Inhibitory Effect of Cranberry Fruit Extracts on Adhesion of Urinary Tract Infection Bacteria

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Abstract

In vitro adhesion of bacteria to uroepithelial cells in the presence of cranberry methanol and water base extracts has been studied. Microorganisms taken from patients with urinary infections were (48%) *Escherichia coli*, (26%) *Klebsiella pneumoniae*, (16%) *Proteus mirabilis*, and (10%) *Staphylococcus aureus*. The adhesion of bacteria to uroepithelial cells in the presence of cranberry methanol base extract with varying concentrations showed high inhibition at concentrated extract (25 mg/ml) with *S. aureus* and *E. coli* being mostly affected and to a lesser extent with *P. mirabilis* and *K. pneumoniae*. Inclusion of water in the extraction of cranberry, showed an inhibition effect of the concentrated extract (25 mg/ml) as being *ca.* 22% less effective than the methanol base extract. Results are discussed in terms of bacterial structure and the disruption of binding between the receptors of uroepitheilal cells.

Keywords: Cranberry extract, Bacterial adhesion, Uuroepithelial cells, Urinary tract infection.

Introduction

UTI is one of the most common bacterial infections that humans are susceptible throughout their life span. UTI occurs in the elderly patients; it has the greatest impact on females of all ages and males as kidney transplant recipients or with structural abnormalities of urinary tract [1,2].

Antibiotic are the main methods to treat UTI s. However, due to the rapid development of bacterial antibiotic resistance and side effects, alternate solutions for infection prevention are of great interest [3].

The American Red Cranberry (*Vaccinium macrocarpon*) has shown antibacterial activity against pathogenic microorganisms and healthy benefits it provides for humans, since these microorganisms are becoming increasingly resistant to pharmacological and antibiotic treatment [4]. Cranberry juice has been widly used to treat and prevent urinary tract diseases, because it inhibits the adherence of *E. coli* to the uroepithelial cells (UC) of the bladder wall [5]. The importance of the affectivity of cranberry juice as having a broader spectrum of anti-bacterial activity has been highlighted by Ofek and Foo [6], and Magarinos *et al*. [7].

The anti-adhesion activity of cranberry juice in decreasing bacterial adhesion has been focused on two areas. First, characterization of the anti-adhesive compound in cranberry juice and refining the dose needed for identifying metabolites. Second, direction towards the elucidation of the anti-adhesion mechanism that cranberry juice imparts on bacteria and uroepthelial cells [8,9]. Liu *et al.* [10] presented quantitative explanation for the role of cranberry juice in disrupting the binding between P-fimbrated *E. coli* and uroepithelial cells receptors through the use of a thermodynamic model and provided evidence of the biophysical effects on bacteria-uroepithelial cells interactions with cranberry juice. The inhibitory effect of cranberry juice cannot be attributed to its low pH but the cranberry juice contains a number of inhibitory bioactive components, mainly flavones and proanthocyanidines [11,12].

The purpose of this study is to investigate the effect of the base extract solution of cranberry and its role in the inhibitory effect on the *in vitro* adhesion of bacteria isolated from UTI patients to uroepithelial cells.

Materials and Methods

Isolation of bacteria samples

A total of 68 urine samples were collected from patients with UTI symptoms from Baghdad and Yarmook Hospitals during a period of 3 months. Urine samples were cultured on blood agar and MacConkey. All plates were incubated for 24hrs at 37 °C. Identification of isolates was done by colony morphology, Gram stain and biochemical tests [13].

Extraction of cranberry material

A batch of cranberry was obtained from local market, washed several times with distilled water (DW) and air dried at room temperature, then ground into powdery form using sterile electric grinder. The methanol base extract of the active ingredients of the cranberry was carried out using the solvent extraction method described by Akroum *et al*. [14]. A quantity of 20 g of air dried powder was mixed with 100 ml of organic solvent (methanol) and placed in Soxhlet apparatus for 6 hrs at 40 °C. The methanolic extracts were evaporated by using rotary evaporator at 40 °C. The final extract was kept at 4 °C for further studies. For the water base extract, 15 g of air dried powder was soaked for 24hrs in 100 ml DW. The extract was filtered using Whatman filter paper No.1. The filtrates were concentrated by rotary evaporator at 40 °C and stored at 4 °C prior to use [15].

Adhesion testing

Uroepithelial cells were collected from urine of healthy persons. The cells were washed three times in phosphate buffer saline (PBS) (pH 7.2) and finally suspended in PBS (pH7.2).

The bacterial cells, were washed in PBS (pH7.2) and finally suspended in PBS (1*8 CFU/ ml). 0.5 ml of uroepithelial cells suspension was mixed with 0.5 ml of bacterial suspension and incubated at 37 °C for 1h. The mixture was then washed four times. Final cell suspension was dyed, fixed on microscopic slide and stained with methylene blue [16].

The mean number of bacteria adherent to the first 30 uroepithelial cells was counted and the mean and standard errors (SE) were calculated for each preparation.

Adhesion inhibition with cranberry extract

One ml of bacterial suspension and 1ml of uroepithelial cells suspension were mixed with 1ml of cranberry extract (methanol or water) with concentrations of 25, 10, or 5 mg/ml for 3hrs at 37 °C. After incubation, the mixture was then washed four times with PBS. Final cell suspension was dyed, fixed and stained with methylene blue [17].

Statistical methods

Experiments were designed according to a completely randomized design with 3 replications. All preparation results were expressed as mean ± SE. Analysis of variance was performed by ANOVA procedures using SPSS version 10 software. Results with $P \leq 0.05$ were regarded as statistically significant.

Results and Discussion

Bacterial samples isolated from 68 patients with UTI are illustrated in Table (1), which identify the bacteria and their prevalence. *E. coli* were found to be the most prevalent in UTI (48 %) followed by *K. pneumoniae* (26 %), *P. mirabilis* (16 %), and *S. aureus* (10 %).

Table (1)
Bacteria isolated from urine samples of patients with UTI.

Bacteria	*No. of isolates*	*% occurrence*
E. coli	32	48
K. pneumoniae	18	26
P. mirabilis	11	16
S. aureus	7	10

Occurrence percentages in Table (1) isolated from the UTI patients appear to be typical when compared with the same bacteria assigned from hospitalized cases [18]. The occurrence of 10% *S. aureus* is relatively high or in the increase trend. This may be the result of the bacteria being resistant to antibiotic which indicate this increase in occurrence. Shigemura, *et al* [19] in a study lasted 20 years have indicated an increase in the frequency of *S. aureus* and concluded that Methicillin resistant *S. aureus* (MRSA) are rather in the increase and this increase is practically remarkable.

The *in vitro* adhesion in terms of mean and ± SE for 4 isolates of each bacterium to uroepithelial cells without (control) and with inclusion of methanol base cranberry extract and water base cranberry extract are shown in Table (2) and (3) respectively. The adhesive capacity of the bacteria for control was in the

following order: *S. aureus* (50.2± 4.2) › *E. coli* (44.2±3.9) › *K. pneumoniae* (39.9±3.1) › *P. mirabilis* (36.7±4.8) and in the presence of methanol base cranberry extract (25 mg/ml) was in the following order: *S. aureus* (11.3±2.8) ‹ *E. coli* (12.1±3.2) ‹ *P. mirabilis* (16.2±2.7) ‹ *K. pneumoniae* (21.5±3.0) ($P \leq 0.005$). The high value of the adhesive capacity of *S. aureus* may be the results of fluctuation in the bacterial distribution in the samples studied. In other relevant study [20], *S. aureus* was found to have high capacity to adhere due to some prevalence factors.

Table (2)
Bacterial adhesion to uroepithelial cells in the presence of different cranberry methanol base extract concentrations in comparison with control.

Bacteria	***Adhesion in methanol base extract Mean± SE***			
	control	***25 mg/ml***	***10 mg/ml***	***5 mg/ml***
E. coli	44.2±3.9	12.1±3.2	20.2±3.1	26.6±3.5
K. pneumoniae	39.9±3.1	21.5±3.0	26.4±2.9	30.2±3.7
P. mirabilis	36.7±4.8	16.2±2.7	22.7±2.4	27.2±3.1
S. aureus	50.2±4.2	11.3±2.8	18.6±2.5	29.8±3.3

Table (3)
Bacterial adhesion to uroepithelial cells in the presence of different cranberry water base extract concentrations in comparison with control.

Bacteria	***Adhesion in water base extract Mean± SE***			
	control	***25 mg/ml***	***10 mg/ml***	***5 mg/ml***
E. coli	44.2±3.9	17.6±3.3	24.1±2.8	29.2±3.0
K. pneumoniae	39.9±3.1	24.3±3.4	30.7±3.1	32.8±2.7
P. mirabilis	36.7±4.8	25.8±2.9	28.9±2.6	35.0±3.2
S. aureus	50.2±4.2	16.9±3.2	23.9±3.0	34.8±2.9

The adhesion in the water base extract (Table (3)) is similar to those for methanol base yet they are in the same order but slightly less effective in inhibition ($P \leq 0.05$) when compared with methanol base; suggesting that water base cranberry extract can be regarded as a candidate for future investigations.

Fig.(1) displays the mean values of adhesion of bacteria for different concentrations of cranberry methanol base extract in comparison with control. Results show that the extract of cranberry concentration is in the following order antibacterial activity against *S. aureus*, › *E. coli*, › *P. mirabilis* and › *K. pneumoniae*.

The inhibition effect of cranberry methanol base extract seems to increase in high concentrations for all types of bacteria but mostly in *S. aureus* and *E. coli*. These results confirm that cranberry extract act as an inhibiter to bacterial adhesion and may be regarded as an alternative non-antibiotic agent for treatment of UTI.

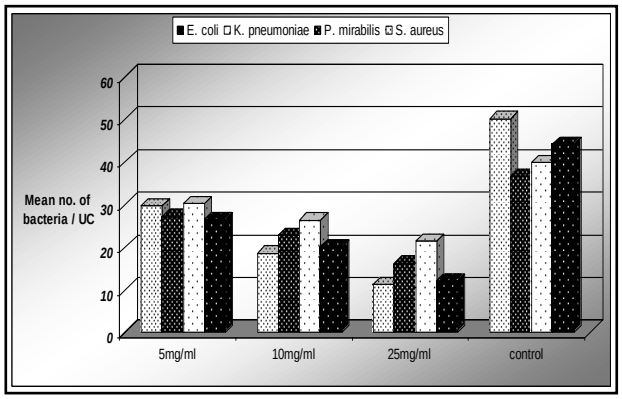

Fig. (1) Mean number of adherent bacteria per uroepithelial cell treated with different concentrations of cranberry methanol bas extract in comparison with untreated bacteria /UC adhesion (control).

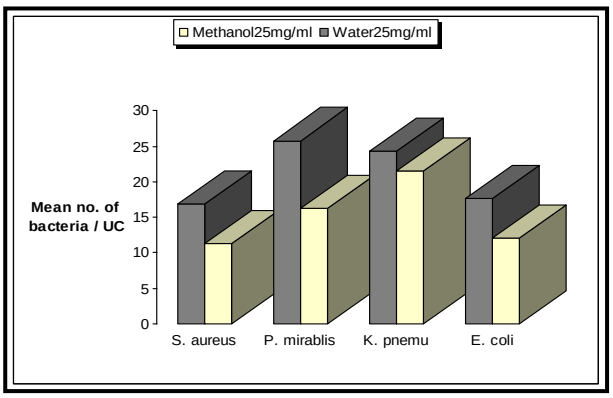

Fig. (2) Mean number of adherent bacteria / UC treated with methanol and water base cranberry extract for a fixed concentration of 25 mg/ml.

The results are in line with those of Magarinos *et al.* [7] who reported that *S. aurous* was more susceptible to cranberry juice inhibition than other microorganisms. Moreover, Akroum *et al.* [14] have found that the cranberry extract has limited the urinary infections caused by *E. coli*, *S. aureus*, and *Candida albicans*.

To monitor the effectiveness of methanol base cranberry extract, water base cranberry was included in the adhesion of bacteria to uroepithelial cells for high fixed concentration of 25 mg/ml, and the bacterial adhesion in the water base extract is shown in Fig.(2). The adhesion of bacteria to uroepithelial cells in the presence of water base extract is less effective by *ca.* 22 % from those of methanol base extract, and also to the hydrophilicity of bacteria to adhere in aqueous of cranberry. The results may be attributed to the difference in the pH of methanol and water base extract.

Our results are in line with Rahbar and Diba [21] who showed that the methanol extract of cranberry plant inhibits the growth of bacterial isolates at varying concentrations.

The anti-adhesive property of cranberries probably works in two ways: first it directly prevents bacteria from adhering to uroepithelial cells, which may be caused by the presence of cell wall structure in *S. aureus* and fimbriae in *E. coli* and *P. mirabilis*, and capsule in *K. pneumonia*; second it has the potency to disrupt the binding between the receptors of uroepithelial cells and fimbriae.

The methanol extract showed more inhibitory effects than the water extract.

Conclusions

- Methanol base cranberry extract at high concentrations inhibits bacterial adhesion to uroepithelial cells with *S. aureus* and *E. coli* being more susceptible to inhibition.
- Active ingredients in cranberry plant are better extracted with methanol than water as manifested by better inhibition affectivity.

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الخلاصة

تم دراسة تثبيط التصاق البكتريا بالخلايا الطلائية البولية خارج الجسم الحي (*In vitro*) بوجود مستخلصي الزعرور الكحولي أو المائي. شخصت الأحياء المجهرية المعزولة من مرضى مصابين بالتهاب المجاري البولية على احتوائها 48% *Escherichia coli* ,26% *Klebsiella pneumoniae* ,16% *Proteus mirabilis* ,10% *Staphylococcus aurous*. أن التصاق البكتريا بالخلايا الطلائية البولية بوجود تراكيز مختلفة من مستخلص الزعرور الكحولي قد اظهر أعلى درجة للتثبيط عند التركيز 25 ملغم/ مل مما عليه للتراكيز 10و 5 ملغم/ مل لكل من بكتريا *S. aurous* و *E. coli* مظهرة تأثرا اكبر للتثبيط مما عليه مع بكتريا *P. mirabilis* و *K. pneumoniae*. في حين يُظهر مستخلص الزعرور المائي بنفس التركيز (25 ملغم/ مل) تأثير تثبيطي للالتصاق اقل من المستخلص الكحولي بنسبة مئوية تقدر بـ22%. نوقشت النتائج على ضوء تركيب البكتريا والتشتيت في الارتباط بين مستقبلات الخلايا الطلائية البولية.