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February 24, 2009

Bacterial Anti-adhesion Activity of Human Urine Following 27% Cranberry Juice Cocktail vs. PACran Capsule Consumption

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Purpose:

To determine the 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) 10 oz 27% Cranberry Juice Cocktail (CJC), 2) 500 mg PACran capsule, measured over a 24-hr time frame with product consumed at the beginning of the test period only.

Methods:

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 and 24 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 (300 mL 27% CJC) was administered in the morning over a 2-day period. On the morning of day 2, following juice ingestion, urines were collected prior to product consumption, at hour 3, 6, 9, and 24 and immediately frozen at -20C. After a 3-day

wash-out period, treatment 2 (one 500-mg PACran capsule) was administered, as stated above, and urines were collected 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-uL drop of each urine was incubated with 10 uL 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-uL 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 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.

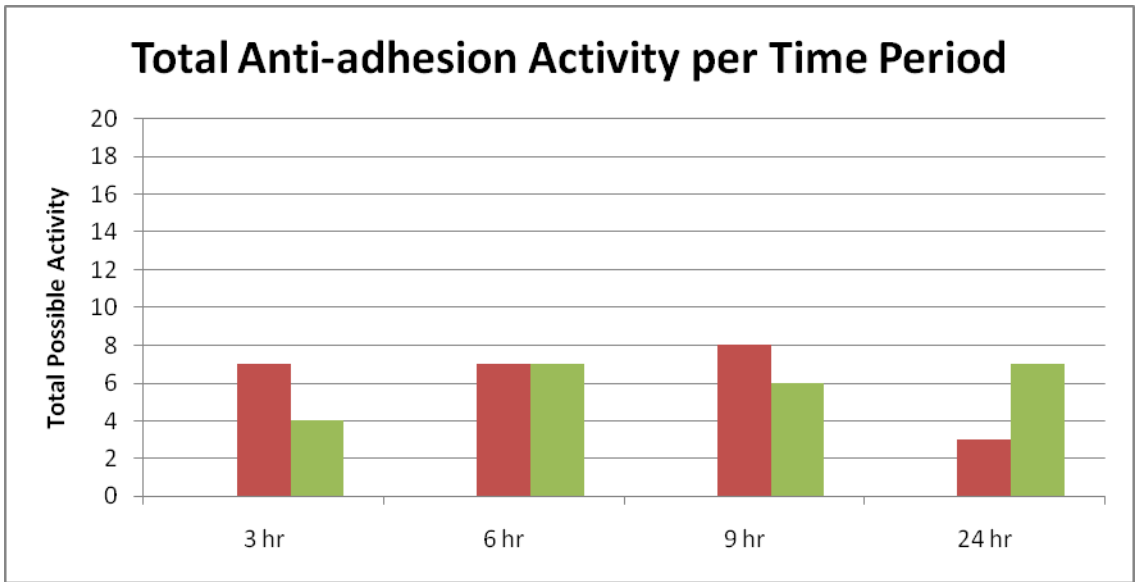
Results and Discussion:

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

27% Cranberry Juice Cocktail vs. PACran: Summing all observed anti-adhesion activity recorded for all participants over every time period yielded nearly identical results for each product (25 out of a possible 80 for CJC, and 24/80 for PACran). This means that the potential efficacy for both products is very similar. By time period, the post-CJC urinary activity increased steadily from 3 to 9 hrs and dropped off significantly at 24 hrs (Fig. 1). The post-PACran urinary activity increased to a high at 6 hrs, dropped off at 9 hrs and then increased again at 24 hrs. This increase at 24 hrs may be the result of PACran processing, as we have found that powders usually take a little longer to transit through secondary metabolism. The PACs in PACran may be higher molecular weight than the PACs in CJC, which could explain the differences in the pharmacokinetic patterns. The overall data for all participants at each time period is presented in Fig. 2. Women responded similarly to each product, as did men. However, overall, there was slightly more anti-adhesion activity recorded for women than men. This was more evident for PACran (Fig. 3).

Overall, the data suggest that CJC and PACran provide similar *ex vivo* urinary anti-adhesion activity over a 24-hr period. Clinical data are necessary to assess whether PACran can prevent urinary tract infections.

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



Red Bars = CJC
Green Bars = PACran

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

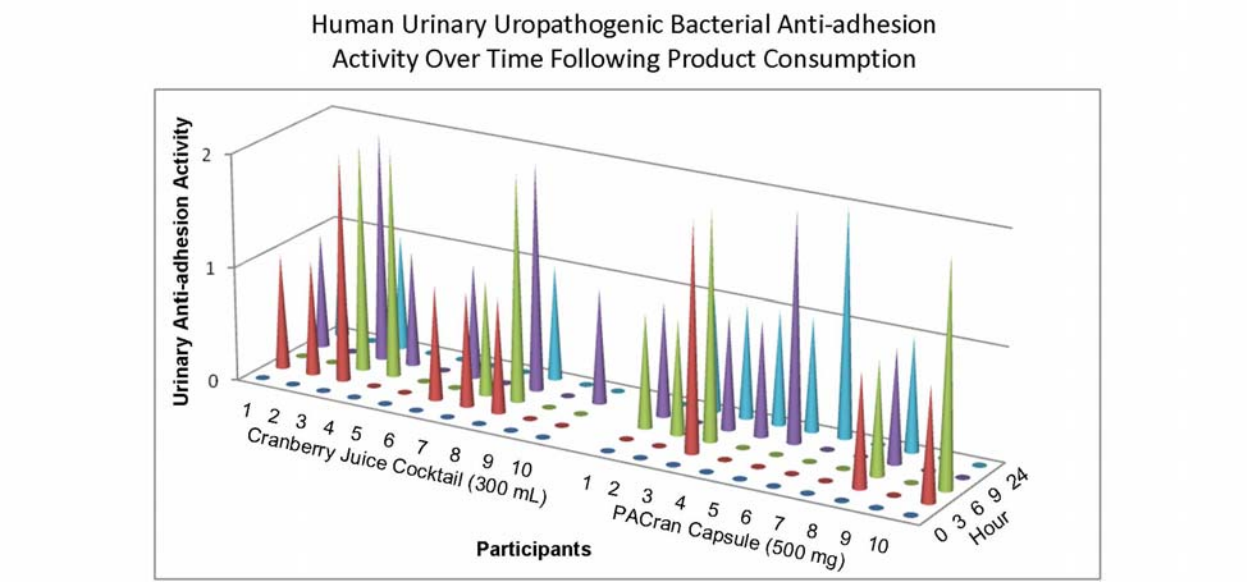
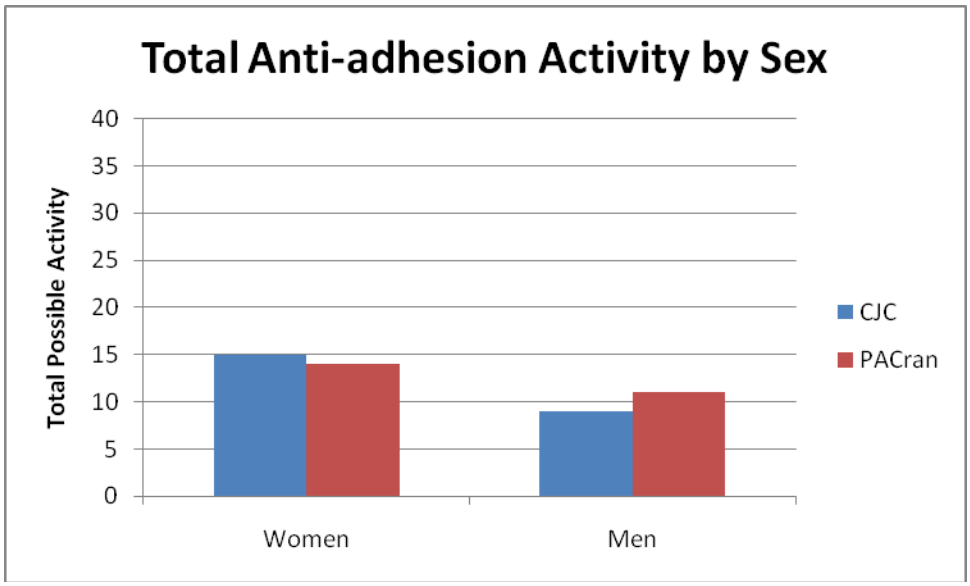


Figure 3 – Total observed urinary anti-adhesion activity recorded for women and men for each product.



Raw Data Set:

	Participant	0 (Background)	3 hr	6 hr	9 hr	24 hr
CJC						
	1	0	1	0	1	1
	2	0	1	0	0	0
	3	0	2	2	2	1
	4	0	0	2	1	0
	5	0	0	0	0	0
	6	0	1	0	1	0
	7	0	1	1	0	0
	8	0	1	2	2	1
	9	0	0	0	0	0
	10	0	0	0	1	0
PACran						
	1	0	0	1	1	0
	2	0	0	1	0	1
	3	0	2	2	1	1
	4	0	0	0	1	1
	5	0	0	0	2	1
	6	0	0	0	0	2
	7	0	0	0	0	0
	8	0	1	1	1	1
	9	0	0	0	0	0
	10	0	1	2	0	0

Participants 1-5 are women
Participants 6-10 are men