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Cysticlean and Recurrent Urinary Tract Infection

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Abstract

Urinary tract infection (UTI) is the most common infection in hospital settings. While antibiotics are quite effective at providing clinical cure for UTI, urogenital pathogen drug resistance is on the increase. In recent decades, cranberry has been shown to be solid means of alleviating and curing several illnesses that previously could only be treated with conventional drugs. The anti-adherence activity of American cranberry products is the main mechanism involved in the preventive actions of UTIs, and it is a dose-dependent. Cysticlean® shows a significant inhibition of E. coli adherence, in vitro and ex vivo, to uroepithelial cells. Cysticlean® is a food product that contains a cranberry extract, that has been formulated as capsules and sachets containing a concentrated cranberry fruit extract. Cysticlean® provides 118 mg of proanthocyanidins (PACs).For people with recurrent uncomplicated UTIs, routine utilization of Cysticlean® products may offer an alternative methodology to antibiotic prophylaxis. The anti-adherence activity of Cysticlean® is greater than other marketed products. The PACs content in Cysticlean® is enough to produce their pharmacological activity, reduce the UTIs recurrences and improve patients symptoms.

Introduction

Urinary tract infection

Urinary tract infections (UTIs) are infections that can happen anywhere along the urinary tract and defined as microbial colonization of the urine and infection of the structures of the urinary tract with or without infection of the adjacent structures [1-3]. UTI is one the most common infectious diseases of the community and of the hospital settings, resulting in high rates of morbidity and high economic costs associated with its treatment [3]. Uncomplicated UTI occurs in patients without any anatomic or functional abnormality in the urinary tract and may reach, on average, 6.1 days of symptoms, 2.4 days of restricted activity and 0.4 bed days [1]. Uncomplicated cystitis (infection of bladder) is the most common UTI and is responsible for 95% of all symptomatic urinary tract infection [2, 4, 5].

90% of UTIs occur in females, except during the neonatal period, in which they are predominant in males [6, 7]. UTIs account for more than 7 million visits to physicians per year (1.2% of all office visits by women) [2, 4]. The financial impact of UTIs, including recurrent and uncomplicated cases, is greater than 1 billion dollars in the United States alone [4, 5]. Over 80% of women who had previous UTIs have recurrent infections over the first 18 months of observation. Of these recurrent infections, three quarters are caused by reinfection with different organisms [1, 2, 5]. Women with frequent reinfections have a rate of 0.13 to 0.22 UTIs per month (1.6 to 2.6 infections per year) [4, 5]. For premenopausal, healthy, and active females, recurrent UTIs are a major healthcare concern [1, 2].

The urinary tract has strong defenses against infection [1, 2, 8]. Urine is usually sterile and both ureters include a mechanism which prevents the urine from returning to the kidney [3, 5, 8]. The immune system of humans is in a constant battle against micro organisms and other damaging invaders [9-11] . Against all odds, urinary tract infection is one of the most common and can

arise at any time during an individual's life [9, 11-13]. Almost 95% of cases are caused by bacteria that normally multiply at the entrance to the urethra and reach the bladder (ascending route) [2, 3, 5, 9]. Less frequently, the bacteria propagate to the kidney via the bloodstream [9, 13].

The most common urinary tract infection (UTI) is cystitis; this infection appears in the lower urinary tract and affects the bladder and the urethra. However, it can also extend to the upper tract (ureters and kidneys) and is then known as pyelonephritis [1, 2, 8, 11, 12]. Sometimes asymptomatic bacteriuria may appear, that is, a patient's having bacteria in the urine without displaying any symptoms [9, 11, 13]. Analysing asymptomatic bacteriuria is not necessary in most medical check-ups, but it is important to do so in pregnant women and patients who have undergone urological surgery, as their conditions may cause significant infections [8, 9, 11-13].

Cystitis is characterised by burning sensation with urination and having to urinate frequently (or an urge to urinate) in the absence of a significant pain [1, 3]. These symptoms may vary from mild to severe with some pain above the pubic bone or in the lower back [9, 11]. People experiencing an upper urinary tract infection, or pyelonephritis, may experience flank pain, fever, or nausea and vomiting in addition to the classic

symptoms of a lower urinary tract infection. Rarely the urine may appear bloody or contain visible pyuria (pus in the urine) [1, 8, 9].

Women are more prone to UTIs than men because, in females, the urethra is much shorter and closer to the anus [9, 11, 14, 15]. As a woman's estrogen levels decrease with menopause, her risk of urinary tract infections increases due to the loss of protective vaginal flora [3, 5, 16]. Frequent sexual activity increases the risk of urinary tract infection [1, 2, 17]. Women who are sexually active have a higher incidence of infection due to a number of different factors. Sexual behavior may influence the risk [1, 16, 17]. The onset or a sudden increase in sexual activities, use of the diaphragm, unlubricated condom and spermicides, may increase the risk of cystitis [3, 17].

Urinary catheterisation increases the risk for urinary tract infections. The risk of bacteriuria (bacteria in the urine) is between three to six percent per day and prophylactic antibiotics are not effective in decreasing symptomatic infections [1, 5, 16, 18]. UTIs are frequent in children. Their prevalence in childhood varies from 2.1% to 8.7% [6, 7] . Approximately 8% of girls and 2% of boys will experience at least one UTI before reaching 7 years of age [1, 6]. In children UTIs are associated with vesicoureteral reflux (an abnormal movement of urine from the bladder into ureters or kidneys) and constipation [6, 7, 11].

The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, infection may also occur via the blood or lymph [7, 9, 12]. E. coli is the cause of 80–85% of UTI's, with Staphylococcus saprophyticus being the cause in 5–10% [9, 12]. Rarely they may be due to viral or fungal infections [9, 10]. Other bacterial causes include: Klebsiella, Proteus, Pseudomonas, and Enterobacter. These are uncommon and typically related to abnormalities of the urinary system or urinary catheterization [5, 7, 16, 19-21]. Urinary tract infections due to Staphylococcus aureus typically occur secondary to blood-borne infections [7, 9]. After gaining entry to the bladder, E. Coli are able to attach to the bladder wall and form a biofilm that resists the body's immune response [5, 9].

A number of measures have not been confirmed but seems to affect UTI frequency including: urinating immediately after intercourse, the type of underwear used, personal hygiene methods used after urinating or defecating, holding one's urine, tampon use, and douching [7, 9-11, 14]. For those with recurrent infections, a prolonged course of daily antibiotics is effective [9, 11, 13, 21]. Medications frequently used as a prophylaxes include nitrofurantoin and trimethoprim/ sulfamethoxazole [9, 13]. In cases where infections are related to intercourse, taking antibiotics afterwards may be useful [17]. In post-menopausal women, topical vaginal estrogen has been found to reduce recurrence [2, 17, 22].

While antibiotics are quite effective at providing clinical cure for UTI, urogenital pathogen drug resistance is on the increase [1, 5, 23-28] . On top of that, drugs have local side effects including disruption of the protective flora of the mouth, anal area, urethra and vagina, which create an increased risk of recurrent infections [23-28]. Also, antibiotics can cause general adverse effects including palpitations, flushes, nausea, vomiting, diarrhoea, abdominal pain, rashes, headache and dizziness [25, 28]. The presence of a natural alternative that could prevent and treat UTI is preferable to any other treatment.

Naturally, the epithelial layer covering the entire urinary tract has intercellular junctions which are formed by glycoproteins [8, 29, 30]. These glycoproteins not only act as glue between cells but also, create a film across the entire epithelium which protects it from bacterial attacks [8, 29]. Glycoproteins naturally deteriorate due to micro-ischaemic, immune system or chemical phenomena but, sometimes they deteriorate for unknown reasons [5, 8, 22].

Microorganisms produce different bioactive substances helping them to adhere to the urinary tract epithelial layer and then gain intracellular entrance, thus, causing inflammation and UTI [9-11, 28]. One of these bioactive substances is called adhesins, which is involved in bacterial adherence to the urinary tract epithelium and triggering the inflammatory response [8, 29, 30]. This process of bacterial adhesion to the epithelium allow them to remain at the urinary tract despite the drag effect of urinary flow [8, 29]. The main adherent structures of E. coli are fimbriae which are filamentous appendages on the surface of the bacteria and they are composed of filaments of protein with adhesions [5, 29, 30]. In turn, after E. coli adhesion, bacterial lipopolysaccharide act on receptor in the urinary tract epithelial cells to synthesis pro-inflammatory cytokines and the inflammatory process follow [23-25, 28].

Urinary tract infection prophylaxis

Different studies have raised serious doubts about the role of antibiotic prophylaxis of UTIs [31, 32]. Antibiotic treatment may cause bacterial resistance and may therefore be ineffective in preventing infection, and it has been associated with some secondary/adverse effects, particularly in the intestinal tract, suggesting

that continuous antibiotic prophylaxis prolonged for months or even years, can be hazardous. The changing pattern of antimicrobial resistance to the causative microorganisms of UTIs is a mounting problem. There is growing concern regarding antimicrobial resistance worldwide, particularly of Escherichia coli [32, 33]. Initially, resistance was described to particular agents, such as ampicillin, trimethoprim, sulphur-based antimicrobials or tetracyclines. More recently, the resistance has broadened to large families of agents, as the resistance to most ß-lactam antibiotics, aminoglycosides and fluoroquinolones [31, 33]. A strong evidence of an association at the individual patient level between the prescribing of antibiotics in primary care and antimicrobial resistance in bacteria in different sites, including the urinary tract, has been established. Effects were strongest in the month directly after prescription but were detectable for up to 12 months [34, 35]. A Cochrane review of 2011 determines that long-term antibiotics appear to reduce the risk of repeat symptomatic UTI in susceptible children but the benefit is small and increases the risk of microbial resistance [31, 36]. Additionally, other Cochrane review of 2011 concludes that there are insufficient data to recommend any specific antibiotic for treatment UTIs during pregnancy [1, 35].

The association between antibiotic use and breast cancer has been investigated in different studies. Although some studies showed no significant relationship [37], a recent meta-analysis

corroborates this possible association. Previously, elevated risk of breast cancer in women linked to the antibacterial treatment for urinary tract infection at premenopausal age. Nevertheless, their underlying nature cannot be defined at present [38]. At any case, this possibility is another factor to question the prophylactic use of antibiotics.

Acidification of urine has been used in traditional medicine for the prevention and treatment of urinary tract infections, although convincing findings for this concept from clinical trials are lacking. Probably other circumstances must occur simultaneously. Some authors speculate that ingestion of nitrate followed some hours later by acidification of urine could be prevent the bacterial conversion of nitrate to nitrite, that automatically convert to toxic nitrogen oxides, thereby leading to self-destruction, but in vivo and clinical studies should be performed. The question then remains if the amounts of nitrate-nitrite and ascorbic acid needed for bactericidal effects are physiologically achievable without troublesome side effects, and if urinary acidity can reach for a sufficient period of time. It is necessary take into account that infected urine often has a higher pH, and therefore, acidification is probably more difficult [39]. Indeed, the administration of one daily gram of ascorbic acid not produces a significant decrease in urine pH [40], and its use in urinary tract infections has not shown clinical benefit in certain studies [41].

In addition, other alternative strategies, including phytotherapeutic products, have gained interest such as the use of probiotics, vaccines, intravaginal estrogen therapy, bacterial immunostimulating fractions from Escherichia coli strains, inhibitors of bacterial adherence and colonization (cranberry and oligosaccharides) and antibacterial (bearberry leaf).

Cranberry

The American cranberry is the fruit of Vaccinium Macrocarpon Aiton [8, 17, 42]. The name "cranberry" comes from the words "crane" and "berry". The long white flower of the cranberry bush was thought to look like a crane. Both the bird and the plant were typical wild species in New England during its colonial period (17th century) [16, 42]. The plant produces small fruit with an intense red colour. These fruits were used by the Wampanoag people of Cape Cod and the Narragansett of what is now southern Massachusetts to "prevent kidney stones"and other urinary ailments [42]. In 1621, the red fruits were served by the Pilgrims with turkey or lobster, and are now a traditional part of the Thanksgiving meal. Sea-men brought the fruit back to Europe to be eaten during the voyage (they have a good, sweet flavor and are rich in vitamin C). When they reached the Old World, they were used in Scandinavia and Russia to produce wines and liquors. They were cultivated in the Netherlands most of all, and spread across northern Europe from there, no one can clearly ascertain when they began to be used to treat urinary tract infections. What is certain, however, is that it happened before the age of antibiotics [8, 29, 42, 43].

In recent decades, cranberry has been shown to be solid means of alleviating and curing several illnesses that previously could only be treated with conventional drugs [8, 16]. For example, decreasing the risk factors of cardiovascular disease, prevent and treat dental caries and periodontal disease, reduction of the anti inflammatory response with antimicrobial activity and the cytotoxic activity against cancer cells (specially against resistant ovarian cancer) [43-48].

Studies on the use of cranberries to fight urinary infections have been published since the 1950s [29, 42, 43]. Since then until now, three different mechanisms of action of cranberry have been

postulated: urine acidification with increased hyppuric acid excretion, increase in urinary salicylates, and inhibition of the bacterial adherence to urinary tract epithelial cells [8, 29, 30, 42, 43, 45, 46]. Initially it was believed that its effect was due to its hyppuric acid increasing urine

acidity. This led to the conclusion that hyppuric acid could be responsible for a bacteriostatic action and urine acidification [8, 29, 42, 46]. It was then shown that massive amounts of liquid (juice) would have to be used to lower urine pH [29, 42]. At a later date, it has been observed an increase in urine salicylates after consumption of cranberry. The impact of this effect is still unknown, although, it could be related to a local anti-inflammatory effect [8, 29, 30, 42, 43].

Cranberry ability to inhibit microbial adherence is due to the presence of polyphenolic tannins called A-type proanthocyanidins (PACs) [8, 16, 29, 30, 42, 43, 45, 46]. The activity of PACs is the most relevant and well-documented mechanism of cranberry, and these compounds are identified as the main responsible for the inhibition of bacterial adherence [8, 29, 30, 42, 45]. These compounds inhibit the adhesion of bacterial fimbriae, thus, preventing bacteriuria from turning into a urinary tract infection [29, 30, 42]. This action could be related to the ability of PACs to bind to proteins, such as the adhesins present on these fimbriae [8, 29, 30, 42]. It produces a length reduction, causing a decrease of adhesion forces between the bacteria and the urinary tract epithelial cells [29, 42]. Moreover, PACs of cranberry could also bind irreversibly to small molecules on the surface of bacterial cells such as lipopolysaccharides affecting its ability to trigger cytokines production and inflammatory process [29, 42, 46].

It was thought that the only limitation for the use of cranberry juice and extracts is its interaction with warfarin causing increased bleeding time in patient using warfarin [29, 30, 49]. Clinical trials confirmed that cranberry does not affect bleeding time or the degradation (metabolism) of warfarin and, so, cranberry juice and extracts are safe to be used by patients who are on warfarin [49-52].

Cysticlean®

Many cranberry extracts available in the market (Table 1), but, Cysticlean® sachets and capsules have shown the highest inhibition on bacterial adherence to urinary tract epithelium, and their use is related to a good tolerability, treatment compliance and efficacy to prevent urinary tract infection [29, 30, 53-56].

Cysticlean® is a food product that contains a cranberry extract. Cysticlean® has been formulated as capsules and sachets. Each capsule or sachet contains a concentrated cranberry fruit extract. Cysticlean® provides 118 mg of proanthocyanidins (PACs), (vanillin-HCl modified method) in one capsule or sachet to ensure their efficacy and for convenience in their intake [29, 30, 53-56]. Currently, Cysticlean® is sold in Europe and Asia mainly.

Cysticlean® capsules and sachets exhibited potent inhibition on bacterial adherence, and it is related to the PACs content in the cranberry extract. In vitro and ex vivo result show that the PACs content in cranberry extract of Cysticlean® produces a remarkable activity on bacterial adherence [29, 30, 53-56]. Cysticlean® has also been clinically proven to be highly effective in helping to reduce UTIs recurrence after 3-6months [30, 53, 55, 56].

What is PACs and how important it is?

A-type proanthocyanidins are identified as the main responsible for the anti-adherence activity of American cranberry. This action could be related to the ability of PACs to bind to proteins, such as the adhesins present on these E. coli fimbriae [57, 58]. A-type PACs from American cranberry differs from B-type PACs found in most berry fruits, and are considered the main active ingredient for inhibiting P-fimbriated E-coli adherence to uroepithelial cells.

The main difference is anti-adhesion effect of these A-type PACs against Escherichia coli, whereas B-type PACs from other berries were devoid of anti-adherence properties [59]. The A-type PACs produces a change of the conformation of the P fimbriae, with a length reduction, causing a decrease of adhesion forces between the bacteria and the uroepithelial cells [60]. Also, the PACs of American cranberry bind irreversibly to small molecules on the surface of E. coli such as lipopolysaccharides [61].

Moreover, the inhibition of adherence activity of the PACs from American cranberry has been observed not only to E. coli and antibiotics sensitive bacteria but also multi-drug resistant bacteria and to asymptomatic bacteriuria [57, 58, 61].

It is not enough to know the amount of cranberry extract, or the PACs content of these extracts. The level of the PACs determines the effectiveness of an American cranberry product. For this reason, it is important to use American cranberry products containing an adequate amount of PACs to achieve an action in the prophylaxis and treatment of UTIs. Furthermore, different studies have shown that the activity of cranberry PACs is dose-dependent. These studies reported a linear regression relationship between the natural logarithm of PACs concentration

and the number of retained P-fimbriated E. coli [53, 62].

Due to the diversity and structural complexity of PACs, their analysis and characterization is difficult and there is no official established method for cranberry PACs quantification [53, 58]. The different analytical methods used to quantify the PACs do not allow correct comparison between different products. Significant differences regarding the results can be obtained depending on the method used. Colorimetric methods are the most commonly used method in quantifying PACs. These assays are advantageous because they are normally inexpensive, quick, and easy to perform [63].

There are certain colorimetric assays for flavan-3-ols and PACs with different chemical reagents that react with specific sites on the PAC molecule. These methods are often used for detection of PACs and include vanillin, 4-dimethylamino-cinnamadehyde (DMACA) and acid-butanol. Variations of these methods have been used to provide quantitative data [63]. However, a lack of appropriate standards and interferences from other sample components, such as anthocyanosides and extraction solvents, can lead to over- and/or underestimation of PACs [64-66].

One of the Method to quantify PACs, is the vanillin-HCl method which is largely specific for flavan-3-ols. This assay has been used extensively to quantify PACs content of various plant materials, whereby vanillin adducts is measured at 500 nm [63]. Another method is the DMACA method, which has been adapted for use in measuring American cranberry PACs. However, DMACA reacts with large polymeric PACs to cause precipitation in the solvent and may not be detected. Therefore, the DMACA method tends to underestimate polymeric PACs [66, 67]. The acid-butanol assay is a classical method but is not well suited for quantification since the native anthocyanins present in the American cranberry samples may interfere with the results, leading to an overestimation of PACs [66].

Since there is no official method established, it is very important that the commercial products provide the analytical method used for PACs quantification and how the compound results are expressed. Accordingly, different commercial products and their content can only be compared if the same quantification method is used. Moreover, it is more important to compare the activity of the cranberry products by establishing pharmacological methods, as in vitro and ex vivo assays for bacterial adhesion.

Cranberry anti-adherent activity

It has been shown that the anti-adherence activity of American cranberry products is the main mechanism involved in the preventive actions of UTIs, and it is a dose-dependent in humans. Moreover, A-type proanthocyanidins are the main responsible for the anti-adherence activity, preventing the adhesion of P-fimbriated E. coli to uroepithelial cells and thus the main compounds responsible for the beneficial effect on UTI prevention [57, 58, 68].

The bacterial adhesion is a critical first step prior to invasion, thus it is a key event to test the pharmacological activity of a cranberry product in the bacterial pathogenesis. Anti-adhesion is the functional concept for prevention of pathogens. Robust in vitro and ex vivo assays for bacterial adhesion on host cells have long been used for the screening of potential therapeutic agents for the ability to minimize pathogen colonization of human tissues. In fact, if bacteria cannot attach to the inner urinary wall it cannot colonize and grow [58, 68].

Some studies cultured P-fimbriated bacteria in the presence of American cranberry products or compounds from American cranberry [29, 61, 69-71]. Human uroepithelial cells and human urinary bladder carcinoma cell line (T24) are often used in experimental in vitro assays. The use of a bladder epithelial cell line increases the reproducibility of the adherence test by comparison with uroepithelial desquamated cells coming from volunteers or patients [62]. Other studies test for the ability to agglutinate red blood cells using a mannose-resistant human red blood cell (HRBC) assay. Others, developed a bioassay to test the adhesion to the T24 bladder epithelial cell line of bacteria grown in urine samples collected after placebo or cranberry preparation drinking in a double-blind procedure [62].

Cysticlean® shows a significant inhibition of E. coli adherence, in vitro and ex vivo, to uroepithelial cells [58, 59]. Many previous bacterial adhesion in vitro assays, after the incubation of P-fimbriated E. coli with American cranberry juice or urine after consumption of American cranberry juice from 20 min [58], 30 min [59], 90 min [72] to 3 h [60], shown a passive obstruction of binding site on P-fimbriae [73]. On the other hand, Cysticlean® exhibited a potent inhibition of adherence of E. coli, pre-incubated in different concentrations of product after the incubation with T24 cells for 1 hour. For these in vitro adherence assays has been used an adaptation of the method proposed to quantify adhesion with T24 cells and using the E. coli strain ATCC 10536 [54, 55]. Statistically significant differences were observed between the control group and all the treated groups with different concentrations

of Cysticlean® (5, 25 and 75 μg/mL of PACs) and a dose-effect relationship is clearly observed. At concentrations of 5, 25 and 75 μg/mL of PACs, Cysticlean® capsules decrease the number of bacteria adhered to epithelial cells by 17%, 52% and 76%, respectively. This demonstrate that the highest inhibition of Cysticlean® capsules was obtained at the concentration of 75 μg/mL of PACs (76%, $p<0.01$). Moreover, Cysticlean® capsules has greater anti-adhesive activity than Cysticlean®

tablets (36%, $p>0.05$ at 25 μg/mL of PACs). Moreover, Cysticlean® has shown greater anti-adhesion activity than other American cranberry products (Urosens®, Urosens Forte®, Urell®, Monurelle® and Cistitus®) (Table 1) [54, 55].

PACs concentrations evaluations have been proportionally chosen according to PACs concentration declared of each product and its daily recommended dose. High, medium and low concentrations of PACs have been analysed. Results confirmed a depending concentration effect of all products evaluated In the same in vitro assay, Cysticlean® decreases the number of attached bacteria at lower concentrations and more powerful than these other products [55]. American cranberry products except Monurelle®, have decreased the number of attached bacteria per cell at high, medium and low concentrations of PACs (Figure 1). Cysticlean® capsules showed the highest activity (inhibition of 52% at 25μg/mL of PACs and 76% at 75μg/mL of PACs). Cysticlean® has been the unique product to show a significant anti-adhesion activity at lowest concentration (Figure 1, 2 and 3).

Urosens Forte® and Monurelle® contain vitamin C, but the daily dosage provided are lower than recommended (40 mg/day and 60 mg/day, respectively). There evidence showed no greater inhibition of bacterial adhesion than Urosens (same product without vitamin C). The difference in

activity of all these products could be related to lack of standardized method of analysis of PACs. Furthermore, some of these methods could measure other products like PACs without anti-adhesion activity [58, 74].

In addition, ex vivo studies with urine samples collected from rats treated with Cysticlean® (tablets and sachets), showed an important inhibitory effect on the adherence of E. coli to uroepithelial cells (Figure 4). The Cysticlean® sachets, at the human doses (118 mg PACs/animal, HCl-vanillin modified method), decreases the number of bacteria adhered to uroepithelial cells (T24) with an inhibition of 83% compared to the control group. At the same dose, the Cysticlean® tablets produced a decrease of the number of bacteria adhered of 52%, suggesting the importance of the product formulation. At lower dose (59 mg PACs/animal) both Cysticlean® products, tablets and sachets, also showed a prominent inhibition of 29% and 40%, respectively [29].

Other ex-vivo studies with healthy volunteers were performed and it has confirmed the anti-adherence activity of American cranberry products [58, 75-78]. This effect is not restricted to a particular group of strains because cranberry intake inhibits the adherence of papC positive and negative, antibiotic-resistant bacteria with different resistance phenotypes and antibiotic sensitive strains [62].

Although some laboratories have previously proposed dose of 36 mg/day of PACs, it is interesting to see how subsequent studies have shown that higher

doses of PACs provide greater activity. Recently, a multicentre, randomized, placebo-controlled and double-blind study confirm the anti-adherence activity ex-vivo and shown the reduction of virulence against E. coli [79]. Samples from volunteers that consumed cranberry product (capsules of cranberry powder) significantly produce dose-dependent anti-adhesion activity of E. coli. The effect is particularly important at dose of 72 mg of PACs (DMACA method) and the effect is prolonged until 24 h (T24 cell line and HRBC assays). In urine samples collected at 24 h, there was a significant difference between the anti-adherence activity of urines belonging to patients who had consumed American cranberry dosages containing 72 mg of PACs and the anti-adherence activity of urines belonging to patients who had consumed 18 or 36 mg of PACs [79]. This is another example of the dose-dependent activity of PACs. However, as already discussed and demonstrated, higher doses as provided by Cysticlean® produce a greater benefit and it can be established the dose of 118 mg as adequate daily amount of PACs. The dose of 118 mg/day of PACs has demonstrated significant efficacy in different clinical studies with Cysticlean® [54, 55, 79].

Additionally, studies to address molecular level adhesion have been performed. It is likely that American cranberry compounds can act on both biospecific and non-biospecific ways to induce changes in either bacteria or the adhering substrate. Several possible mechanisms to explain the interactions between American cranberry and the surfaces of bacteria were suggested. It is also possible that more than one mechanism act simultaneously [60, 70, 73, 80]. Some of these mechanisms include, Alteration of the conformation of the P-fimbriae,

binding adhesins in the P-fimbriae, decrease in the adhesion forces of the fimbriae between the cell surface and the bacteria, loss of the P-fimbriae from E. coli surface, changes at genetic level in bacteria with P-fimbriae, causing non-expression of the fimbriae and disruption of the bacterial ligand and uroepithelial cell receptor binding [70, 73].

Alterations to the growth rate of bacteria were implicated in contributing to the overall antimicrobial activity, in addition to the anti-adherence action [81]. The antimicrobial mechanisms of cranberry are not as well studied or clearly defined as the anti-adherence properties. PACs were the major contributing factor to the impact of American cranberry juice on reduction of the growth rate of bacteria [81]. The inclusion of PACS from American cranberry juice in bacterial growth media was found to significantly impact the duplication time of E. coli. The gene expression results revealed altered expression of genes associated with iron transport (dmsA, dmsB, ydhV, ydhX, ydhY and tdcG) causing iron depletion and, to a lesser degree, to direct disruption of metabolic enzymes, as well as with ATP synthesis (fumB, atpD) and fumarate hydratase in these cultures [81]. These results are consistent with the strong iron chelating capability, dose-dependent, of PACs [81]. The impact of iron depletion on the growth of bacteria is well documented [82, 83]. It is known that under aerobic conditions, microbes need iron for a variety of functions including reduction of the ribonucleotide

precursor of DNA and formation of heme [81]. Furthermore, against bacteria, sugars and organic acids from cranberry caused visible osmotic stress, while phenolics and anthocyanins caused disintegration of the outer membrane [74].

Clinical trials and medical literature data

Clinical evidence supports that the consumption of American cranberry products prevents the recurrent UTIs [84-87]. This effect has mainly been studied in women, but has also shown a significant reduction in the frequency of these infections in men and in children.

All clinical trials studied the effect of American cranberry in preventing urinary tract symptoms. In some of them, the primary parameter tested was UTI, and in other studies, bacteriuria was the primary endpoint. Added to that, American cranberry treatment is a safe, well-tolerated supplement that does not have significant drug interactions [88].

A Cochran review concluded that there was preliminary evidence supporting the efficacy of cranberry for the prevention and treatment of UTI [85]. The findings highlighted that American cranberries are effective for the prevention of recurrent UTI, especially in young sexually active women. Meta-analysis was performed using the data from four randomized controlled trials [89-92], where women, subjects with spinal cord injury or the elderly people were involved, the results show that American cranberry products significantly reduced the incidence of symptomatic UTIs in 12 months compared with placebo or control, particularly in women with recurrent UTIs [85].

Other reviewers also demonstrated the effectiveness of American cranberry juice in women with recurrent UTIs [84, 86]. Although, some limitations such as the lack of uniformity with regard to the intervention, (type of American cranberry product, concentration or content, dosage and duration of the intervention) they all concluded that the number and severity of infections could be reduced in patients with frequent recurrent UTI using American cranberries [86].

Medical literature data about the use and safety of American cranberry during pregnancy and lactation supports the use of American cranberry for UTIs during pregnancy [93, 94]. The study surveyed 400 women using American cranberry fruit juices as the most commonly used herbal therapy during pregnancy, and did not uncover any adverse events with regular consumption [94]. The effect of regular intake of American cranberry products on bacteriuria and pyuria in elderly has also been studied and the reduction of UTIs was observed [95, 96].

Compared to antibiotics, trimethoprim (100 mg/day) has only a very limited advantage over American cranberry extract (500 mg/day) in the prevention of recurrent UTIs in older women and has more adverse effects. The median time to recurrence of UTI was 84.5 days for the American cranberry groups and 91 days for the trimethoprim [97]. Moreover, it has been shown that American cranberry does not alter the pharmacokinetics of oral antibiotics

[98]. Although these studies did not include a control group, their results are equivalent or similar to those reported for other American cranberry products [99].

Cysticlean® has shown a beneficial effect as a prophylactic treatment of UTIs in women [100, 101] and in children [102].

There are two observational studies performed using Cysticlean® tablets (118 mg/day of PACs) after treatment with prescribed antibiotic (10 days average). The first observational study included women (18-65 years, n=83) with a history of at least 2 UTIs in the last 6 months. Three visits were established: initial visit, at three weeks and at three months. At three months, the

UTI incidence rate was significantly lower than usual rate (8.4% vs. 20%), representing a significant reduction (90%) in habitual recidivism. Added to that, a great improvement of the main parameters: decrease urinary frequency, dysuria, burning during urination, haematuria and leukocyturia in sediment test (Figure 5). The E. coli sediment was also decreased because in the beginning of the study, all patients had positive E coli sediment and only 8 patients (9.6%) had positive E. coli sediment at three weeks of treatment. Of these 8 patients, 4 left the trial and only 1 had positive E. coli sediment at three months of treatment, and 6 other women had reinfections [100].

The other observational study included women (18-65 years, n=78) with a history of recurrent UTIs, and three visits were also established: initial visit,

at three weeks and at three months. A significant reduction of UTI symptoms was observed immediately after starting treatment with Cysticlean (Figure 6). At three weeks it is observed an improvement of the main parameters: decrease of the E. coli sediment, urinary frequency, dysuria, burning, haematuria and leucocytosis, and effects are maintained until the end of treatment (three months). After three months of the Cysticlean treatment produces UTI incidence rate was lower than usual rate (14% vs 20%) [101].

Previously, in placebo-controlled studies, cranberry extract was found to be significantly more effective than placebo in reducing the occurrence of UTIs in women with a reduction of spent antibiotics in both treatment groups [89, 103]. Another important observational study confirmed the high effectiveness and good tolerance of Cysticlean® tablets in children with frequent urinary tract infections. Study enrolled 62 children (5-17 years), with a history of at least 2 UTIs in the last year and with no congenital malformation, systemic pathologies, neuropathic bladder, lithiasis or renal failure. This study concluded that daily intake of Cysticlean® (118 mg/day of PACs) produces a total prevention of acute pyelonephritis and 92% absence of symptomatic infections. Furthermore, Cysticlean® treatment is highly accepted by parents and patients regarding a long-term treatment, and a very low rate of dropouts [102].

Recent observational study used Cysticlean® to study its prophylactic efficacy in women (18-45 years, N=20) with recurrent postcoital urinary tract infections and its impact on their quality of life. 100.0% of patients were positive to the presence of bacteria causing postcoital urinary tract infections at baseline, and only 15.0% at month 6. The number of postcoital urinary tract infections decreased from 2.8 at the baseline visit to 0.6 at Month 3 and 0.2 at Month 6. The number of postcoital urinary tract infections for which patients went to the doctor decreased, as well as the number of infections for which an antibiotic was taken. At baseline, the mean score on the VAS scale for assessing the quality of life was 62.4, increasing to 71.1 at Month 3 and 78.2 at Month 6 [17].

Discussion

Cysticlean® capsules, tablets and sachets are food products that contain a cranberry extract. Its efficacy in UTIs prophylaxis has been demonstrated clinically and exhibited potent inhibition on bacterial adherence. The use of Cysticlean® products exhibits a good tolerability, treatment compliance and efficacy to prevent recurrent urinary tract infection. Cysticlean® provides 118 mg of PACs (vanillin-HCl modified method) in one tablet or sachet to ensure their efficacy and for convenience in their intake.

Urinary tract infections (UTIs) are among the most common types of bacterial infection in outpatient medicine. After respiratory tract infections, they are the most common reason why antibiotics are prescribed. Rising rates of antibiotic resistance and a better understanding of the adverse effects or collateral damage of antibiotics warrant a re-evaluation of the treatment recommendations for uncomplicated UTI. Antibiotic prophylaxis of UTI may cause bacterial resistance and may therefore be ineffective in preventing infection, and it has been associated with some negative effects. Different studies have raised serious doubts about the role of this treatment and recommended alternative strategies, including cranberry extracts.

The inhibitory effect on adherence of E. coli to urothelium is highly desirable in children because UTI is particularly common and recurrence worsening with vesicoureteral reflux. In those cases, continuous antibiotic prophylaxis, prolonged for months or even years, causes undesirable effects, particularly in the intestinal tract, and increases bacterial resistance. Therefore, the availability of an effective cranberry extract to replace antibiotic prophylaxis is a great advance.

Cranberry products significantly reduce the incidence of symptomatic UTIs, particularly in women with recurrence. Their main mechanism of action is the inhibition of the bacterial adherence to uroepithelial cells and PACs are identified as the main responsible for this activity. PACs produces a change of the conformation of the P fimbriae, with a length reduction, causing a decrease of adhesion forces between the

bacteria and the uroepithelial cells. Moreover, the inhibition of adherence activity of the PACs from cranberry has been observed not only to sensitive but also multi-drug resistant bacteria and to asymptomatic bacteriuria.

Since the level of the PACs determines the effectiveness of a cranberry product, it is very important to know the PACs content of these extracts.

Colorimetric methods, mainly vanillin and DMACA, are the most commonly used in quantifying PACs, but there is no official established method. The different analytical methods do not allow correct comparison between different products and pharmacological assays on the adherence of bacteria could be a good alternative to compare different cranberry products. Due to lack of a standard accepted analytical method to measured PAC concentration and PAC composition, cranberry extract anti-adhesion activity should be the best way to guarantee the preventive effect of cranberry extracts.

In vitro and ex vivo, tested concentrations of Cysticlean® have demonstrated dose-effect inhibition of bacterial adherence and agree with published literature data. At dose of 118 mg PACs/animal, the urine samples from rats treated with Cysticlean® capsules and sachets showed a significant inhibition of bacterial adherence. At lower dose Cysticlean® decrease the number of bacteria adhered to epithelial cells, demonstrating the importance of using the necessary amount of PACs.

Cysticlean® has shown greater in vitro activity than other cranberry products. It decreases the number of attached bacteria at lower concentrations and more powerful then these other products. Other ex-vivo studies demonstrated greater benefit observed with doses of PACs higher than those used in preliminary studies. These findings confirmed that the activity of PACs from cranberry is dose-dependent. This shows the need for products with an adequate amount of PACs, such as Cysticlean® that provided 118 mg/day of PACs, with a single capsule or sachet. A single daily dose usually predicts a good compliance and a better tolerability.

Clinical evidence supports that the consumption of the American cranberry products prevents the recurrent UTIs and their use is safe and well-tolerated, without significant drug interactions. The use of American cranberry treatment is supported by European Scientific Cooperative on Phytotherapy (ESCOP) monograph, meta-analysis and different published reviews.

Clinical data have shown the high efficacy and good tolerance of Cysticlean® in children and women with frequent urinary tract infections. Also, the use of Cysticlean® could reduce the antibiotic use, the recurrence of postcoital urinary tract infections and the quality of life of those patients.

Conclusions

There is strong in vitro, ex vivo and clinical evidence confirming the hypothesis that UTIs can be prevented by decreasing bacterial adherence to uroepithelial cells, which is the main mechanism of cranberry extracts.

A-type PACs is the main compound responsible for the anti-adherence activity and thus may be the compound responsible for the beneficial effect on UTI prevention and it is dose-dependent.

American Cranberry products must declare the amount of PACs, and their method of analysis, sufficient to ensure its effectiveness and its anti-adhesion activity. A single capsule or sachet of Cysticlean® provides adequate daily

amount of PACs (118 mg/day) that has shown a greater effect than lower doses.

For people with recurrent uncomplicated UTIs, routine utilization of Cysticlean® products may offer an alternative methodology to antibiotic prophylaxis. The anti-adherence activity of Cysticlean® is greater than other marketed products. The PACs content in Cysticlean® is enough to produce their pharmacological activity, reduce the UTIs recurrences and improve patients symptoms.

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Illustrations

Illustration 1

Figure 1

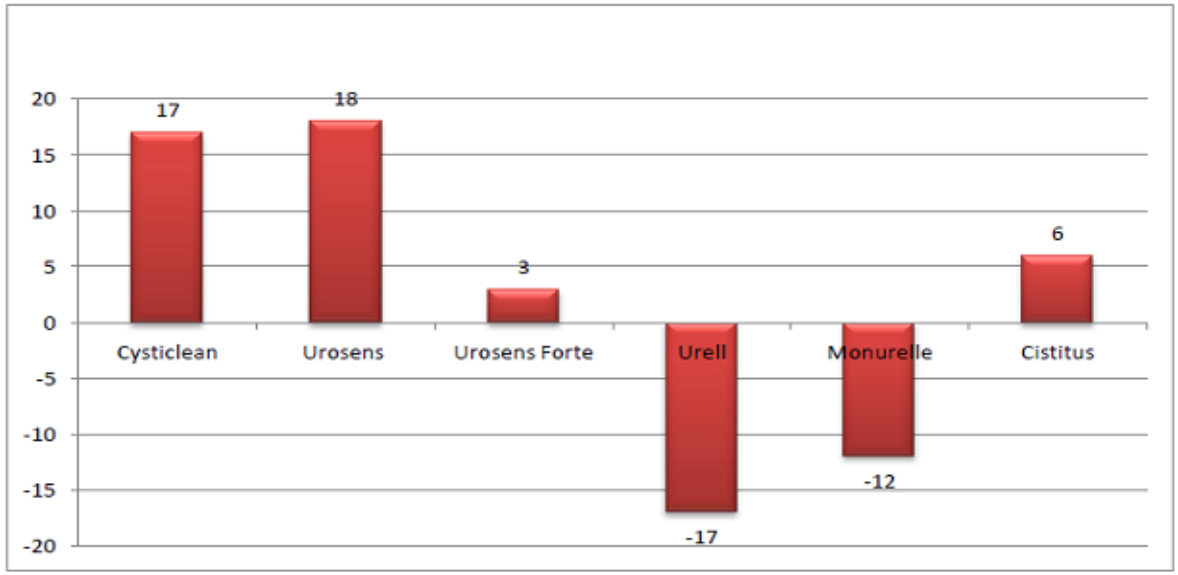

Figure 1: Inhibitory effect of different American cranberry extracts at low PACs concentration on the number of bacteria adhered to T24 uroepithelial cells.

Illustration 2

Figure 2

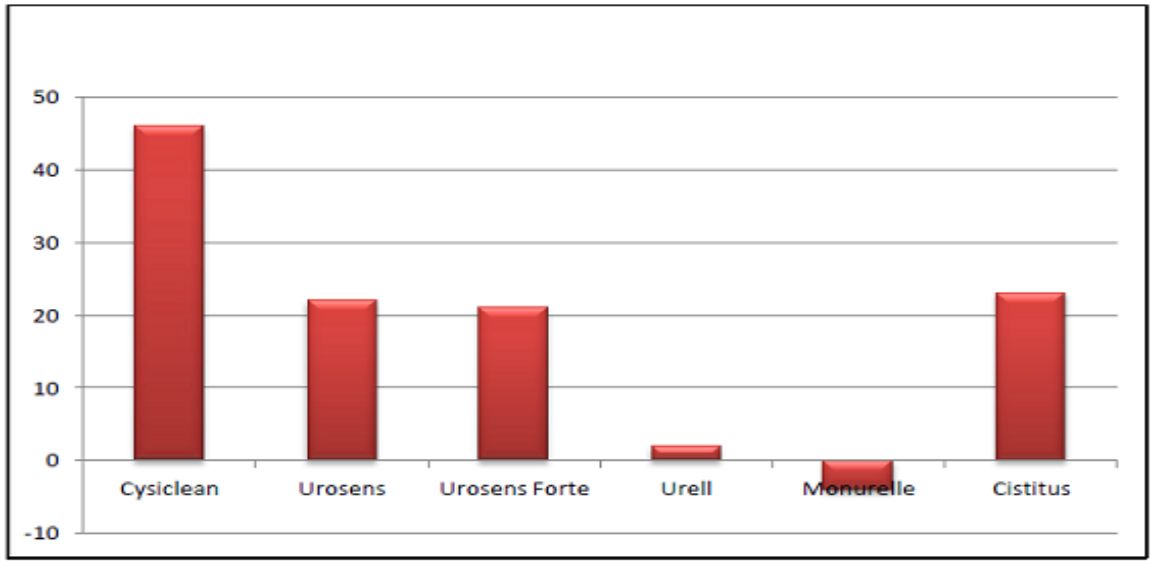

Figure 2: Inhibitory effect of different American cranberry extracts at medium PACs concentration on the number of bacteria adhered to T24 uroepithelial cells.

Illustration 3

Figure 3

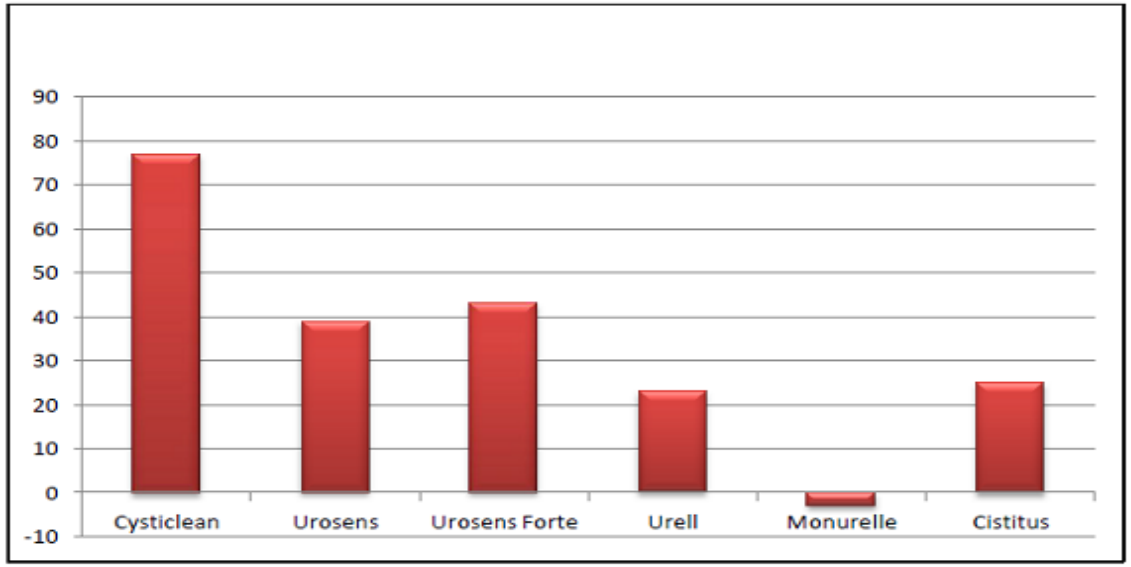

Figure 3: Inhibitory effect of different American cranberry extracts at high PACs concentration on the number of bacteria adhered to T24 uroepithelial cells.

Illustration 4

Figure 4

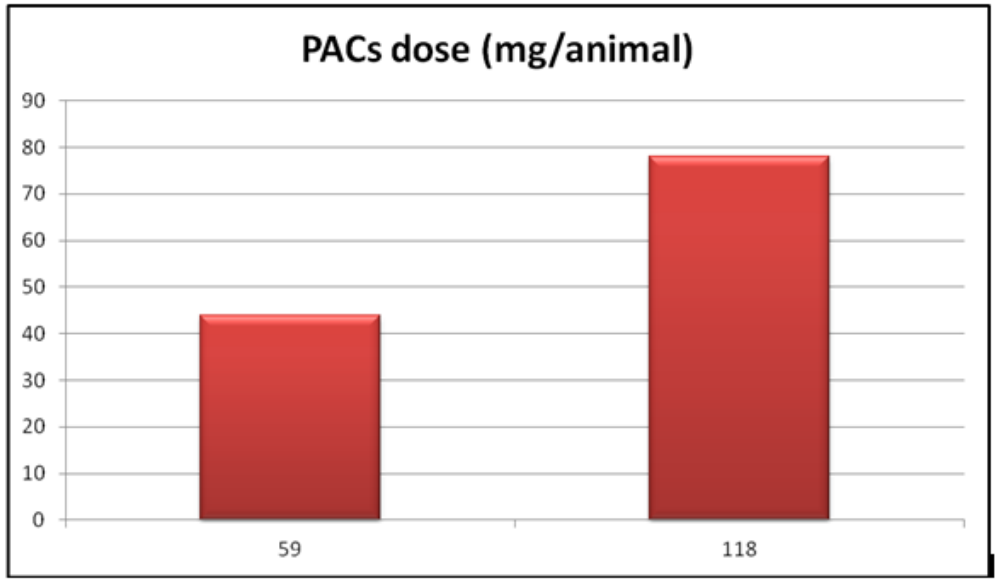

Figure 4: Inhibitory effect of Cysticlean® sachets at doses of 59 and 118 mg of PACs/rat on the number of bacteria adhered to T24 uroepithelial cells

Illustration 5

Figure 5

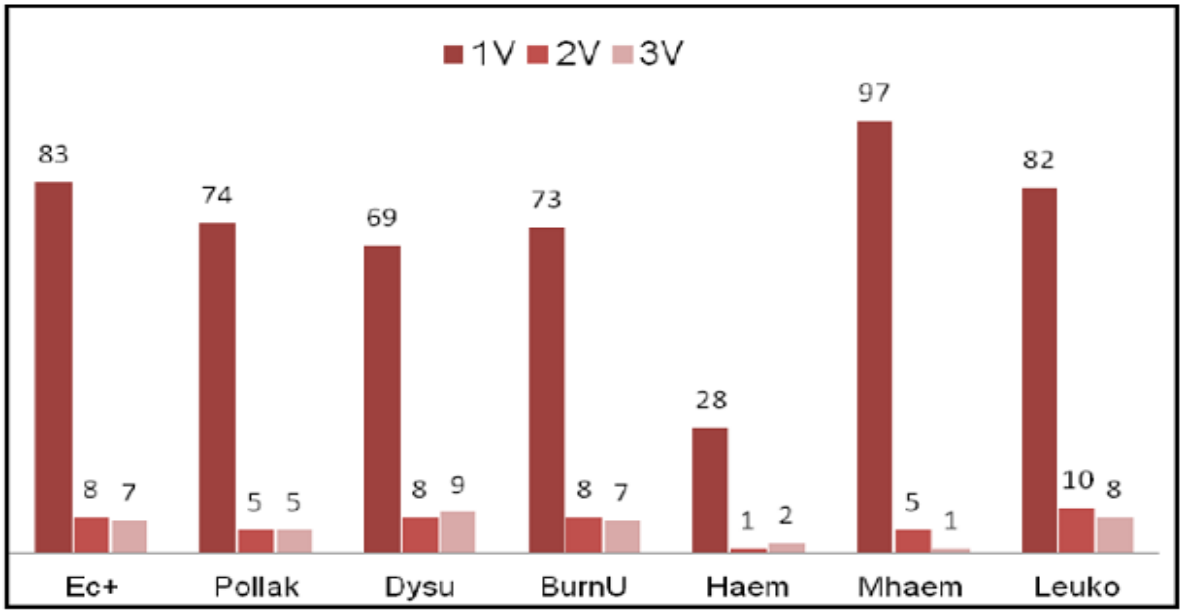

Figure 5: Symptoms and signs in women treated with Cysticlean® tablets (118 mg/day of PACs). V= visit, Ec+ = E Coli sediments, Pollak= pollakuria, Dysu= dysuria, BurnU= burning urination, Haem= macroscopic haematuria, Mhaem= microscopic haematuria, Leuko= leukocyturia in sediment.

Illustration 6

Figure 6

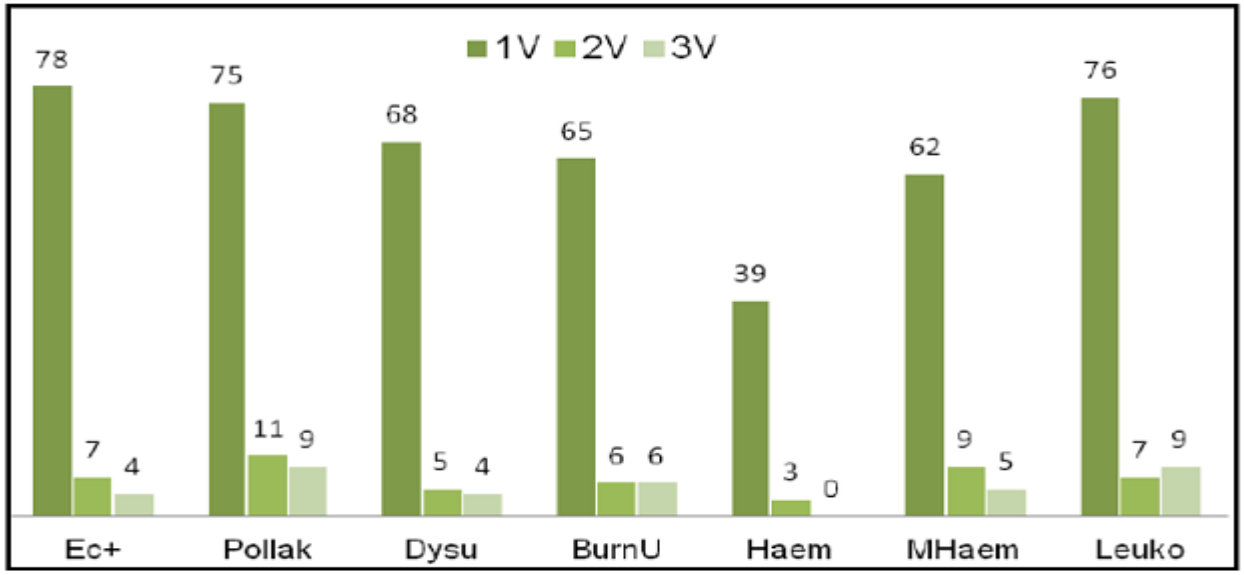

Figure 6: Symptoms and signs of cystitis in women treated with Cysticlean® tablets (118 mg/day of PACs). V= visit, Ec+ = E Coli sediments, Pollak= pollakuria, Dysu= dysuria, BurnU= burning urination, Haem= macroscopic haematuria, Mhaem= microscopic haematuria, Leuko= leukocyturia in sediment.

Illustration 7

Results of the in vitro assay used for the evaluation of the effect of commercially available cranberry capsules on the adherence of E. coli to T24 urinary tract epithelial cells.

Treatment	Dose µg/ml of PACs	Mean number of bacteria adhered/cell	Inhibition %
Cysticlean® Capsules 118mg PACs/ capsule	0	32.4 ± 0.95	
	5	26.8 ± 0.58	17.3
	25	15.5 ± 0.44	52.2
	75	7.8 ± 75.9	75.9
Urosens® 120mg PACs/ capsule	0	31.7 ± 1.74	
	5	26.1 ± 1.92	17.75
	25	24.0 ± 1.83	24.47
	75	19.1 ± 1.35	39.92
Urosens Forte® 120mg PACs/ capsule	0	39.1 ± 1.73	
	5	38.4 ± 1.39	1.9
	25	29.8 ± 1.16	23.9
	75	21.1 ± 1.37	46.1
Urell® 36mg PACs/ capsule	0	31.7 ± 1.74	
	1.5	36.9 ± 2.08	-16.39
	8	30.8 ± 2.84	2.84
	23	22.9 ± 1.51	27.84
Monurelle® 36mg PACs/ capsule	0	39.1 ± 1.73	
	1.5	44.3 ± 1.11	-13.3
	8	41.2 ± 1.29	-5.3
	23	39.8 ± 1.60	-1.7
Cistitus® 36mg PACs/ capsule	0	39.1 ± 1.73	
	2	36.4 ± 1.68	7.0
	10	29.4 ± 1.74	24.9
	30	26.9 ± 1.21	31.3
P<0.05 Recommended dose: 1 capsule/ day			

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