

# L-Methionine associated with *Hibiscus sabdariffa* and *Boswellia serrata* extracts are not inferior to antibiotic treatment for symptoms relief in patients affected by recurrent uncomplicated urinary tract infections: Focus on antibiotic-sparing approach

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**Summary** *Objective: To evaluate the efficacy of a phytotherapeutic combination of L-Methionine associated with *Hibiscus sabdariffa* and *Boswellia serrata* for treatment of acute episodes of uncomplicated urinary tract infections (UTI) in women affected by recurrent UTIs.*

*Materials and methods: In this randomized phase III clinical trial, adult females with uncomplicated UTI were enrolled into one of the following treatment groups: Group A: phytotherapeutic combination 1 tablet in the morning and 1 tablet in the evening for 7 days; Group B: Short term antibiotic treatment according to international guidelines recommendations. At baseline, all patients were evaluated by a urologist and quality of life (QoL) questionnaires and mid-stream urine culture. Same clinical and laboratory investigations were repeated at each follow-up visit. Results: Forty-six patients were enrolled in Group A and 47 in Group B. At the first follow-up (30 days), both groups showed a statistically significant improvement in quality of life scores as compared with baseline assessment [Group A: (QoL 94.3 vs 98.5  $p < 0.001$ ); Group B: (QoL 94.5 vs 98.7  $p < 0.001$ )]. An improvement from baseline was also seen at the second follow-up evaluation after 3 months [Group A: (QoL 94.3 vs 99.1  $p < 0.001$ ); Group B: (QoL 94.5 vs 98.1  $p < 0.001$ )]. At the second follow-up visit, a statistically significant difference in QoL was reported between the two groups (99.1 vs 98.1;  $p < 0.003$ ) and a transition from UTI to asymptomatic bacteriuria (ABU) was observed 12 of 46 (26%) patients in Group A, while no patients in Group B demonstrated ABU ( $p = 0.007$ ).*

*Conclusions: Here, we demonstrated that this phytotherapeutic combination is able, in comparison to antibiotic treatment, to improve patients quality of life, reducing symptoms in acute setting and preventing the recurrences. Interestingly, a significantly higher proportion of patients in the phytotherapy group had ABU after three months. Our findings are of great interest in an antibiotic stewardship perspective.*

**KEY WORDS:** Urinary tract infection; L-Methionine; *Hibiscus sabdariffa*; *Boswellia serrata*; Plant extracts; Antibiotic stewardship; Treatment.

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## INTRODUCTION

Urinary tract infections (UTIs) represent one of the most common community-acquired infectious diseases with

significant impact on patients' quality of life and public health care costs (1). More than half of women report having had at least one UTI during their lifetime, and almost 11% of women suffer from a UTI every year (2-3). In particular, 20-30% of women experience another UTI within 6 months after their first episode (1).

The most common management of recurrent UTIs is a short course of oral antimicrobial therapy, with possible alteration of the normal microbial flora of the vagina and/or gastrointestinal tract. Moreover, frequent use of antibiotics for treatment and prevention of UTIs increases healthcare costs and the spread of multidrug-resistant microorganisms (4-7). Nowadays, there are no universally accepted recommendations on prophylactic antibiotic management to prevent recurrent UTI (3). Reduced antibiotic prescription would not only prevent resistance but will also lower public health care costs. In this scenario, the use of phytotherapy for symptom relief and decrease of symptomatic recurrences in UTI is an intriguing alternative (3). The aim of the present study was to compare the effectiveness of a combination of L-Metionina associated with *Hibiscus sabdariffa* and *Boswellia serrata* (*Acidif plus*®) and standard antibiotic treatment of acute episodes of uncomplicated UTI in women affected by recurrent UTI, focusing on QoL improvement and the capability to reduce antibiotic usage.

## MATERIALS AND METHODS

### Study design

This is a single Centre, randomized and controlled phase III study. From January to June 2017, we enrolled all women affected by recurrent uncomplicated UTI seen in our referral centre. Written informed consent was obtained from all subjects before proceeding with the enrollment. Baseline assessment comprised evaluation by urologist, completion of QoL questionnaires and a mid-stream urine culture. All patients in the antibiotic arm were treated according to an empirical approach, in line with the

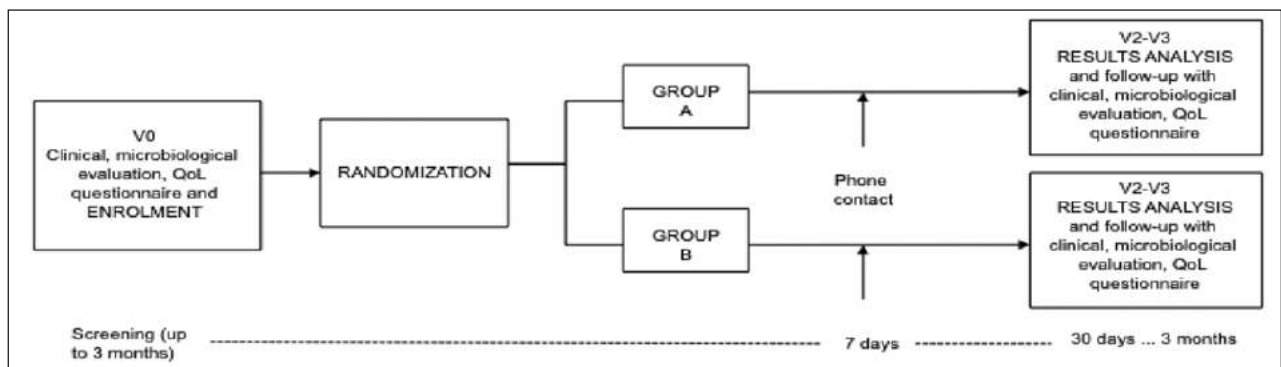
European Association of Urology (EAU) guidelines recommendations (8). The results of mid-stream urine culture were used only for confirmation of the diagnosis and not for tailoring the treatment. All selected patients were, then, randomly allocated into one of the two following groups:

- Group A: one-week therapy of *Acidif Plus*<sup>®</sup>, 1 tablet in the morning and 1 tablet in the evening for 7 days.
- Group B: Short term antibiotic therapy according to the trialist's choice based on the EAU guidelines on urological infections (8).

After one week from the start of treatment, all patients were contacted by phone in order to ensure compliance to the treatment in both arms. The follow up schedule included urologic visit after one and three months with QoL questionnaires and mid-stream urine cultures. The study schedule is shown in Figure 1.

**Figure 1.**

The figure shows the study schedule. QoL: quality of life evaluation. Group A: one-week therapy of *ACIDIF PLUS*<sup>®</sup>, 1 tablet in the morning and 1 tablet in the evening for 7 days. Group B: Short term antibiotic therapy according to the trialist's choice and EAU guidelines on urological infections.



**Composition and characterization of the phytotherapeutic extracts used**

All patients who were randomized to the *Acidif Plus*<sup>®</sup> group received oral administration of 1 tablet of *Acidif Plus*<sup>®</sup> in the morning and 1 tablet in the evening, in line with the manufacturer's instructions (*Biohealth srl, Rivoli - TO, Italy* (<http://www.biohealth.it/it/prodotti/linea-acidif/25-acidif-plus>). Each dose contained a combination of 400 mg of *L-Methionine* associated with 100 mg of *Hibiscus sabdariffa* and 100 mg of *Boswellia serrata* (L.) Roxb.

**Inclusion and exclusion criteria**

Exclusion criteria comprised: evidence of overactive bladder; anatomical abnormalities or previous surgery of the urinary tract; complicated UTI; intake of antibiotics within 4 weeks before enrollment; pregnancy; indwelling urinary catheter or stent.

**Microbiological and clinical considerations**

Treatment failure in group A was defined as microbiological colonization of at least 10<sup>5</sup> colony-forming units per milliliter (CFUs/mL) of a single uropathogen in the mid-stream urine culture associated with the persistence of urinary tract symptoms. Transition from symptomatic UTI to asymptomatic bacteriuria was not considered as treatment failure, but was registered as an improvement in symptoms. In case of treatment failure in group A, patients

underwent a short course of antibiotic therapy according to the antibiogram and guidelines recommendations.

**Outcome measures and statistical analysis**

The primary endpoint was the rate of responders defined as the demonstration of urinary pathogens < 10<sup>3</sup> CFU/mL in a mid-stream urine culture on the second and third follow up visit (after one and three months respectively from the beginning of the treatment) as well as improvement in symptoms scores. Data about the compliance to treatment, frequency of UTI recurrences or transition from symptomatic UTI to asymptomatic bacteriuria at subsequent follow up visits were also recorded. In order to obtain valid results for analysis, sample size calculation was based on the following assumptions. Difference between the groups, 3 ± 1 score

points in the QoL questionnaire; α error level, 0.05 two-sided; statistical power, 80%; anticipated effect size, Cohen's d = 0.5. The calculation yielded 2×41 individuals per group. Taking into account a drop-out rate of 10%, the final sample size was set to 90 patients in both groups. Statistical analysis was performed by using SPSS.

**RESULTS**

Ninety-three female patients with clinical evidence of uncomplicated UTI entered the study. Forty-six and forty-seven patients were enrolled in Group A (mean age 46.5 range: 23-62) and Group B (mean age 47.1 range: 22-63), respectively. Demographic and clinical characteristics of the study population are shown in Table 1.

The most commonly isolated bacterial strain at the time of enrollment was *Escherichia Coli* (*E. coli*) in 86.9% (40/46) of patients in Group A and in 82.9% (39/47) in Group B, respectively.

**Treatment compliance and treatment related adverse effects**

After 7 days treatment compliance was complete in Group A (100%), while within Group B 14.9% (7/47) of patients discontinued the prescribed therapy because of drug-related adverse effects. No side effects occurred in the phytotherapy group.

**Table 1.**

Demographic, clinical and microbiological characteristics of all enrolled and analysed women, at the baseline.

Group A: one-week therapy of Acidif Plus<sup>®</sup>, 1 tablet in the morning and 1 tablet in the evening for 7 days.

Group B: Short term antibiotic therapy according to the trialist's choice and EAU guidelines on urological infections.

Demographic and clinical characteristics of the study population at baseline	Group A	Group B	P
Enrolled patients	46	47	
Age (mean)	46.5	47.1	
Previous UTI per year (mean)	4.3	4.7	
Number of sexual partners			
1	44 (95.6)	43 (91.4)	
> 1			
Marital status			
Married	31 (67.3)	29 (61.7)	
Single	15 (32.7)	18 (38.3)	
Sexual encounters per week (mean)	2.1	2.0	
Bacterial strains			
E. coli	40 (86.9)	39 (82.9)	
E. faecalis	2 (4.4)	2 (4.3)	
E. faecium	1 (2.2)	1 (2.2)	
Klebsiella spp.	3 (6.5)	5 (10.6)	

### First follow-up evaluation (30 days)

At one month follow up visit, a statistically significant clinical improvement was reported in 95.7% (44/46) of patients in Group A (QoL 94.3 vs 98.5  $p < 0.001$ ) and 100% (47/47) in Group B (QoL 94.5 vs 98.7  $p < 0.001$ ), respectively. No statistically significant differences emerged when comparing QoL results in the two groups. A sterile urine culture was reported in 80.4% (37/46) and in 95.7% (45/47) of patients in Group A and Group B, respectively. Notably, in the remaining 19.6% (9/46) of

**Table 2.**

Outcome variables before and after treatment, in the two groups. Group A: one-week therapy of Acidif Plus<sup>®</sup>, 1 tablet in the morning and 1 tablet in the evening for 7 days. Group B: Short term antibiotic therapy according to the trialist's choice and EAU guidelines on urological infections. ABU: asymptomatic bacteriuria; UTI: symptomatic urinary tract infection; n.s.: not statistically significant.

Outcomes variable	Baseline	Follow-up 30 days	Follow-up 90 days	P
Clinical improvement				
Group A	0/46	44/46 (95.5)	44/46 (95.5)	< 0.001
Group B	0/47	47/47 (100)	45/47 (95.7)	< 0.001
p (between the groups)		n.s.	n.s.	
QoL (questionnaire)				
Group A	94.3	98.5	99.1	< 0.001
Group B	94.5	98.7	98.1	< 0.001
p (between the groups)		n.s.	< 0.003	
Microbiological improvement				
Sterile urine				
Group A	0/46	37/46 (80.4)	34/46 (73.9)	n.s.
Group B	0/47	45/47 (95.7)	44/47 (93.6)	n.s.
p (between the groups)		n.s.	n.s.	
Transition to ABU from UTI				
Group A	-	9/46 (19.6)	12/46 (26)	n.s.
Group B	-	2/47 (4.3)	0/46	n.s.
p (between the groups)		n.s.	0.007	

patients within Group A a transition from symptomatic UTI to asymptomatic bacteriuria (ABU) was observed and also a change in microorganism (from *E. coli* to *Enterococcus faecalis*). On the contrary, only 4.3% (2/47) of patients among Group B showed a transition from UTI to ABU. No treatment failures were recorded in Group A. All findings at the first follow-up are shown in Table 2.

### Second follow-up evaluation (90 days)

At the 3 month follow up visit, 95.7% (44/46) of patients in Group A showed a clinically significant improvement (QoL 94.3 vs 99.1  $p < 0.001$ ), as well as 95.7% (45/47) patients in Group B (QoL 94.5 vs 98.1  $p < 0.001$ ). A statistically significant difference emerged in terms of QoL between the two groups ( $p < 0.003$ ; 99.1 vs 98.1).

In Group A, 73.9% (34/46) of patients demonstrated a second sterile mid stream urine culture, while the remaining 26% (12/46) of patients showed a transition from symptomatic UTI to ABU and a change of microorganism (from *E. coli* to *Enterococcus faecalis*) ( $p = 0.007$ ). Again, no treatment failures were recorded in Group A while 2 patients in Group B showed symptomatic UTI with microbiological demonstration of *Escherichia coli*. No patients in Group B showed transition from UTI to ABU. Table 2 shows all findings at the second follow-up visit.

## DISCUSSION

Although antimicrobials remain the mainstay of treatment for UTI, the spread of multidrug resistant microorganisms among community-acquired isolates is worrying and calls for stronger surveillance and new preventive approaches. Today, inappropriate antibiotic use and antimicrobial resistance have become major issues worldwide. Unfortunately, there is no "gold standard" prophylactic regimen to prevent recurrent UTI. Actually, EAU guidelines present several non-antibiotic measures for preventing recurrent UTIs but only few are based on well-designed randomized clinical trials (8).

Furthermore, many women have signs of UTIs without evidence of bacterial presence, a clinical condition known as ABU. Although ABU is usually treated in women with recurrent UTIs there is no clear indication for this treatment. A significant body of recent evidence (6-7, 9) has demonstrated that antibiotic treatment of ABU is not only pointless, but could even be harmful. Particularly, in a randomized controlled study, Cai *et al.* showed that after a 12-month period of antimicrobial therapy of ABU in women affected by recurrent UTIs, the rate of symptomatic UTIs was higher in patients treated with antibiotics. There was also a higher prevalence of multidrug-resistant *E. coli* after antibiotic treatment of ABU due to *Enterococcus faecalis* (9). These data suggest that ABU could play a protective role in preventing symptomatic recurrences, interfering with the settlement in the urinary tract of many enteric pathogens, such as *E. coli*. In this sense, a non-antibiotic approach should be preferred because it doesn't modify the normal commensal bowel flora. As a result, research on non-antibiotic prophylaxis of recurrent UTIs has gradually grown during recent years. Particularly, the use of phytotherapy and nutraceuticals could represent a feasible alternative

approach for reducing the use of antibiotics and decreasing the rate of symptomatic recurrences (3, 10-11). In this light, we wanted to evaluate an oral supplementation of *L-Methionine* combined with *Hibiscus sabdariffa* and *Boswellia serrata* (named *Acidif plus*<sup>®</sup>). *L-Methionine* is a sulfur donor which is necessary for the biosynthesis of cysteine, a sulfide amino acid. A physiological pH is obtained by the action of *L-Methionine* which creates unfavorable conditions for bacterial colonization since a large number of Gram-negative bacteria are able to alkalinize the urine by enzymatic degradation of urea. Furthermore, dry extract of *Hibiscus sabdariffa* is a phyto-complex containing Sambubiosides, which seems to have an anti-inflammatory activity by lowering the levels of inflammatory mediators including iNOS, NO, IL-6, MCP-1, and TNF- $\alpha$  induced by LPS (12). Moreover, several preliminary studies demonstrated that *Hibiscus sabdariffa* is able to prevent recurrent uncomplicated urinary tract infections due to its inhibitory activity against *Escherichia coli* and *Candida albicans* (13). Finally, *Boswellia serrata* also seems to inhibit some inflammation mediators, in particular 5-lipoxygenase, with a subsequent decrease in inflammatory response mediated by leukotrienes (14). In our knowledge, this is the first study evaluating the prophylactic effect of *L-Methionine* associated with *Hibiscus sabdariffa* and *Boswellia serrata* extracts in women suffering from recurrent uncomplicated UTIs. In a recent multicenter observational study Passaro *et al.* (15) had already assessed the effectiveness of *Acidif Plus*<sup>®</sup> in reducing the bacterial load and improving symptoms in pregnant women affected by symptomatic UTI. In our experience, *Acid Plus*<sup>®</sup> was well tolerated with optimal compliance and no side effects. As many as 95.7% (44/46) of patients demonstrated relevant clinical improvement with a statistically significant difference compared to women treated with standard antibiotic therapy 3 months after the beginning of treatment. Moreover, at first follow up visit almost 20% of patients within Group A showed a transition from symptomatic UTI to ABU with lasting results at 3 months. Noteworthy, the fact that *Acidif Plus*<sup>®</sup> was able to establish an ABU is a tangible proof of absence of effect on the normal urine microbiota. Increased understanding of the action of phytotherapy and nutraceuticals will enable us to develop appropriate non-antibiotic treatment and prevention strategies for recurrent UTIs (16). Larger high-quality studies are required to confirm the findings in our study.

## CONCLUSIONS

Urinary tract infections affect a large number of women and many suffer from recurrent UTIs. Actually, current antibiotic treatments select multi-drug resistant microorganism, both in the gut and in the bladder, and this practice is no longer sustainable. Use of phytotherapy and nutraceuticals is an intriguing approach to reduce the excessive prescription of antibiotics. In the present study we could demonstrate that *Acidif Plus*<sup>®</sup> was able to improve patients QoL, lower symptoms in the acute setting and prevent UTI recurrences in women with recurrent UTIs as compared to antibiotic treatment. Further prospective studies are needed to validate our results.

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