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Use of aqueous extract of cranberry in the treatment of urinary tract infection during pregnancy.

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ABSTRACT

Asymptomatic bacteriuria presents a considerable risk to the mother and may lead to onset of acute pyelonephritis in about 5% of pregnant women and also increase the risk of fetal mortality. In the present study 250 urine samples were collected from pregnant women attending antenatal clinic of Somaiya Hospital, Sion, Mumbai. The frequency of asymptomatic bacteriuria was 7.6% in our study. Prolonged use of antibiotics in urinary tract infection cases may cause side effects and resistance in bacteria against antibiotics. Therefore, we need alternative herbal and non-antibiotic therapy for urinary tract infection such as cranberry fruit. Thus, the study further continued to better understand effects of cranberry juice on uropathogenic strains in cases of urinary tract infection. D-mannose, a simple sugar found in cranberries and other fruits, is included in this formula to provide additional concentrated support. A total of 18 gram positive and gram negative bacteria and 1 fungal isolate, isolated from urine samples were tested against different concentrations of cranberry juice to examine the inhibitory activity invitro. All isolates showed sensitivity to concentrated cranberry juice while in antibiotic therapy Cefazolin, Norfloxacin, ciprofloxacin, co-trimoxazole, Levofloxacin and Cefotaxime were found to be most effective antibiotics against urinary isolates. It seems that cranberry has potent antibacterial activity against some uropathogens.

KEYWORDS : Urinary tract infection, Asymptomatic bacteriuria, Cranberry, D-mannose, Antibiotics.

INTRODUCTION:

Infection of the urinary tract, so called "The Problem Tract", is one of the commonest of all infections. UTI are the most common medical complications of pregnancy. Due to the increase in sex hormones and the anatomic and physiologic changes during pregnancy, bladder and kidney infection is more likely and may result in hypertension, preeclampsia, low birth weight, prematurity, septicaemia and maternal death[1]. Asymptomatic bacteriuria (ASB) is defined as significant bacterial count ($> 10^5$ organisms or colony forming units present per millilitre) in the urine of a person without symptoms and is present approximately in 5 to 10% of the pregnant women and if untreated, it leads to the development of symptomatic cystitis and pyelonephritis in upto 50% of patients[1,2]. However, with the advent of modern medicine, the use of selected antibacterial agents and antibiotics was validated in clinical practice. Although the effectiveness of antibiotics was validated in clinical practice, the continued use of selected agents in the prophylaxis and therapy of UTIs led to emergence of antibiotic resistant uropathogens. This triggered an interest in the application of alternative, non-antibiotic approaches for preventing and controlling UTIs during pregnancy. These include cranberry, blueberry, berberine, bearberry, cinnamon, cornsilk and other herbs[3]. In our study we deal with cranberry fruit. Proanthocyanidins (PACs) - in the American cranberry extract block fimbriae, which bacteria use to attack predominately the kidneys. D-Mannose – a monosaccharide found in the fruit of many plant types, after entering the urinary tract, pathogenic bacteria stick to its surface with the help of fringelike projections called fimbriae. Together D-Mannose and the proanthocyanidins block over 95% of the fimbriae of the bacteria. Therefore D-Mannose and the proanthocyanidins combined provide a much better and reliable urinary tract protection[3,4].

AIMS AND OBJECTIVES: The aim of our study is to screen the pregnant women attending the antenatal clinic for asymptomatic bacteriuria. To study the antibiotic resistance pattern of uropathogens in pregnant women associated with urinary tract infection. To investigate the antibacterial effects of cranberry against uropathogens during pregnancy.

MATERIALS AND METHODS:

Location of study: This study was carried out in the Department of Microbiology, K.J.Somaiya College of Science and Commerce. A total of 250 pregnant women with asymptomatic bacteriuria attending Somaiya hospital, were enrolled for the study.

Collection and Processing of specimen: Urine samples were collected by standard mid-stream "clean catch" method from all the pregnant women, in a sterile, wide-mouthed container that can be covered with a tightly fitted lid and transported to laboratory without delay. The samples were processed using standard microbiological procedures [2,3]. Culture was put-up by standard loop method on sheep blood agar and Mac-conkey agar and by pour plate method on nutrient agar. The inoculated plates were incubated at 37°c for 24 to 48 hrs [3]. Culture results were interpreted as being significant and insignificant, according to the standard criteria. Count of $> 10^5$ CFU/mL was taken as significant bacteriuria. Positive cultures with colony count less than 10^5 CFU/mL was considered as not significant and if no growth was observed after 48 Hrs of incubation; cultures were reported as no growth [9, 10]. Identification of isolates was done by colony morphology, gram staining and standard biochemical tests [1, 3].

Collection and Identification of Plant Material and plant extraction: Plant materials, cranberry fruit were obtained from the local market. The sample were washed with distal water and then squeezed to obtain pure juice, filtered by filter paper and serial of dilutions were made by sterile distilled water (D.W.). The isolates were tested against different dilutions of cranberry juice (100%, 100%+ D-mannose (2% w/v), 1:1, 1:5) to examine the inhibitory activity in vitro[4,5].

Antibacterial activity: By using well assay, all isolates were cultured in Muller-Hinton agar plate, each plate were cultured with overnight growth from nutrient broth by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 5.0 mm was used to make five wells in each agar plates. Five dilution were made with sterile distilled water (100% cranberry and 100% D-mannose, 100% cranberry + D-mannose (2% w/v), 1:1, 1:5 with cranberry juice). A 50µL volume of each dilution was applied by micropipette in each well into Muller-Hinton Agar plate. The plates were allowed to stand for 1h or more at refrigerator for diffusion to takes place to avoid evaporation and then incubated at 37°C for 24hrs to examine inhibitory effect. The zone of inhibition was recorded in mm [4, 5].

Antibiotic susceptibility test: Bacterial susceptibility to antimicrobial agents was determined by the Kirby Bauer disk diffusion method on Mueller-Hinton agar. Isolates were categorised as susceptible and resistant based upon interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI). Antibiotic discs (Hi-Media) Ampicillin (10mcg), Cefazolin (30mcg), Nalidixic acid (30mcg), Norfloxacin (10), Ciprofloxacin (5mcg), Co-Trimoxazole (25mcg), Levo-

floxacin (5mcg), Nitrofurantoin (300mcg) were used for antimicrobial susceptibility tests [1,3].

RESULTS and DISCUSSION:

Table 1: Uropathogens isolated in the study group.

Sr No.	Organisms	No. of organisms	Percentage (%)
1.	*Escherichia coli*	13	41.9
2.	*Klebsiella pneumonia*	04	12.9
3.	*Proteus mirabilis*	06	19.3
4.	*Pseudomonas aeruginosa*	02	6.4
5.	*Staphylococcus aureus*	02	6.4
6.	*Candida albicans*	01	3.22
7.	*Streptococcus pyogenes*	03	9.67
	Total	31	100

Table 2: Prevalence of ASB in the study population based on age distribution.

SR. No.	Colony forming units (CFU/ml)				
	Age group	10^5	10^2-10^4	$<10^2$	Total
		Positive %	Suspected (Doubtful) %	Negative %	
1.	15-20	1 (5.26)	2 (33.33)	2 (33.33)	5 (16.12)
2.	21-26	5 (26.31)	3 (50.00)	3 (50.00)	11 (35.48)
3.	27-32	10 (10.00)	1 (16.66)	1 (16.66)	12 (38.70)
4.	33-38	3 (15.78)	0 (0.00)	0 (0.00)	03 (09.67)
5.	39-44	0 (0.00)	0 (0.00)	0 (0.00)	00 (00.00)
	Total	19 (61.29)	06 (19.35)	06 (19.35)	31 (100.00)

Table 3: Antimicrobial Susceptibility testing.

Sr. No.	Antibiotics	For all isolates (%) n=19	
		Resistance	Sensitive
1.	Ampicillin (AMP)	44	56
2.	Cefazolin (CZ)	06	94
3.	Nalidixic acid (NA)	28	72
4.	Norfloxacin (NX)	06	94
5.	Ciprofloxacin (CIP)	-	100
6.	Co-trimoxazole (COT)	11	89
7.	Levofloxacin (LE)	11	89
8.	Nitofurantoin (NIT)	28	72
9.	Ceftazidime (CAZ)	22	78
10.	Cefotaxime (CTX)	-	100
11.	Cefpodoxime (CPD)	44	56

Table 4: Antibacterial activities of cranberry juice.

Sr.No.	Isolates	Zone of inhibition (mm)				
		100% cranberry	100% D-mannose	100% + D-mannose	1:1	1:5
1.	*Escherichia coli*	15	14	18	R	R
2.	*Escherichia coli*	16	15	19	R	R
3.	*Klebsiella pneumoniae*	18	17	20	R	R
4.	*Escherichia coli*	15	15	19	R	R
5.	*Proteus mirabilis*	R	R	R	R	R
6.	*Escherichia coli*	16	15	18	R	R
7.	*Candid albicans*	R	R	R	R	R
8.	*Escherichia coli*	16	16	20	R	R
9.	*Klebsiella pneumoniae*	18	18	20	R	R
10.	*Proteus mirabilis*	R	R	R	R	R
11.	*Pseudomonas aeruginosa*	19	17	21	R	R
12.	*Staphylococcus aureus*	18	15	20	R	R
13.	*Streptococcus pyogenes*	15	14	17	R	R
14.	*Escherichia coli*	16	15	18	R	R
15.	*Proteus mirabilis*	R	R	R	R	R
16.	Streptococcus pyogenes	15	14	17	R	R
17.	Escherichia coli	15	14	19	R	R
18.	Proteus mirabilis	R	R	R	R	R
19.	Escherichia coli	17	15	20	R	R
20.	Control	R	R	R	R	R

Table 1 describes the pattern of isolates out of the total 31, *Escerichia coli* was the predominant organism isolated in 41.9% of pregnant women followed by other uropathogens such as *Klebsiella pneumonia* (12.9%), *Proteus mirabilis* (19.3%), *Pseudomonas aeruginosa* (6.4%), *Staphylococcus aureus* (6.4%), *Candida albicans* (3.22%), *Streptococcus pyogenes* (9.67%). Table 2 describes that Out of 250 pregnant women examined for asymptomatic bacteriuria, 219 urine samples had no growth when plated and were found to be sterile. Significant bacteriuria was found in 19 (7.6%) cases and insignificant bacteriuria in 6 (2.4%) cases. This study revealed that Cefazolin, Norfloxacin, ciprofloxacin, Co-trimoxazole, Levofloxacin and Cefotaxime were effective against most of the urinary isolates. Nalidixic acid, Nitrofurantoin, Ceftazidime were moderately effective against the urinary isolates. Ampicillins, Cefpodoxime were least effective against the isolates (table3). All isolates showed sensitivity to concentrated cranberry i.e. 100% and 100% concentrated cranberry + D-mannose (2%w/v) and 100% D-mannose except *Candida albicans* and *Proteus mirabilis* isolates, while inhibitory effect stopped from1:1 dilution against all isolates (table4).

Fig1: *Escerichia coli* **on MacConkey's agar.**

Fig2: *Klebsiella pneumoniae* **on MacConkey's agar.**

Fig3: *P.aeruginosa* **on Nutrient agar .**

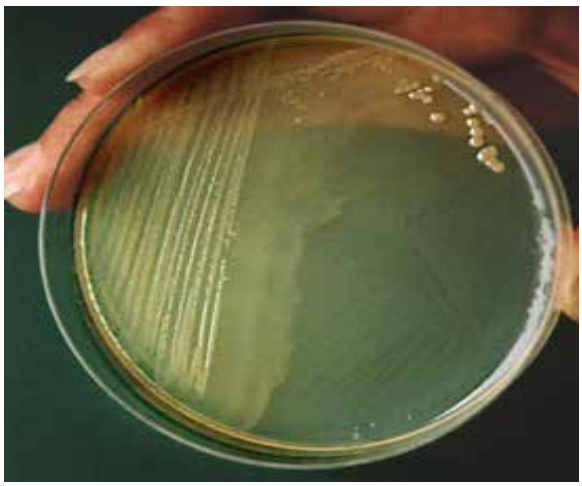

Fig4: *Proteus mirabilis* on Nutrient agar.

Fig5: *S.aureus* on Nutrient agar .

Fig6: Antibiotic susceptibility test.

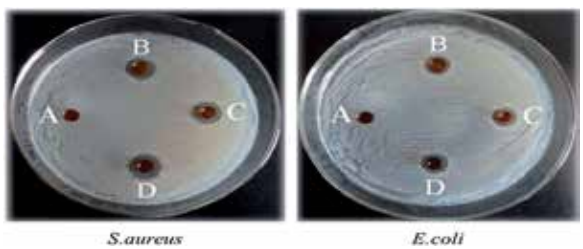

Fig7: Antibacterial activity of cranberry juice. (A=1:1, B=100% cranberry, C= 100% D-mannose, D= 100% cranberry+2% w/v D-mannose).

CONCLUSIONS: These studies indicate a need for continued surveillance of antimicrobial resistance among uropathogens during pregnancy, so as to increase positive outcomes of clinical interventions and prevent preterm birth and low birth weight. With rising concerns of antibiotic resistance among uropathogens, cranberry could serve as an effective alternative in controlling UTIs during pregnancy.

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