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Evaluation of direct *in-vitro* effect of *Vaccinium macrocarpon* against *E. coli* in urinary tract infection patients

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Abstract

Cranberry extract is a famous crude drug used in urinary tract infection all over the world and research is being carried out on its different aspects in urinary tract infection treatment. In patient with recurrent urinary tract infection and prophylaxis use of anti-microbial is required for prolong period. It is an effective way but on the cost of adverse effects and increasing resistance among uro-pathogens has pursued the researchers to dig out other alternative anti-microbial agents for treatment of recurrent urinary tract infection. This fact has created interest in Cranberry (*Vaccinium macrocarpon*) for its use in recurrent urinary tract infection. One hundred patients from two hospitals were studied. Using well diffusion method *E. coli* resistance against Cranberry was checked in-vitro. *E. coli* showed resistance against CB extract powder in both centers. Cranberry has no direct effect on *E. coli* but its effectiveness in urinary tract infection along with antibiotics is due to its anti-motility, anti-adherence effects.

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Introduction

Urinary tract infection (UTI) is among the most common bacterial infections occurring in population and there is an estimate of incidence 18 people per thousand annually (Nicolle, 2008). UTI is important cause of death in population (Acharya *et al.*, 2011). It is estimated that on average 6 million patients visit out-patient department in the world every year and 300,000 are treated for UTI (Akortha and Ibadin, 2008).

UTI is defined as invasion of any part of urinary tract by microorganisms and causing disease. Disease may be in the form cystitis, urethritis, pyelonephritis and asymptomatic bacteriuria. Mostly UTI symptoms are found in women frequently while having negative effects on their life specifically on physical, medical, psychological, social and economic aspects (Bilgic and Beji, 2010). Asymptomatic bacteriuria may be defined as: "isolation of bacteria in significant quantities from urine without symptoms". Significant bacteriuria is considered when microbiologist fined 10^5 colony forming units (CFU) per milliliter of urine.

Commonly the patients visiting the out-patients departments and clinics are diagnosed based on their urinalysis and clinical presentation(Lane and Takhar, 2011). UTI is divided into complicated and uncomplicated based on pathology. Uncomplicated UTI is acquired from community and normally found in urinary tract. More than 80% cases are due to *E. coli*. Approximately 1.6 billion dollars is the cost every year on urinary tract infections (Foxman *et al.*, 2000).

It is required to explore alternative options for UTI management due to high incidence, high economic burden and drug resistance (Ullah *et al.*, 2009). Many studies strongly suggest that cranberry (*Vaccinium macrocarpon*) use in UTI is beneficial (Minardi *et al.*, 2011; Hisano *et al.*, 2012; Micali *et al.*, 2014). Cranberry was in use of native Americans as medicine prior to 1620 and it remained in use as urinary antiseptic for more than 200 years.

There were limited clinical studies on cranberry effect but still they showed promising results in many cases. For example, many studies showed that bacteriuria and pyruria were reduced in patients treated with cranberry juice compared to those of receiving placebo drugs. Despite of the fact that cranberry promotes healthy urinary tract, detailed understanding of how cranberry works to improve health is still lacking. In start it was thought antibacterial activity of cranberry is due to acidity of cranberry due to hippuric acid however more experiments showed that pH of urine shows only slight decrease (Liu *et al.*, 2006) Proanthocyanidins (PACs) are active compounds in cranberry that adhere to fimbriae of bacteria i.e., *E. coli* and hinder their attachment to urinary tract epithelium so they get washed away (Lavigne *et al.*, 2008; Pérez-López *et al.*, 2009). Many questions are unanswered toward role of cranberry that how it works. Current study was aimed to investigate whether Cranberry has direct anti-microbial effect on *E. coli*orin direct action by its constituents on uro-epithelium.

Material and methods

It was a lab based experiment. The study was conducted the microbiology Departments of Abbas Institute of Medical sciences and Combined Military Hospital Muzaffarabad Azad Kashmir. The urine samples obtained from OPDs (outpatients departments) and also from indoor (admitted patients of different departments) during Nov 2014 to Jun 2015. After explaining the method to the patients and taking proper ethical consent sample were collected in sterile container. For microscopy we observed 50ml of urine under high powers objectives. For culture purposes criteria was presence of pus cell >5 under high power field. Further bacterial load $\geq 10^5$ cfu/mL confirmed presence of UTI. Sterile platinum loop was used inoculate urine sample to the CLED media and incubated at 37°C for 24 hours.

Identification and Maintenance of Pure Bacterial Isolates

Colony morphology was done, Pure colonies>**15** were considered as significant growth. The isolated bacteria were maintained in nutrient agar and incubated at 37°C for 24 hrs.

Cranberry Powder and Analysis

The CB (*Vccinium macrocarpon*) powder was obtained from Flow pharmaceuticals Lahore The company imported it from Hangzhous Star Shine Pharmaceutical Ltd China.

This powder was analyzed and its solution was prepared as per requirement of study in the quality control lab of Well mark pharmaceuticals Hittar. UV method was adopted and its absorption was noted at 980nm and on calculation its percentage was found 101% while limit is 98-102%. This confirmed the purity of CB powder of the study.

Preparation of Cranberry Solution

An amount of 125mg of this fine, free flowing light reddish powder was dissolved in 100ml of water at 10c (as per instructions of Artemis International, April 2010.) This yielded concentration of 1.25mg/ml which was required because 1.25mg/ml is already approved MIC of CB against. *E. coli.*

This solution was allowed to stand for 72 hr, and sterilized by filtration through filter paper of 0.45micrometer diameter. Than Kept it at 4c for further use in my experiment (Al-Khafaji, 2014).

Agar Well Diffusion Method for in-vitro effect of CB against E. coli

E. coli was taken with the help of sterilized wire lope from colonies of bacteria and suspended it in normal saline. The turbidity of diluted bacterial suspension was adjusted with normal saline by comparing it with 0.5 Mc Farl and standard for preparing $1.5x10^8$cfu/ml concentration of bacteria in this suspension. Sterilized cotton swab was dipped in this uniform prepared suspension of tested bacteria. Streaked it on whole surface of Mueller-Hinton agar plate and kept in room temperature for 15 minutes for drying. The wells of 5mm diameters were made into this agar plate with the help of sterile cork borer. Then added 20 micro liter of CB solution into these wells and incubated at 37^oC for overnight. The effect of CB on inhibition of *E. coli* growth was observed in term whether zone around well is inhibited or not.

Results

Gender Pattern of UTI at AIMS

The UTI was found predominant in female that was 66%.and patients having age 20months-68 years were suffering from this disease.

Gender Pattern of UTI at CMH Muzaffarabad

The UTI was found predominant in female that was 68%.and patients having age 20months-68 years were suffering from this disease.

In-Vitro effect of Cranberry on E. coli growth

Procedure for in-vitro susceptibility of CB has been carried out for my samples in micro-biology department of both hospitals of my study work. In spite of performing procedure several times but no inhibitory effect of this compound was seen.

Table 1. Sensitivity pattern of *E.coli* against CB.

No. of Samples	Resistant	Sensitive	Percentage of Resistant
100 (50 at each hospital)	100	0	100%

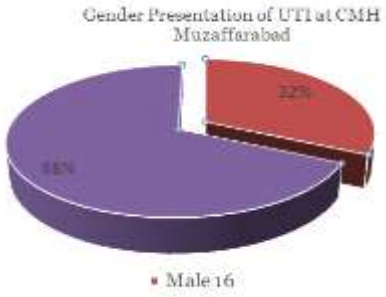

Fig. 1. Gender representation at AIMS.

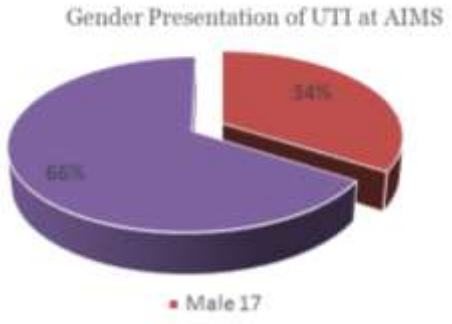

Fig. 2. Gender presentation of UTI pattern in CMH Muzaffarabad.

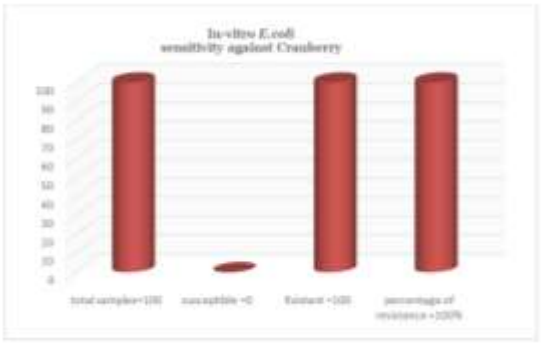

Fig. 3. *In-vitro* Cranberry sensitivity against *E. coli*

Discussion

Currently cranberry (*Vaccinium macrocarpon*) is used widely for the prevention of UTI. It is used in the form of juices, capsules and extract. Further work is also required for the cranberry role for the management of urinary tract infection. Most common pathogen isolated in our study was *E. coli*. Our study clearly indicated that in vitro there is no effect on *E. coli* and it showed 100% resistance against it. This study showed that there is no antibacterial activity of cranberry.

It means beneficial effect of CB in UTI is attributed to its other in-direct actions that prevent establishment of infection by uro-pathogens in urinary tract. Different researches having full support for following of its indirect actions on uropathogens that helps in treatment and prophylaxis in recurrent UTI.

The motile capability of pathogen has a main step in establishing colonies on the uro-epithelium surface. This provides basis for establishing infection (Hidalgo *et al.*, 2011). CB contains Tannic acid that blocks the motility of *E. coli* (Howell, 2007). Tannic acid has ability to inhibit binding of Pilli of *E. coli* with epithelium cells of bladder(Brooks, 1990).

In pathogenesis of UTI, the first step is adherence of uropathogens to epithelial cells of urinary tract with help of fimbriae of *E. coli* (Raz *et al.*, 2004). The adherence of *E. coli* with uro epithelium is of two types mannose sensitive and mannose resistant (Gupta *et al.*, 2007). CB contains two compounds Fructose and pro-anthrocynadin, both of these having similar structure as of *E. coli* to compete with receptors site of uro-epithelium.

These two compounds blocks the receptors and this prevents the attachment of fimbriae of bacteria with uro-epithelium which is a first step for establishing UTI (Sharma and Tiwari, 2012). Fructose inhibits mannose sensitive fimbriae adherence while pro-anthro cynadins inhibits mannose resistant fimbrial adherence of *E. coli* (Zafriri *et al.*, 1989). Cranberries are rich in Benzoic acid, after bio- transformation Hippuric acid is excreted in urine (Blatherwick, 1914). In human Hippuric acid is synthesized from Benzoic acid.

This process occurs in liver with the help of ATP and Co-enzyme A (Toromanović[1] *et al.*, 2008). It was thought that Cranberries juice is beneficial due to Hippuric acid excretion in urine, the bacteriostatic agent (Moen, 1962). Decreasing in PH of urine from 6.4-4.5, there is increased in excretion of Hippuric acid from 0.77-4.74gm in urine of individual who had eaten 350gm of cooked berries (Blatherwick and Long, 1923).

It was noted that Hippuric acid was bacteriostatic at a minimum concentration of 0.02mmol/l at PH 5 and antimicrobial activity of Hippuricacid decreased 5 fold as slight increase in PH that is from 5-5.6 (Bodel *et al.*, 1959).

Conclusion

This experiment proved that CB has no direct in-vitro activity against *E. coli* but its beneficial effect in UTI is due to its anti-motility, anti-adherence on uro-pathogen that prevent colonization which is first step for establishment of infection and bacteriostatic effects of its metabolite that is Hippuric Acid, which kills bacteria directly when passing through Urinary tract system after ingestion of this product but concentration of this valuable metabolite is very low in normal doses that cannot be claimed for this drug to use independently in UTI.

Hence these facts prove that CB can be used as prophylaxis in re-current UTI and as adjuvant therapy in UTI with standard regimen of antibiotic to achieve therapeutic goal efficiently in minimum time.

References

Acharya A, Gautam R, Subedee L. 2011. Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. Nepal Med Coll J **13**, 30-33.

Akortha E, Ibadin O. 2008. Incidence and antibiotic susceptibility pattern of Staphylococcus aureus amongst patients with urinary tract infection (UTI) in UBTH Benin City, Nigeria. African Journal of Biotechnology **7**.

Bilgic D, Beji NK. 2010. Lower urinary tract symptoms in women and quality of life. International Journal of Urological Nursing **4**, 97-105.

Blatherwick N. 1914. The specific role of foods in relation to the composition of the urine. Archives of Internal Medicine **14**, 409-450.

Blatherwick N, Long ML. 1923. Studies of urinary acidity II. The increased acidity produced by eating prunes and cranberries. Journal of biological chemistry **57**, 815-818.

Bodel PT, Cotran R, Kass EH. 1959. Cranberry juice and the antibacterial action of hippuric acid. The Journal of laboratory and clinical medicine **54**, 881-888.

Brooks D. 1990. The management of suspected urinary tract infection in general practice. Br J Gen Pract **40**, 399-401.

Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. 2000. Urinary tract infection: self-reported incidence and associated costs. Annals of epidemiology **10**, 509-515.

Gupta K, Chou M, Howell A, Wobbe C, Grady R, Stapleton A. 2007. Cranberry products inhibit adherence of p-fimbriated Escherichia coli to primary cultured bladder and vaginal epithelial cells. The Journal of urology **177**, 2357-2360.

Hidalgo G, Chan M, Tufenkji N. 2011. Inhibition of Escherichia coli CFT073 fliC expression and motility by cranberry materials. Applied and environmental microbiology **77**, 6852-6857.

Hisano M, Bruschini H, Nicodemo AC, Srougi M. 2012. Cranberries and lower urinary tract infection prevention. Clinics **67**, 661-668.

Howell AB. 2007. Bioactive compounds in cranberries and their role in prevention of urinary tract infections. Molecular nutrition & food research **51**, 732-737.

Lane DR, Takhar SS. 2011. Diagnosis and management of urinary tract infection and pyelonephritis. Emergency medicine clinics of North America **29**, 539-552.

Lavigne JP, Bourg G, Combescure C, Botto H, Sotto A, 2008. *In vitro* and *in vivo* evidence of dose dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon (cranberry) capsules. Clinical Microbiology and Infection **14**, 350-355.

Liu Y, Black MA, Caron L, Camesano TA. 2006. Role of cranberry juice on molecular scale surface characteristics and adhesion behavior of Escherichia coli. Biotechnol. Bioeng. **93**, 297-305.

Micali S, Isgro G, Bianchi G, Miceli N, Calapai G, Navarra M. 2014. Cranberry and recurrent cystitis: more than marketing? Critical reviews in food science and nutrition **54**, 1063-1075.

Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. 2011. Urinary tract infections in women: etiology and treatment options. Int J Gen Med **4**, 333-343.

Moen D. 1962. Observations on the effectiveness of cranberry juice in urinary infections. Wisconsin medical journal **61**, 282-283.

Nicolle LE. 2008. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urologic Clinics of North America **35**, 1-12.

Pérez-López FR, Haya J, Chedraui P. 2009. Vaccinium macrocarpon: an interesting option for women with recurrent urinary tract infections and other health benefits. Journal of Obstetrics and Gynaecology Research **35**, 630-639.

Raz R, Chazan B, Dan M. 2004. Cranberry juice and urinary tract infection. Clinical infectious diseases **38**, 1413-1419.

Sharma D, Tiwari S. 2012. Use of cranberry in Urinary Tract Infection of E. coli. International Journal of Pharmacy & Life Sciences **3**.

Toromanović[1] J, Kovač-Bešović E, Šapčanin A, Tahirović[1] I, Rimpapa Z. 2008. Urinary hippuric acid after ingestion of edible fruits. Bosnian Journal of Basic Medical Sciences **8**, 38-43.

Ullah F, Malik S, Ahmed J. 2009. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial Escherichia coli from urinary tract infections in Pakistan. African Journal of Biotechnology **8**.

Zafriri D, Ofek I, Adar R, Pocino M, Sharon N. 1989. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. Antimicrobial agents and chemotherapy **33**, 92-98.