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DOES BLACK GRAPE JUICE INHIBIT BACTERIAL ADHERENCE AND BIOFILM PRODUCTION BY UROPATHOGENIC ESCHERICHIA COLI JUST AS CRANBERRY JUICE?

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ABSTRACT

Background and objectives: Cranberry juice is known to prevent urinary tract infections. One of the important mechanisms of pathogenesis by uropathogens is the production of biofilms and bacterial adherence which is usually prevented by cranberry juice. However, Cranberry is mainly found in America and an alternative source needs to be found. This study was therefore undertaken to study the effect of black grape juice on bacterial adherence and biofilm formation and conclude if it could be an alternative to cranberry juice.

Methods: The effect of cranberry juice and black grape juice on bacterial adherence was studied by using the method of Jackson and Fowler. Production of biofilms by the uropathogens was done by the method of O'Toole and Kolter and the effect of cranberry juice and blackgrape juice on biofilm production was studied.

Results: There was a significant reduction in the number of adherent bacteria after exposure to Cranberry juice as well as to black grape juice. Likewise, there was a significant decrease in biofilm production in the presence of cranberry juice and black grape juice.

Interpretation and Conclusion: Cranberry juice and blackgrape juice cause significant decrease in biofilm production and prevent adherence to epithelial cells. Therefore this study suggests that black grape juice may also be used in the prevention of UTI

Key Words: Cranberry juice, Black grape juice, Biofilm, Urinary tract infection, Bacterial adherence

INTRODUCTION

Escherichia coli is the predominant cause of urinary tract infections. The organism produces intracellular bacterial communities or biofilms within the bladder epithelium and this could be an important cause for recurrent urinary tract infections. One of the preventive measures against urinary tract infections especially in the United States has been the consumption of Cranberry (*Vaccinium macrocarpon*) juice. Cranberries are Vaccinium berries which are known to contain proanthocyanidins and anthocyanidins[1,2]. Cranberry juice has been used for generations to prevent recurrent urinary tract infections and has been postulated to inhibit bacterial adherence due to the presence of proanthocyanidin. A number of clinical studies have shown the clinical use of cranberry juice as a preventative measure for UTI caused by *Escherichia coli* [3-5]. Rane *etal* have demonstrated that cranberry derived proanthocyanidins prevent the adherence of *Candida albicans* to both polystyrene and silicone[6]. Likewise, black grape (*Vitis vinifera*) skin contains anthocyanidins while the seeds contain proanthocyanidins and these substances are also thought to possess anti-adhesive properties[7]. Cranberry is a native fruit of North America and is not commonly found in India. Therefore an alternative needs to be identified. Hence if grape juice can act in a manner similar to cranberry, it could be a promising solution for persons who suffer from recurrent urinary tract infection

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in places where cranberry is difficult to be procured. The aims and objectives of this study were to find the effect of cranberry juice and black grape juice on bacterial adherence of *E.coli* to epithelial cells and also on the biofilm production.

MATERIALS AND METHODS

Cranberry juice extract was obtained from USA and the source of black grape juice was commercially available fruit juice of Indian origin. Strains of *E. coli* isolated from cases of urinary tract infection (n=48) were used in the study.

Bacterial adherence to buccal epithelial cells was studied by the method of Jackson and Fowler [8]. Effect of cranberry juice on bacterial adherence was done by performing the adherence assay in the presence of cranberry juice extract while the effect of black grape juice on bacterial adherence was studied by performing the adherence assay in the presence of black grape juice. The number of bacteria adherent per cell was counted.

Estimation of biofilm production was done by the microtitre plate method of O'Toole and Kolter [9]. Overnight broth culture of the organism (200μl) was inoculated into microtitre wells. After incubation at 37°C for 24h the contents of each well was gently aspirated. The wells were washed with 200μl phosphate buffered saline (pH 7.2), fixed with Bouin fixative and stained with crystal violet. The plates were washed, dried and OD_{570} was recorded using an ELISA reader. Effect of cranberry juice and black grape juice on biofilm production was studied by adding 100 μl of cranberry juice and black grape juice respectively to 100μl broth culture in the microtitre plate method. 100μl distilled water was added as control to 100 μl broth culture in the microtitre plate method.

Tests used for Statistical analysis were the Wilcoxon Signed rank sum test and Kruskal-Wallis test

RESULTS

The mean number of bacteria adherent per epithelial cell was 18.04 $\pm$ 8.7. There was a significant reduction in the number of adherent bacteria after exposure to cranberry juice as well as to black grape juice (Table I and II).

Table 1: Effect of cranberry juice extract on bacterial adherence

Bacterial adherence	Before exposure	After exposure
No. of bacteria/cell + SD	18.04 + 8.7	4.83 + 2.4*

*P< 0.01 - significant

Table 2: Effect of black grape juice on bacterial adherence

Bacterial adherence	Before exposure	After exposure
No. of bacteria/cell + SD	18.04 + 8.7	7.34 + 2.8*

*P< 0.01- significant

41 out of the 48 strains of *E.coli* produced significant amount of biofilm. There was a significant decrease in biofilm production in the presence of cranberry juice and black grape juice. ($P < 0.01$)

DISCUSSION

Certain studies have shown that cranberry juice and certain other juices like grape juice have a high molecular weight inhibitor that cause inhibition of haemagglutination by urinary and non urinary isolates of *E.coli* expressing P fimbriae [9]. It has also been suggested that the antiadhesive agents in juice may act in preventing colonization of the host tissue. Previous investigations on the usefulness of cranberry juice in the treatment of urinary tract infections have focused on the potential of cranberry juice to increase the acidity of the urine and to increase urinary excretion of hippuric acid, a strong bacteriostatic agent associated with the ingestion of cranberry juice [10]. A particular study has also suggested the total reducing capacity of ascorbic acid which is present as a component of cranberry which facilitates nonenzymatic generation of nitric oxide [11]. It has also been suggested that proanthocyanidins of cranberry have both non-biospecific activity against adherence, perhaps due to steric hindrance [12], and biospecific activity against adherence, including decreased expression of *fliC* which is the adhesion gene [13]. The potential use of cranberry juice in the treatment of urinary tract infections might be particularly beneficial in the management of patients who suffer from recurrent infections. Our study also suggests that black grape juice can be used as a substitute for cranberry juice. The presence of the proanthocyanidin and anthocyanidins in these juices with anti adherence effects also suggests their beneficial nature if used in other infections involving bacterial adherence [14]. Hence the present study suggests the use of blackgrape juice as an alternative to cranberry juice.

CONCLUSION

The results of our study suggest that since both Cranberry as well as blackgrape juice cause reduction in biofilm production and prevent bacterial adherence, black grape juice can be used as an alternative to cranberry juice especially in places where cranberries cannot be grown.

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