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Bacterial Anti-adhesion Activity of Human Urine: CystiCran (360 mg) vs. 27% Cranberry Juice Cocktail Consumption

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Objective:

1) Determine the *ex vivo* uropathogenic bacterial (P-type *E. coli*) anti-adhesion activity in human urine following consumption of two treatments in succession with a wash-out period between each treatment: 1) 360 mg CystiCran capsule, 2) 300 mL of 27% Cranberry Juice Cocktail (Ocean Spray), measured over a 36-hr time frame with product consumed for two days at the beginning of the test period only.

Ex vivo Urine Study Method:

Pre-Visit Subject Preparation:

Participant inclusion and exclusion criteria: 5 women and 5 men, healthy, between the ages of 25 and 60, no current urinary infections, no diabetes, or antibiotic use for 6 months.

Dietary restrictions: participants refrained from consuming all cranberry, blueberry, pomegranate, grape, chocolate and other high-flavonoid products for a 3-day wash out period prior to consuming test products and throughout testing period.

Study Design

- 3-day wash out period prior to consuming test products and throughout test period
- On urine collection days, additional fluid consumption standardized participants to 240 mL every 3 hours to avoid dilution of urine samples and allow for detection of anti-adhesion activity, if present
- On test days, products were administered in the morning
- Urine (approximately 25 ml) was collected (clean-catch) by each participant prior to product consumption (time 0) and at 3, 6, 9, 24 and 36 hrs following product consumption
- Urine was centrifuged, filtered (.45 micron filter) and immediately frozen at -20C

Urine Protocol Specifics

Background urine samples were taken from all 10 participants prior to consumption of treatment products. Treatment 1 (one 360 mg CystiCran capsule) was administered in the morning over a 2-day period. On the morning of day 2, following CystiCran ingestion, urines were collected after product consumption and immediately frozen at -20C. After a 3-day wash-out period, a background urine was collected and treatment 2 (300 mL of 27% Cranberry Juice Cocktail (CJC)) was administered, as stated above. Urines were collected at hour 3, 6, 9, 24 and 36 and frozen at -20C.

Thawed urines were tested full strength for bacterial anti-adhesion activity utilizing an HRBC hemagglutination assay specific for uropathogenic P-fimbriated *E. coli* according to Foo et al. (*Phytochemistry*, 2000). A 30- μ L drop of each urine was incubated with 10 μ L of bacterial suspension on a 24-well polystyrene plate for 10 min at room temperature on a rotary shaker. Freshly drawn HRBCs (A1, Rh+) were suspended (3%) in PBS and added separately (10- μ L drops) to test suspensions, which were then incubated for 20 min on a rotary shaker at room temperature and evaluated microscopically for the ability to prevent agglutination.

Anti-adhesion activity of each urine sample was scored visually based on a quantitative estimation of percent agglutination of each sample using the following scale: 0 = no anti-adhesion activity, 1 = 50% anti-adhesion activity, 2 = 100% anti-adhesion activity. A score of 2 indicates significant anti-adhesion activity in the urine, whereas a score of 1 indicates moderate activity. The detection limits of the anti-adhesion assay are not high enough to allow quantification of the activity in each urine sample via a dilution series; therefore the result is presented as either a positive or a negative for the activity of each sample. Anti-adhesion assays were repeated four times per sample and the results averaged. Controls included wells containing bacteria + PBS, HRBC + PBS, bacteria + test material, HRBC + test material, and bacteria + HRBC.

Data were analyzed statistically using ANOVA.

Results and Discussion:

Ex vivo Urine Study

No anti-adhesion activity was detected in urines prior to product consumption. Urinary pH averaged 6.5, eliminating a bacteriostatic effect.

360 mg CystiCran vs. 300 mL CJC: Summing all observed anti-adhesion activity recorded for all participants over every time period yielded 48 out of a possible 120 for CystiCran, and 41/120 for CJC. The differences between the products were not statistically significant. By time period, the post-CJC urinary activity was significantly greater (P Value 0.0015) at the 3-hr time period than the activity for CystiCran, whereas at 24 and 36 hours CystiCran was significantly greater than CJC (P Values 0.002 and 0.025, respectively) (Fig. 1). This suggests that CJC has a more rapid and substantial effect in the first 3-6 hours, which it maintains at 9 hours, but diminishes thereafter. Peak activity for CJC is at 6 hours. The CystiCran activity is substantial from 6-24 hours and reaches peak activity at 9 hours. CystiCran had some residual activity at 36 hours. CystiCran is a powdered product and may take longer to metabolize than the juice, which could explain the shift in the pharmacokinetic patterns. Further research is needed to determine what activity levels at each time period correspond to a biologically relevant decrease in urinary tract infections. The overall data for all participants at each time period is presented in Fig. 2. Women responded similarly to each product, as did men (Fig. 3).

Overall summary: There was no significant difference in overall *ex vivo* urinary bioactivity between CJC and CystiCran. CJC had a significantly higher spike of activity in the first 6 hours following ingestion, whereas CystiCran spiked at 9 hours and then began to drop off, however some residual activity was evident at 36 hours post-ingestion. The activity at 36 hours was minimal and may not be sufficient to prevent bacterial adhesion. Additional work needs to be done to determine the level of activity needed at each time period to prevent UTIs.

Figure 1 – Comparison of all observed urinary anti-adhesion activity recorded per time period by all 10 participants.

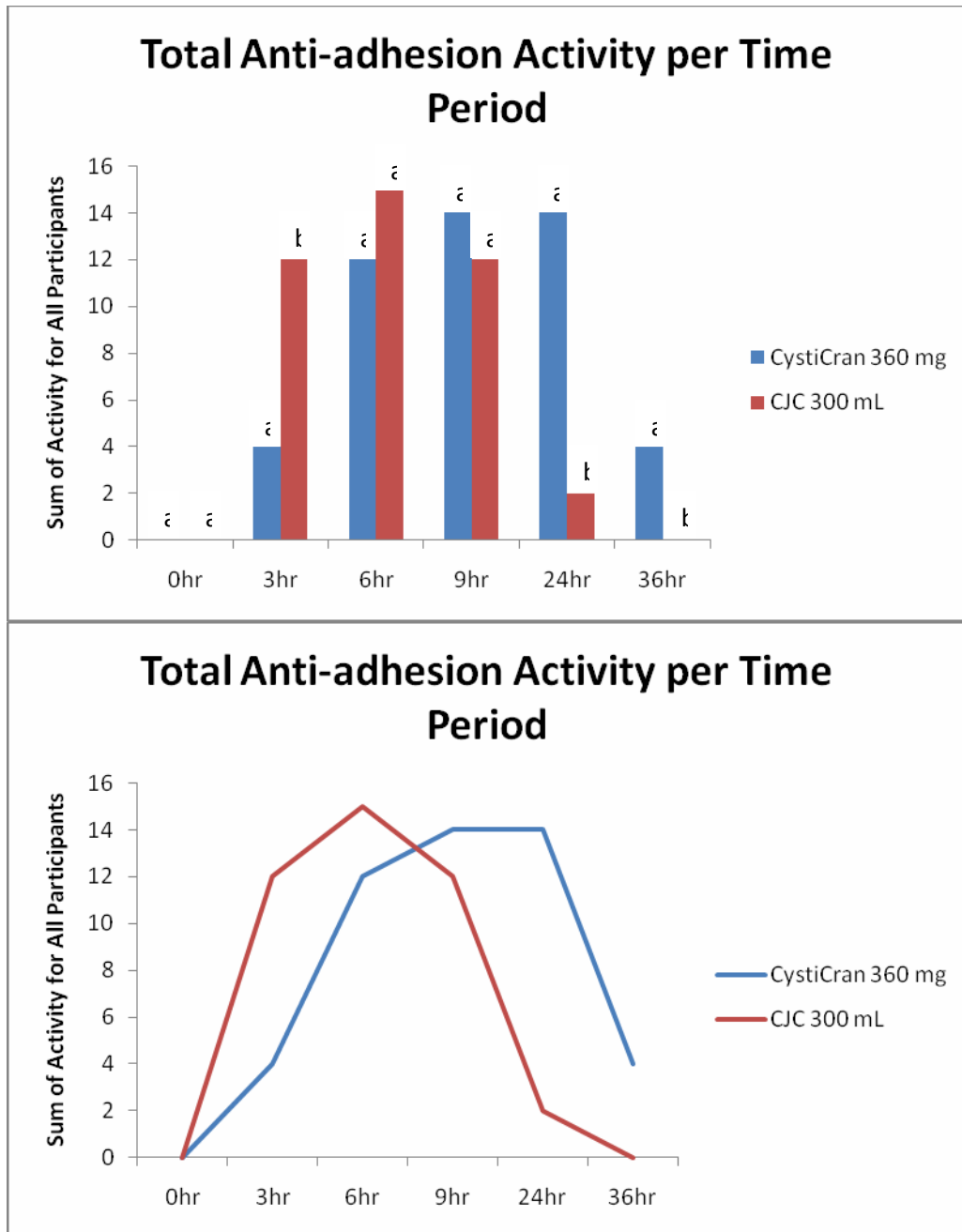


Figure 2 – Comparison of observed urinary anti-adhesion activity for each participant over each time period.

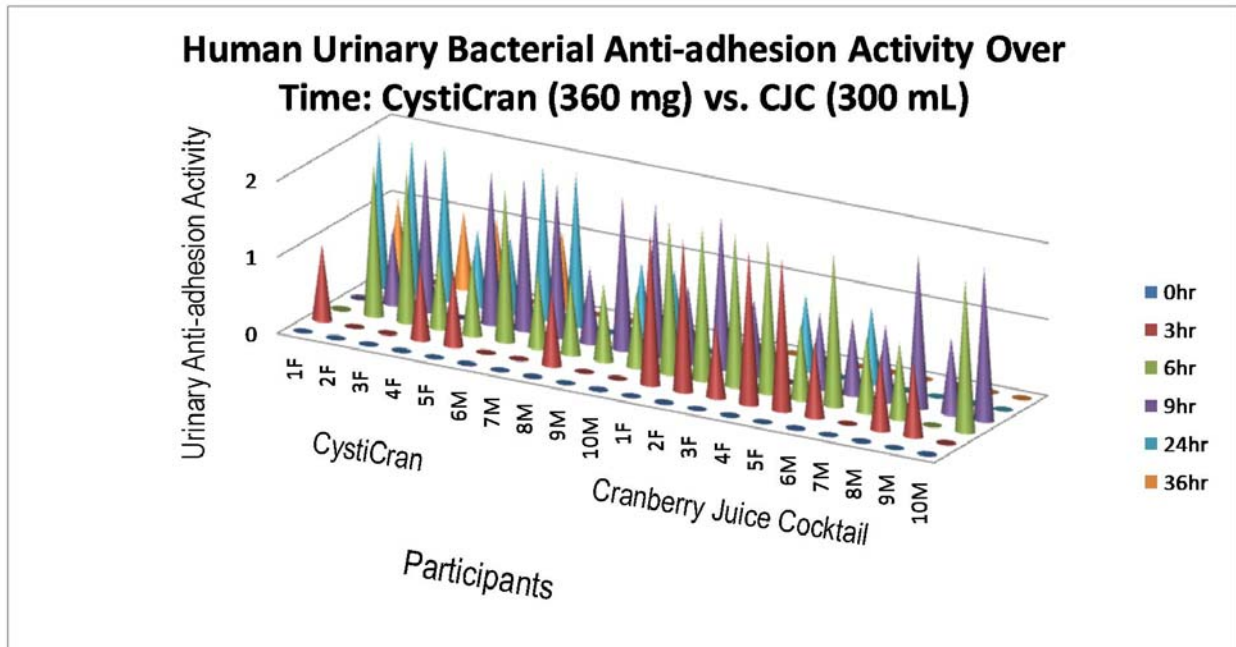
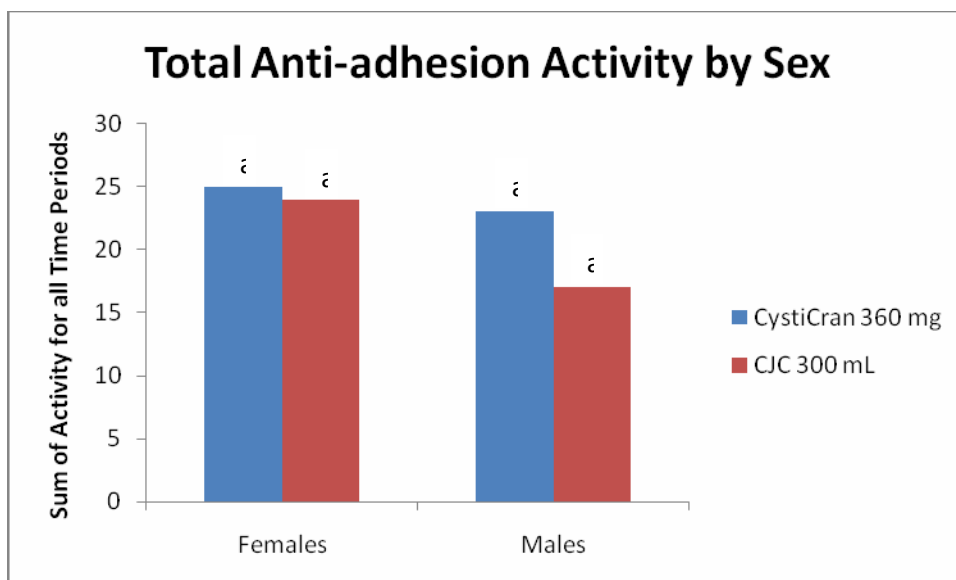


Figure 3 – Total observed urinary anti-adhesion activity recorded for women and men for each product.



Raw Data Set:

360 mg		0hr	3hr	6hr	9hr	24hr	36hr
treat	rep						
CystiCran	1F	0	1	0	0	2	1
CystiCran	2F	0	0	2	1	2	0
CystiCran	3F	0	0	2	2	2	1
CystiCran	4F	0	1	1	0	1	1
CystiCran	5F	0	1	1	2	1	0
CystiCran	6M	0	0	2	2	2	1
CystiCran	7M	0	0	1	2	2	0
CystiCran	8M	0	1	1	1	0	0
CystiCran	9M	0	0	1	2	1	0
CystiCran	10M	0	0	1	2	1	0
CJC	1F	0	2	2	1	0	0
CJC	2F	0	2	2	2	0	0
CJC	3F	0	1	2	1	0	0
CJC	4F	0	2	2	0	1	0
CJC	5F	0	2	1	1	0	0
CJC	6M	0	1	2	1	1	0
CJC	7M	0	0	1	1	0	0
CJC	8M	0	1	1	2	0	0
CJC	9M	0	1	0	1	0	0
CJC	10M	0	0	2	2	0	0

Participants 1-5 are women

Participants 6-10 are men