Effectiveness of an Association of a Cranberry Dry Extract, D-mannose, and the Two Microorganisms *Lactobacillus plantarum* LP01 and *Lactobacillus paracasei* LPC09 in Women Affected by Cystitis

*A Pilot Study*

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**Background:** Urinary tract infections (UTIs) are the most common bacterial infection in women. Most UTIs are acute uncomplicated cystitis caused by *Escherichia coli* (86%). This study was undertaken to assess the effectiveness of an association of a cranberry dry extract, D-mannose, a gelling complex composed of the exopolysaccharides produced by *Streptococcus thermophilus* ST10 (DSM 25246) and tara gum, as well as the 2 microorganisms *Lactobacillus plantarum* LP01 (LMG P-21021) and *Lactobacillus paracasei* LPC09 (DSM 24243) in women affected by acute uncomplicated cystitis.

**Materials and Methods:** Thirty-three premenopausal, nonpregnant women diagnosed with acute uncomplicated cystitis were enrolled in a pilot prospective study and completed the treatment protocol. Subjects were instructed to take 2 doses per day during the first month, and then to continue with 1 sachet per day until the sixtieth day. Nitrates and leukocyte esterase on urine dipstick testing were used as indicators of cystitis, with analysis performed at enrollment, after 30 and 60 days, and after 1 month of follow-up. Typical UTI symptoms, namely dysuria, frequent voiding of small volumes, urinary urgency, suprapubic pain, and gross hematuria were scored 0 to 3 and evaluated at each visit.

**Results:** Positive results for the presence of nitrates and leukocyte esterase were found in 14 and 20 subjects after 30 days and in 9 and 14 women after 60 days, respectively (*P* < 0.001). At the end of the follow-up period, positive results for nitrates and leukocyte esterase were recorded in only 4 and 3 of 24 and 19 subjects (16.7%, *P* = 0.103; 15.8%, *P* = 0.325, respectively), with negative results after 60 days. Typical symptoms of cystitis, specifically dysuria, frequent voiding, urgency, and suprapubic pain were significantly improved as well. No significant differences were recorded in the incidence and severity of hematuria at any visit.

**Conclusion:** The long-term ability of an association of cranberry, D-mannose, an innovative gelling complex, and the 2 microorganisms tested to significantly improve the uncomfortable symptoms reported by women with acute cystitis has been suggested.

**Key Words:** acute cystitis, cranberry extract, gelling complex, lactobacilli, barrier effect

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Recurrent UTI (rUTIs) are reported in 16% to 25% of women within 6 months of a UTI episode and in 40% to 50% of women within 1 year of an UTI episode despite antimicrobial treatment.14-16

The social and financial implications of UTIs are very important. In fact, their total costs include the cost of outpatient doctor visits, antimicrobial prescription, and hospital expenses as well as nonmedical cost associated with sick days and morbidity.2

The main pharmacological treatments of cystitis usually involve the use of antibiotics, in particular quinolones (such as ciprofloxacin and levofloxacin), fosfomycin, second-generation and third-generation cephalosporins, and β-lactam antibiotics associated with β-lactamase inhibitors.27 Because of the formation of bacterial biofilms, unfortunately, the results of antibiotic treatment are often not satisfying. In fact, the infection is not always eradicated and bacteriuria and recurrences may persist. Biofilms have recently been advocated as playing a possible role in the pathogenesis of rUTI, and it has been speculated that they have a role in persistent colonization by harmful or pathogenic bacteria.18,19

Several molecular and genetic studies concerning mechanisms involved in E. coli biofilm formation, with particular reference to E. coli K12, have led to identification of many factors for biofilm establishment.20-22 Biofilms are one of the most important factors underlying the inability of antibiotics to offer significant protection towards relapses. Furthermore, treatment with antibiotics, in addition to not being decisive, often leads to infection by yeasts, which are not susceptible to antibiotic treatment, or to the development of antibiotic-resistant infectious species.23

As alternatives or in addition to traditional treatments, there are some natural ingredients with a recognized effectiveness in countering the onset, persistence, or propagation of a UTI caused by E. coli.

A dry extract of cranberry (Vaccinium macrocarpon) is able to establish a natural physical-mechanical contrast against the adhesion of E. coli to the surface of the epithelial cells of the bladder and urinary tract.24 Its main activity is related to the proanthocyanidin (PAC) fraction containing oligomers that compete with the adhesions located on type-P fimbriae (FimH, mannose-sensitive) used by E. coli to mediate its anchorage to the epithelial cells.25 The profile of cranberry bioactives is distinct from that of other berry fruits, as they are rich in A-type PACs in contrast to the B-type PACs present in most other fruit.26

D-mannose is a simple sugar with no real metabolic importance to which UPEC can bind through P-type fimbriae, thus remaining in a free form in urine and, therefore, being more easily eliminated with urination.27 In vitro research has identified a mannose-specific lectin on the surface of adherent strains of E. coli.28 Another in vitro research project has elucidated the adherence mechanism. D-mannose is apparently the primary bladder cell receptor site for uropathogenic E. coli. The first step in adhesion involves the mannose-sensitive binding of FimH (adhesion at the tip of type 1 pili of E. coli) to bladder epithelium.29 One in vitro study found aromatic α-glycosides of mannose to be more effective inhibitors of E. coli adherence than α-methyl-mannoside.30

This study was undertaken to assess the effectiveness of an association of a cranberry dry extract, α-mannose, a gelling complex composed of the exopolysaccharides (EPS) produced by S. thermophilus ST10 (DSM 25246) and tara gum, as well as the 2 microorganisms L. plantarum LP01 (LMG P-21021) and L. paracasei LPC09 (DSM 24243), characterized by specific activities against E. coli31 and by other ancillary properties, in women affected by acute uncomplicated cystitis.

MATERIALS AND METHODS

Study Design

Thirty-five premenopausal, nonpregnant women diagnosed with acute uncomplicated cystitis were enrolled in this pilot study between April and May 2013 at the Gynecological Associated Medical Practice “Vicariotto-De Maria” (Milan, Italy). Informed written consent was obtained from all participants involved in the study. This study was carried out in accordance with the Helsinki Declaration (2000) of the World Medical Association.

Eligible subjects were at least 18 years old and had active, uncomplicated cystitis diagnosed by urine dipstick testing and an evaluation of the presence of typical related symptoms. In particular, frequency (frequent voiding of urine), urgency (the urge to void immediately), dysuria (painful voiding), suprapubic pain, and gross hematuria were taken into account and scored according to the UTI Symptoms Assessment questionnaire (UTISA), a 14-item instrument querying the severity and bothersomeness of 7 key UTI symptoms.32

The exclusion criteria were acute cystitis symptoms for >1 week before the first visit, diabetes mellitus, congenital urinary tract abnormality, female subjects who were pregnant or lactating, or those with a positive urine pregnancy test or who were intending to become pregnant during the study or within 3 months after the completion of the study, vaginal discharge, fever (>37.5°C), sexually transmitted diseases, use of a postvoiding catheter or an intermittent self-catheterisation program, neurogenic bladder, and treatment with antibiotic agents or other lactobacilli-containing products in the previous 2 months.

After enrollment, subjects were given 90 sachets of the active formulation and were instructed to take 2 doses per day during the first 30 days (acute treatment), and then to continue with 1 sachet per day until the 60th day (long-term treatment). Each dose contained 2.5 billion live cells of L. plantarum LP01 (LMG P-21021) and 1 billion viable cells each of L. paracasei LPC09 (DSM 24243) and S. thermophilus ST10 (DSM 25246), a strain able to synthesise EPS in the gut lumen, and 250 mg of tara gum (General Trading & Consulting Ltd., Milan, Italy). The formulation included also 500 mg of a high PACs cranberry extract (Naturex SA, Avignon, France) and 250 mg of α-mannose (Hebei Huaxu Pharmaceutical Co., Shijiazhuang, China). A copy of UTISA was provided to each subject along with some simple instructions about how to answer the questions provided.

Patients were prohibited from using topical vaginal or other systemic antibiotic agents at any time during the study. They were also prohibited from using other products containing lactobacilli or bifidobacteria, even if taken orally, throughout the duration of the study.

Urine dipstick testing was performed at the Gynecological Associated Medical Practice “Vicariotto-De Maria” at enrollment (d0), after 30 days (d90), at the end of the second month of treatment (d180), and after 1 month of follow-up (d360). UTISA were collected from each patient at each visit starting from d0.
The active formulation used in this study was manufactured and provided by Probiotical Ltd., Novara, Italy.

Quantification of Nitrites and Leukocyte Esterase in Urine Samples

Nitrites and leukocyte esterase on urine dipstick testing (Multistix 10 SG Reagent Strips; Siemens AG, Erlangen, Germany) were used as indicators of cystitis, with analysis performed at the gynecology laboratory at enrollment, after 30 and 60 days, and after 1 month of follow-up. In detail, freshly voided urine was collected in a clean container, mixed well just before testing, and analyzed within 2 hours after voiding or otherwise refrigerated immediately for subsequent testing, still to be carried out within 24 hours. A first-morning specimen was preferred, although random collections were considered acceptable. One strip was then removed from the bottle and all the test pads were dipped into the urine. Each test pad was then compared to the corresponding row of color blocks on the bottle label and read by the gynecologist at the time shown on the label. The results were expressed as positive or negative according to the specific chromatic threshold defined for each of the 2 parameters analyzed (≥ 10 leukocytes/μL and 0.075 mg/dL nitrite ion, respectively).

Assessment of the Most Typical Symptoms of Cystitis

At each visit the presence of the most common and recognized symptoms of cystitis, namely dysuria (pain or burning when passing urine), frequent voiding of small volumes, urinary urgency (a strong and uncontrollable urge to pass urine), suprapubic pain (pain or uncomfortable pressure in the lower abdomen/pelvic area), and gross hematuria (blood in the urine) were quantified according to the UTISA questionnaire. In detail, each symptom was graded “none/not at all,” “mild/a little,” “moderate,” or “severe/a lot,” and scored 0 to 3. Possible changes in the UTI symptoms, could be used as an indication of a recurrence during the follow-up.

Statistical Analysis

Paired t tests were used to weigh the results and compare them between $d_{0}, d_{30}, d_{60}, d_{90}$, and $d_{30}, d_{60}, d_{90}$, $d_{0}, d_{60}$ and $d_{90}$. In particular, UTI-associated symptoms were scored 0 to 3, whereas nitrites and leukocyte esterase were reported as positive or negative according to the specific chromatic threshold defined. Differences were considered significant at $P \leq 0.05$.

RESULTS

Quantification of Nitrites and Leukocyte Esterase in Urine Samples

Thirty-three female subjects of 35 initially enrolled in the study completed the treatment according to the instructions provided. Our results reported the presence of nitrites and leukocyte esterase in 14 and 20 subjects after 30 days and in 9 and 14 women after 60 days, respectively ($P < 0.001$). At the end of the 1-month follow-up period, positive results for nitrites and leukocyte esterase were recorded in only 4 and 3 of 24 and 19 subjects (16.7%, $P = 0.103$; 15.8%, $P = 0.325$, respectively), with negative results after 60 days (Table 1). This parameter, considered together with UTI symptoms scores, could be used as an indication of a recurrence during the follow-up.

Assessment of the Most Typical Symptoms of Cystitis

The UTISA was found to comprise 3 four-item domains named “urination regularity,” “problems with urination,” and “pain associated with UTI.” Two questions asking about hematuria provided information about a fourth factor. Our results highlighted a significant improvement of 4 of 5 symptoms typically associated with UTI, namely dysuria, frequent voiding, urgency, and suprapubic pain. Hematuria remained almost unaltered throughout the study (Table 2 and Fig. 1).

TABLE 1. Number of Subjects With a Positive Result for the Presence of Nitrites and Leukocyte Esterase in the Urine at Each Time (Time Zero, Day 30, Day 60, and Day 90)

<table>
<thead>
<tr>
<th>Time zero</th>
<th>Nitrites</th>
<th>$P \ (d_{00}, d_{60}, \text{ and } d_{90})$</th>
<th>$P \ (d_{30}, d_{60}, \text{ and } d_{90})$</th>
<th>Leukocyte Esterase</th>
<th>$P \ (d_{00}, d_{60}, \text{ and } d_{90})$</th>
<th>$P \ (d_{30}, d_{60}, \text{ and } d_{90})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>33</td>
<td>**</td>
<td>—</td>
<td>33</td>
<td>**</td>
<td>—</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>&lt; 0.001</td>
<td>—</td>
<td>20</td>
<td>&lt; 0.001</td>
<td>—</td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td></td>
<td></td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>&lt; 0.001</td>
<td>**</td>
<td>14</td>
<td>&lt; 0.001</td>
<td>**</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td></td>
<td></td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>&lt; 0.001</td>
<td>0.103</td>
<td>17</td>
<td>&lt; 0.001</td>
<td>0.325</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td></td>
<td></td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of negative result</td>
<td>60.6</td>
<td>&lt; 0.001</td>
<td>48.5</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. recurrences</td>
<td>4</td>
<td>16.7</td>
<td>15.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Comparison reference time.

$P$ values were calculated using Student $t$ test and considered significant when $P < 0.05$. 
The majority of women had moderate or severe dysuria (n = 25, 75.8%), frequency (n = 27, 81.8%), and urgency (n = 27, 81.8%) at baseline clinical evaluation. Suprapubic pain was less common, with the majority of women (n = 26, 78.8%) rated as having either mild or moderate suprapubic pain. Hematuria was reported as absent in 18 women (54.5%).

After 30 and 60 days, significant improvements were recorded in dysuria (n = 14 and n = 7 females with moderate or severe score, respectively), frequent voiding (n = 21 and n = 6 subjects with moderate or severe score, respectively), urgency (n = 17 and n = 10 females with moderate or severe score, respectively), and suprapubic pain (n = 8 and n = 2 subjects with moderate or severe score, respectively). Hematuria was absent in 17 (51.5%) and 16 (48.5%) subjects, respectively.

At the end of the 1-month follow-up, a significant worsening of dysuria (P = 0.048), frequent voiding (P = 0.021), and suprapubic pain (P = 0.018) was reported. No significant differences were registered for urgency and hematuria.

**DISCUSSION**

UTIs are the most common bacterial infections in women, with one half of all women experiencing at least 1 UTI in their lifetime. Most UTIs in women are acute uncomplicated cystitis caused by *E. coli* (86%) and by other coliforms such as *Enterobacter*, *Citrobacter*, and *Klebsiella*. During menstruation, elevated protein concentrations and increase in oxygen and carbon dioxide concentrations with a decrease in vaginal lactobacilli all contribute to UTIs.33

In recent years, natural approaches alternative to traditional pharmacological treatments have been investigated. Some studies have acknowledged the prospective efficacy of probiotics, especially lactobacilli, in counteracting acute cystitis when administered orally or directly in the vagina. Lactobacilli could potentially replace low-dose, long-term antibiotics as a safer prophylactic for rUTI.

In order for oral probiotic supplementation to benefit UTI risk, the bacteria must be able to colonize the intestinal tract and/or the urovaginal region. In a study of 10 women, *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 given twice daily for 14 days resulted in bacterial recovery from vaginal tissue within 1 week of commencing supplementation.34 Oral probiotic supplementation has been shown to benefit pediatric populations. In a multicentre randomised control trial (RCT) in 12 neonatal intensive care units, 585 preterm newborns were randomised to oral *L. rhamnosus* GG (n = 295) or placebo (n = 290) once daily until discharge (average 47.3 and 48.2 d, respectively). Incidence of UTIs in the *Lactobacillus* group was 3.4% compared to 5.8% in the placebo.35

A random-effects model meta-analysis was performed by Grin et al36 using a pooled risk ratio, relating incidence of rUTI in patients receiving *Lactobacillus* to control. MEDLINE and EMBASE were searched from inception to July 2012 for RCTs using a *Lactobacillus* prophylactic against rUTI in premenopausal adult women. Data from 294 patients across 5 studies were taken into account. No statistically significant difference was registered in the risk for rUTI in patients receiving *Lactobacillus* versus controls, as indicated by the pooled risk ratio of 0.85 (95% confidence interval, 0.58-1.25, P = 0.41). In any case, an additional sensitivity analysis was completed to omit studies using ineffective strains and studies testing for safety. Data from 127 patients in 2 studies were included, thus highlighting a statistically significant decrease in rUTI in patients given *Lactobacillus* strains, denoted by the pooled risk ratio of 0.51 (95% confidence interval, 0.26-0.99, P = 0.05). In conclusion, lactobacilli could be regarded as safe and effective in preventing rUTI in adult women. However, more RCTs are required before a definitive recommendation can be formulated, as the patient population contributing data to the meta-analysis was relatively small.36
This pilot study demonstrates the long-term ability of an association of cranberry dry extract, α-mannose, a gelling complex composed of the EPS produced by S. thermophiles ST10 and tara gum, as well as the 2 microorganisms L. plantarum LP01 and L. paracasei LPC09, to significantly improve the uncomfortable symptoms reported by women with acute cystitis. To our knowledge, this is the first study assessing the effectiveness of an association of lactobacilli with a cranberry dry extract in women with acute uncomplicated cystitis. A relatively low number of recurrences were recorded during the 1-month follow-up, according to the parameters taken into account, therefore suggesting a long-term barrier effect exerted by the product. The compliance and overall tolerability profile were very good, with only 2 dropouts registered during the first and second months of treatment, due to protocol deviation and not to significant adverse events.

A previous study by Mogna et al31 reported the ability of L. plantarum LP01 to antagonize the growth of different E. coli biotypes, thus providing an in vitro rationale supporting the possible effectiveness of the product in women with acute uncomplicated cystitis.

Both cranberry (V. macrocarpon extract) and α-mannose have been explored in several human studies suggesting their effectiveness in mechanically counteracting the adhesion of E. coli strains to the urothelium, the epithelium lining much of the urinary tract. This evidence may represent a natural tool aimed as progressively reducing the severity of cystitis, thus helping to alleviate related symptoms. In any case, cranberries are relatively high in oxalic acid, a substance best avoided by individuals with a tendency to form calcium-oxalate kidney stones.37 The strain L. paracasei LPC09 is significantly able in vitro to metabolize oxalates (Mogna L, Pune M, Nicola S, et al, data under publication, 2014), therefore offering potential protection from the risk of gut inflammation arising from prolonged supplementation with a cranberry dry extract.

The gelling complex made of tara gum and S. thermophiles ST10 is able to extend the mechanical barrier effect normally provided by the gum to the whole intestine, therefore protecting the organism against the possible onset or maintenance of a chronic low-grade inflammatory status (data under publication).

In this study, urine dipstick testing was used with symptoms scoring to diagnose UTI and to quantify the efficacy of the product tested. The convenience and cost-effectiveness of urine dipstick testing makes it a common diagnostic tool, and it is an appropriate alternative to urinalysis and urine microscopy in diagnosing acute uncomplicated cystitis.38 Furthermore, it is known that combining positive dipstick test results, particularly tests for nitrates, with symptoms scoring increases posttest probability of an UTI.3,8 In particular, the presence of nitrates and leukocyte esterase in urine samples were regarded as the paramount factors for ruling in (both positive) and ruling out (both negative) an UTI. In fact, a dipstick positive for either leukocyte esterase or nitrate and negative for the other provides inconclusive diagnostic information, and further testing is therefore required in these patients.39

Normally, no nitrite is detectable in urine. Many enteric gram-negative bacteria such as coliforms are able to synthesize nitrite, thus enabling the detection of their presence by means of a simple test. In contrast, an increase in leukocytes is an indication of pyuria and is found in nearly all diseases of the kidney and urinary tract. Leukocyte esterase is a reliable indicator of leukocytes in urine.9 Urine cultures are recommended only for patients with suspected acute pyelonephritis, patients with symptoms that do not resolve or that recur within 2 to 4 weeks after the completion of treatment, and patients who present with atypical symptoms.5

A parallel decrease in the incidence of positive results for nitrates and leukocyte esterase and the severity of UTI symptoms, with particular reference to dysuria, frequent voiding, urgency, and suprapubic pain, was registered. It is remarkable to note that hematuria remained almost unchanged throughout the study, probably because a longer time is needed for the healing of the urothelium to occur and become clinically evident. In any case, despite the encouraging results registered in this pilot study, future trials will be needed to further investigate the possible usefulness of this new association of natural ingredients in the alleviation of UTI-associated symptoms and in the long-term prevention of possible recurrences.

REFERENCES


