baseline characteristics.

Reasons for dropout in the experimental group included relocation (1), feeling better prior to the end of treatment (4), contrary advice from a family doctor (1), and a family perception of Oximacro® ineffectiveness (1). Reasons for dropout in the placebo group included acute pain (12), contrary advice from a family doctor (2), and a family perception of Oximacro® ineffectiveness (1). The median follow-up time in both groups was 4 weeks.

The mean capsule intake was 97% (95% CI: 96.6–97.6%) and was similar between the experimental and placebo groups. After 7 days of Oximacro® and placebo administration, a contingency table was calculated based on recovered vs. not recovered volunteers (**Table 3**); this table showed a significant difference (Fisher's exact test: $P < .001$) between the Oximacro® and placebo groups. A general Mann-Whitney *U*-test test further showed a highly significant difference between the placebo and Oximacro® groups (1191; $P < .001$; n = 70), with most of the placebo group unable to recover from UTI. Eventually, all placebo volunteers had to be treated with antibiotics (Monuril®, trometamol salt of fosfomycin) to reduce pain. **Figure 5** shows the boxplot of female and male scores; a significant difference was found between the placebo and Oximacro® groups for both females (Mann-Whitney *U*-test = 875; $P < .001$; n = 60) and males (Mann-Whitney *U*-test = 24; $P = .016$; n = 10). Significant differences were also found when the age ranges were analysed. In particular, between the placebo and Oximacro® groups, the female age ranges of 19-24, 25-30, 36-40, 41-50 and over 51 years showed

highly significant differences (for each age range: Mann-Whitney *U*-test = 25; *P* = .008; n = 10); the age range from 31-35 years showed barely significant differences (Mann-Whitney *U*-test = 20.5; *P* = .095; n = 10) (**Figure 6 left panel**). For males, the only age range (over 51) showed a significant difference between the placebo and Oximacro® groups as reported above (**Figure 6 right panel**). Finally, considering the CFU/mL counts from the urocultures, a significant difference (*P* < .001) was found in the comparison between the experimental group and the placebo group (SD difference = 51688; df = 34, t = -10.27; Dunn-Sidak Adjusted *P* < .001, Bonferroni Adjusted *P* < .001).

Overall, these results show that the administration of Oximacro® significantly ameliorated UTI in the treatment group. When a multivariate binary logistic regression was performed to examine the independent effect of Oximacro® on healing (dependent variable) based on sex and age (covariates), no significant effect (*P* > .900) for categorical variables was found for the treatment outcomes (data not shown).

DISCUSSION

The efficacy of cranberry in preventing UTIs remains controversial primarily due to contrasting results indicating either a nonsignificant effect (as in the case of cranberry juice drinking)[20-23] or an extended duration therapy requirement (e.g., 12 months of drinking cranberry juice).[24] In individuals with recurrent UTIs, low-dose antibiotic prophylaxis for several months is usually recommended.[25] However, extended use of antibiotics may lead to the development of antibiotic resistance. Indeed, several *E. coli* isolates are resistant to antimicrobial treatment, and the interest in non-antibiotic methods for the prevention of UTIs is growing.[26] If the dosage of non-antibiotic methods is not standardized, the cost/effect ratio may be higher than antibiotic treatment, as recently shown with a cranberry prophylaxis regimen for preventing UTIs in which the PAC-A treatment was far below (18.2 mg/day) the recommended dosage (72 mg). In this case, the cranberry treatment was less effective and more expensive than (dominated by) trimethoprim-sulfamethoxazole prophylaxis.[27] The use of concentrated cranberry extract with a high PAC content has been successfully proven to prevent UTIs in women who are subject to recurrent infections.[7,9,10,18,28-31] Despite these positive results, one of the major concerns is the quantification of PACs-A, which are the only bioactive compounds thus far demonstrated to exert a significant uropathogenic bacterial anti-adhesion effect.[9,19] A recent survey on the PAC content of some cranberry extract products via both BL-DMAC and HPLC found that BL-DMAC values for the PAC content per unit were below those declared by the manufacturers. In particular, some cranberry extract medicinal products showed a A-type PAC content so low that they would have no chance of providing health benefits;[32] the availability of these extracts was likely the result of overestimation of the PAC content provided by the Bates-Smith and the European Pharmacopoeia methods. On the one hand, these methods grant a high percentage value; on the other hand, by overestimating the real PAC content, these methods limit the health benefits of cranberry extracts. The cranberry industry is currently using BL-DMAC as a standard method;[13] however, the BL-DMAC method is unable to discriminate between A- and B-type PACs.[13] It is thus important to combine accurate timing and the kinetics of the method with the HPLC-MS authentication of PAC types. The results of this work show that the presence of PAC-B in cranberry extract can overestimate the total PAC content based on a PAC-A calibration curve. For instance, a cranberry extract with a high percentage of PAC-B (as is typical in some cranberry cultivars) may yield a high total PAC value with the BL-DMAC method despite a PAC-A standard calibration curve. Furthermore, in our conditions (see the Materials and Methods), 20 min of reaction were required for an accurate PAC-A determination (complete saturation of the reaction). Therefore, the standardization of PACs using the BL-DMAC method and the authentication of PAC-A with LC-MS is a prerequisite in preparing cranberry dosages for the prevention of UTIs.

Our results showed that the cranberry extract Oximacro® contains a high total PAC content and a high percentage of bioactive PAC-A dimers and trimers. When administered to volunteers, the extract was particularly suitable for UTI prevention, and 112 mg Oximacro® (equivalent of 36 mg PACs-A) twice per day for 7 days was significantly effective in reducing the total urobacterial CFU counts in both the female and male groups with respect to placebo. The age ranges were unaffected by treatment with the sole exception of the 31-35 year age range in the female group. This group did not differ in baseline characteristics with respect to the other age groups; thus, the reason for the reduced effect of Oximacro® in this group requires further investigation. A literature search on age-related responses to cranberry treatment did not provide any reported cases, although further studies will focus on this aspect.

CONCLUSIONS

The results of this work are in agreement with previous randomized, double-blind versus placebo multicentre studies examining the effects of 72 mg of PAC-standardized cranberry.[2,10,13] Furthermore, our results show that 72 mg PAC-A is highly effective, and we suggest the use of dosages based on PACs-A instead of the total PACs in UTI treatment. Due to the impossibility of BL-DMAC in discriminating between PAC-A and PAC-B, the sole total PACs quantification may not be sufficient in providing the required amount of PAC-A needed to significantly inhibit UTIs. Further studies will assess the recurrence of UTIs in Oximacro®-supplemented volunteers.

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CONFLICTS OF INTEREST

None declared

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