

Hibiscus extract, vegetable proteases and Commiphora myrrha are useful to prevent symptomatic UTI episode in patients affected by recurrent uncomplicated urinary tract infections

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Summary *Objective: To evaluate the efficacy of a combination of Hibiscus extract, vegetable proteases and Commiphora myrrha extract in the prophylaxis of symptomatic episode in women affected by recurrent urinary tract infections (rUTIs).*

Materials and methods: In this phase II clinical trial, all patients with history and diagnosis of rUTI were enrolled. All patients underwent the following treatment schedule: 1 tablet in the morning and 1 tablet in the evening for 7 days and, then, 1 tablet in the evening for 10 days (1 cycle every each month, for 6 months) of a combination of Hibiscus extract, vegetable proteases and Commiphora myrrha extract. At the baseline, all patients underwent urologic visit with quality of life (QoL) questionnaires and mid-stream urine culture. After 3 and 6 months, all patients underwent urologic visit, urine culture and QoL questionnaires evaluation.

Results: Fifty-five women were enrolled (mean age 49.3; range: 28-61). At the enrollment time, the most common pathogen was Escherichia coli (63.7%). The median number of UTI per 6 months was 5 (IQR: 4-9). At the end of the second follow-up evaluation, 25 women did not reported any symptomatic episode of UTI (49%), 18 reported less than 2 episodes (35.3%), while 8 reported more than 2 episodes (15.7%). However, at the first and second follow-up evaluation the clinical statistically significant improvement (QoL) was reported by 38/51 (74.5%) ($p < 0.001$ from baseline) and 43/51 (84.3%) ($p < 0.001$ from baseline) women, respectively. The median number of UTI decreased to 2 (IQR: 0-3). At the end of the follow-up period, 30/51 had sterile urine (58.8%), while 21/51 (41.2%) reported a transition from symptomatic UTI to asymptomatic bacteriuria.

Conclusions: In conclusion, this treatment, in motivated patients, is able to prevent symptomatic UTI symptomatic episode and improve patient's QoL.

KEY WORDS: Urinary tract infection; Ellirose; Protelix; Serrazimes; Myrliq; Plant extracts; Antibiotic stewardship; treatment.

Submitted 11 August 2018; Accepted 24 August 2018

INTRODUCTION

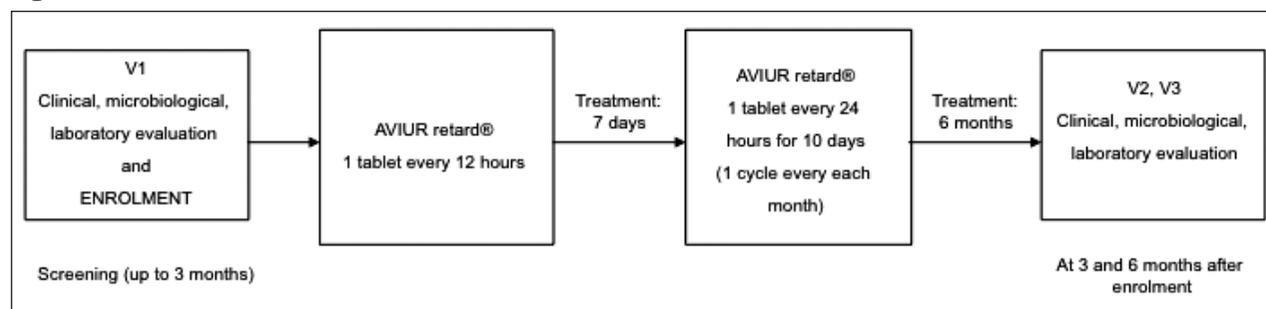
Urinary tract infections (UTIs) represent the most frequently reported community-acquired infection and carry a significant burden for patients' quality of life and

healthcare costs (1). Overall, more than 50% of women report having had at least one UTI at some point in their lifetime, and it is estimated that almost 11% of women aged over 18 suffer from a UTI each year (2-3). In particular, UTIs recur approximately in 20-30% of women within 6 months after their first episode (1, 4) and in around 35 to 53% of women within 12 months (5). The management of recurrent uncomplicated UTIs in women is still object of great discussion. To date, there are not well-established recommendations for a 'standard' prophylactic protocol to prevent recurrent UTI (6). Since the mainstay for the management of recurrent UTI still remains a short course of oral antimicrobial therapy, on the other hand the frequent use of antibiotics has led to the spread of multidrug-resistant microorganisms (7-10). Possible side effects, although rare, increasing healthcare costs, and rising bacterial resistance have progressively marked the downfall of classic antibiotic prophylaxis and, over years, paved the way to consider alternative prophylactic methods (4). In this regard non-pharmacological oral supplements, including the phytotherapy and nutraceuticals, seem to be an attractive alternative to antibiotic therapy to ameliorate symptoms related to UTIs and lower the rate of symptomatic recurrences. These considerations led us to conduct a prospective non-randomized phase II clinical trial in order to evaluate the efficacy of a combination of Ellirose[®], Protelix[®], Serrazimes[®] and Myrliq[®] (AVIUR[®] retard) in the prophylaxis of symptomatic episode in women affected by recurrent UTIs focusing on both the capability to reduce the number of symptomatic recurrences and the efficacy in improving QoL.

MATERIALS AND METHODS

Study design

From September to December 2017, we enrolled in a prospective, non-randomized phase II study, all women affecting by recurrent uncomplicated UTI and attending our Centre. Written informed consent was obtained

Figure 1.

from all subjects before proceeding with the enrollment. Preliminary evaluation was performed by administering QoL questionnaires and by performing a preliminary mid-stream urine culture, in line with Cai *T et al.* (11). Before enrolment all patients were treated with antibiotics in line with trialist's choice in order to obtain the infection free condition at baseline. The last isolated bacterial strain before the antibiotic treatment was collected and consider for this analysis. The main outcome measure was, then, the recurrence-free condition at the follow-up visit. All enrolled patients underwent the following treatment schedule: AVIUR retard® 1 tablet in the morning and 1 tablet in the evening for 7 days and, then, 1 tablet in the evening for 10 days (1 cycle each month, for 6 months). After 30 days from the beginning of the treatment, all patients have been contacted by phone in order to ensure compliance to the treatment. Subsequent follow up schedule included urologic visit with QoL questionnaires and mid-stream urine culture performed after 3 and 6 months from the beginning of the therapy. In case of acute symptomatic UTI episode all patients were treated with antibiotics in line with *European Association of Urology (EAU) guidelines* on urological infections (12) and in line with the antibiogram. The Figure 1 shows the study schedule.

Clinical and microbiological considerations

In accordance with the EAU guidelines, the diagnosis of UTI was defined according to the following parameters: patient reported symptoms and patient interview, physical examination, bed-side dip-stick urinalysis and urine culture (12). All microbiological and laboratory analyses have been described previously (13). In brief, all clean-catch midstream urine samples collected at room temperature were immediately taken to the laboratory under refrigerated conditions and analyzed. All urine samples were analyzed for common bacteria and yeasts, aliquoted for DNA extraction and polymerase chain reaction testing for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *urogenital Mycoplasma*. Microbiological culture was performed according to the procedure described by Hooton *et al.* (14). All other microbiological procedures and DNA extraction have been performed in line with Mazzoli *et al.* (15). For microbiological diagnosis, a colony count ≥ 105 units/mL was considered the cutoff for significant bacterial growth.

Inclusion and exclusion criteria

We included in this study all patients with recurrent

UTI, defined as ≥ 2 infections in six months or ≥ 3 infections in one year. Relevant exclusion criteria comprised: evidence of overactive bladder; anatomical abnormalities or previous surgery of the urinary tract; complicated UTI; pregnancy; urinary catheterization. Moreover, we excluded all patients with active infection by STD pathogens. We did not include any other exclusion criteria in order to make the results clinically relevant.

Composition and characterization of the extracts used
All patients who were treated in line with the manufacturer's instructions (Anatek Health Italia s.r.l. Via A. Caroncini, 5 - 20137 Milano; www.anatekhealth.com). Each administration contained a combination of 200 mg Ellirose™, 100 mg Protexil®SP750, 100 mg Serrazimes®, 60 mg Myrliq®PWD.

Outcome measures and statistical analysis

The primary outcome of this study was the rate of recurrence at the end of the study period. A good response to treatment was defined as a significant reduction of recurrences (< 2 in six months). Treatment failure was defined as the absence of recurrence reduction at the end of the study period. Transition from symptomatic UTI to asymptomatic bacteriuria was not considered as treatment failure. In order to obtain significant results to analyze, sample size calculation was based on the following assumptions: difference in terms of recurrence between baseline and follow-up visit: -3 ± 1 ; α error level, 0.05 two-sided; statistical power, 80%; anticipated effect size, Cohen's $d = 0.5$. The calculation yielded 43 individuals. Taking into account a drop-out rate of 10%, the final sample size has been set to 49 patients in the both groups. Statistical analysis was performed by using SPSS.

RESULTS

Fifty-five women were enrolled (mean age 49.3; range: 28-61). At the time of enrollment, the most common isolated pathogen was *Escherichia coli* (63.7%). All patients obtained sterile urine after antibiotic before enrollment. The median number of UTI per 6 months was 5 (IQR 4-9). Demographic, microbiological and clinical characteristics at the time of enrollment are reported in Table 1.

Clinical and microbiological data at the first follow-up evaluation (3 months)

After 3 months from the beginning of the treatment, 43 out of 51 patients (84.3%) reported a clinical improvement in terms of QoL from the baseline ($p < 0.001$),

Table 1.
Patient's sociodemographic anamnestic, clinical characteristics at enrolment time.

No. of total patients	55
Median age (range)	49.3 (28-61)
Educational level	
Primary school	-
Secondary school	25 (45.5)
Post-secondary education	30 (54.5)
Sexually active (past month)	48 (87.3)
Current smoker	
No	21 (38.2)
Yes	34 (61.8)
Number of sex partners	
1	49 (89.1)
≥ 1	6 (10.9)
Number of UTI in the past 6 months	
2	39 (70.9)
≥ 2	16 (29.1)
Hormonal status	
Pre-menopausal	35 (63.7)
Post-menopausal	20 (36.3)
Isolated strains before ABT treatment	
Escherichia coli	35 (63.7)
Klebsiella spp.	10 (18.2)
Enterococcus spp.	7 (12.7)
Others	3 (5.4)

while 42 out of 51 patients (82.3%) reported a restore to pre-UTI situation. From a microbiological point of view, 40 patients (78.4%) showed sterile urine and 11 (20%) showed a transition from UTI to ABU (from *Escherichia coli* to *Enterococcus Faecalis*).

Clinical and microbiological data at the second follow-up evaluation (6 months)

At the end of the second follow-up evaluation, 25 women did not reported any acute episode of UTI (49%), 18 reported less than 2 episodes (35.3%), while 8 reported more than 2 episodes (15.7%).

However, at the first and second follow-up evaluation the clinical statistically significant improvement (QoL) was reported by 38/51 (74.5%) ($p < 0.001$ from baseline) and 43/51 (84.3%) ($p < 0.001$ from baseline) women, respectively. Moreover, the median number of UTI decreased to 2 (IQR 0-3). From a microbiological point of view, at the end of the follow-up period, 30/51

Table 2.
All patient's sociodemographic anamnestic, clinical characteristics at enrolment time. ABT: antibiotic treatment.

Outcomes variable	Baseline	Follow-up 180 days	P
Clinical improvement			
No episode of UTI	0/51	25/51 (49)	
< 2 episodes of UTI	0/51	18/51 (35.3)	< 0.001
> 2 episodes of UTI	51/51	8/51 (15.7)	
QoL (questionnaire)	91.3	99.0	< 0.001
Microbiological improvement			
Sterile urine	0/51	30/51 (58.8)	< 0.001
Transition to ABU from UTI	0/51	21/51 (41.2)	< 0.001

sterile urine samples (58.8%) and 21/51 (41.2%) reported a transition from symptomatic UTI to ABU.

The Table 2 shows all clinical and microbiological data at the second follow-up evaluation.

Adherence results and treatment related adverse effects At the end of the study period, the adherence to the therapy was very high.

Only one patient (1.8%) discontinued the prescribed therapy because of the onset of drug-related adverse effects, nausea in this particular case.

DISCUSSION

The inappropriate use of antibiotics during last years has been paralleled by the growing onset of multidrug-resistant pathogenic strains among community-acquired isolates. The evolution of antimicrobial resistances has resulted into a global public health challenge, with rising costs and greater risk of poorer patient outcomes. Nowadays antibiotic stewardship protocols represent a precise imperative need. Unfortunately, to date there is not a "gold standard" prophylactic management to prevent recurrent UTI. Actually, *European Association of Urology* guidelines report several non-antibiotic measures for preventing recurrent UTIs but only a few are based on well-designed randomized clinical trials (13). However it cannot be denied that during last years research in non-antibiotic prophylaxis of recurrent UTIs has gradually grown. Particularly the use of phytotherapy and nutraceuticals might represent a feasible alternative approach for reducing the use of antibiotics and decreasing the rate of symptomatic recurrences (4, 16-17). In this light, in this phase II clinical trial we evaluated the efficacy and safety of a new nutraceutical agent named containing *Ellirose*[®], *Protexil*[®], *Serrazimes*[®] and *Myrliq*[®] in the prophylaxis of symptomatic episodes in women affected by recurrent UTIs. In our experience *AVIUR*[®] *retard* was well tolerated, resulting in optimal compliance since only one patient experienced adverse drug related effect which forced the discontinuation of the therapy. Moreover a statistically significant improvement in QoL was reported in almost 74.5% of patients and in 84% at first and second follow up visit respectively. Also from a microbiological point of view *AVIUR*[®] *retard* showed promising results since almost 60% of patients at the end of the follow-up period showed sterile urine and more than 40% reported a transition from symptomatic UTI to ABU. To this regard, a significant body of evidence advocated that ABU could play a protective role in preventing symptomatic UTI recurrences, interfering with the establishment of many enteric pathogens, such as *E. coli* (8). Noteworthy the fact that *AVIUR*[®] *retard* is able to establish an ABU is a tangible proof of the absence of its effects on the normal microbiota. In this sense, the use of non-antibiotic approach should be preferred since it doesn't interfere with the normal commensal bowel flora. *AVIUR*[®] *retard* is made of a combination of several nutraceutical agents playing different roles in the prevention of symptomatic UTI recurrences. *Ellirose*[®], an *Ibiscus* dry extract, is a phyto-complex containing Sambubiosides, which seem to have an anti-inflammatory activity by reducing the levels of

inflammatory mediators such as iNOS, NO, IL-6, MCP-1, and TNF- α induced by LPS (18). *Protexil*[®] is a form of concentrated protease derived as an exogenous production from controlled fermentation of maltodextrins through the *Aspergillus oryzae*.

The protease, once absorbed into the blood stream, effectively performs anti-inflammatory and anti-edema functions (19-20). Particularly, the proteases from *Protexil*[®] are bound to alpha 2-macroglobulines, which are thus modified into an activated form. Once activated, the alpha-2-macroglobulines bind the cytokines TNF-alpha (tumor necrosis factor alpha) and TGF-beta (transformative growth factor beta), modulating their activity in an anti-inflammatory way. Similarly, *Serrazimes*[®] is a proteolytic blend composed of enzymes derived from non-pathogenic enterobacteria belonging to genus *Serratia* species E-15. *Serrazimes*[®] contributes to the maintenance of a balanced immune system response by regulating protein-based immune cell inflammation mediators. It seems that *Serrazimes*[®] has also an additional analgesic activity due to its capability to block the release of biogenic amines. (19) Finally, *Myrliq*[®] is a *Commiphora myrrha* extract with a standardized content of curzerene, furanoeudesma-1,3-diene, and lindenstrene and a high total furanodiene content (20) Recent evidence showed that myrrh extract inhibits the production of interleukin-6 (IL-6) and interleukin-8 (IL-8) in human fibroblast cells. In addition it is involved in the blockage of the proteins involved in the inflammatory process such as Cox and also inhibits the formation of NO, ROS, TNF- α , PGE2, NF-kB and MAPK (21-22). Our results showed that *AVIUR*[®] *retard* can be taken into consideration as a potential effective prophylactic agent for symptomatic recurrent UTIs. However, the present study was not devoid of several limitations. Possible shortcomings of the study lie in the lack of randomization as well as in the small cohort size. Each of these variables might have introduced statistical bias and weaken the overall reliability of the reported findings. Acknowledged the limitations, the present study represents the largest series so far evaluating the prophylactic effect of *Ellirose*[®], *Protexil*[®], *Serrazimes*[®] and *Myrliq*[®] in women suffering from recurrent uncomplicated UTIs. Although antimicrobials remain the mainstay of treatment for acute UTI, the spread of multidrug resistant microorganisms among community-acquired isolates is worryingly increasing and needs for stronger surveillance and new preventing approaches. Only advancing our understandings about alternative treatments, such as phytotherapy and nutraceuticals, will enable us to develop appropriate non-antibiotic approaches and prevention strategies for recurrent UTIs. To this regard, further phase III randomized studies and larger series will certainly be needed to confirm and validate our preliminary results.

CONCLUSIONS

Recurrent urinary tract infections afflict a large number of young women nowadays, with a high impact on patient's quality of life. Currently, several strategies have been purposed and used in order to reduce the number

of recurrence and improve QoL, without any significant achievement.

Here, considering the available evidence, the use of phytotherapy seems a feasible antibiotic-sparing approach for reduce the number of recurrence.

In our experience, the treatment with *AVIUR retard*[®], in motivated patients, is able to prevent symptomatic UTI episode and improve patient's QoL. Moreover, the use of *AVIUR retard*[®] is able to improve the antibiotic stewardship, reducing the use of antibiotic prophylaxis in these patients. However, further prospective studies are needed to validate our results in larger series.

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