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Bacterial Anti-adhesion Activity of Human Urine: Cranberex (Ethical Naturals)

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Objectives:

Determine the *ex vivo* uropathogenic bacterial (P-type *E. coli*) anti-adhesion activity (AAA) in human urine measured over a 24-hour time frame following consumption of a single course of two 240-mg Cranberex capsules (36-mg PAC each) administered BID at the beginning of the test period only. Measure urinary pH to determine if a bacteriostatic effect on bacterial growth is elicited by the treatment.

Product Specifications

Cranberex (Ethical Naturals) Capsules (each capsule contains 240 mg Oregon cranberry powder with 15% PAC (36 mg). Lot# R1021. Expires Nov. 2018

Ex vivo Urine Study 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 product
- On urine collection day, 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

- Background urine samples taken in the evening from all 10 participants prior to consumption of treatment product (time 0)
- Capsule #1 administered following the background urine collection
- The following morning, capsule #2 administered in the morning
- Urine (approximately 25 ml) was collected (clean-catch) by each participant at times 0 and then in the morning at 3, 6, 9, 12, 24 hours following administration of capsule #2
- Urine was centrifuged, filtered (0.45 micron filter) and immediately frozen at -20C

Urine Protocol Specifics:

Thawed urines were tested full strength for bacterial anti-adhesion activity (AAA) utilizing an HRBC hemagglutination assay specific for uropathogenic P-fimbriated *E. coli* according to Howell et al. (*Phytochemistry*, 2005). 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.

AAA 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.

Results and Discussion:

Changes in Urine pH:

Urinary pH averaged 6.1, eliminating a bacteriostatic effect. Cranberry consumption has historically not resulted in decreases in bacterial growth, as the urinary pH must be reduced to 5.5 or lower.

Overall AAA Response of the Treatment:

Over all time periods from 3 hours to 24 hours, the overall percent urinary AAA response by all participants to two 240-mg doses of Cranberex (containing 36 mg PAC each) given BID in one administration cycle was 43% (Fig. 1). The male participants exhibited an overall response of 46% compared to a 40% response by the women. All participants exhibited an anti-adhesion response to Cranberex in at least two time periods.

Urinary AAA by Time-period:

Urinary AAA response increased rapidly and reached a peak of 75% at the 3-6 hour collection time (Fig. 2). AAA response decreased to 25% at the 9-12 hour collection period and decreased further to 15% after 24 hours. There was a similar urinary AAA response for men and women at each time period (Fig. 3).

Overall Summary:

The *ex vivo* urinary bacterial AAA after consuming a single course of two 240-mg capsules of Cranberex BID followed a bell-shaped curve, peaking 3-6 hours after the second capsule was consumed. Activity was exhibited at 12 and 24 hours. All participants responded with AAA in at least two urine collection periods and the overall response at all time periods was high at 43%. Men and women responded similarly to the Cranberex. Further research is needed to determine what activity levels at each time period correspond to a biologically relevant decrease in urinary tract infections. In addition, this treatment regime did not reduce the pH of urine enough to produce a bacteriostatic effect on bacterial growth.

Figure 1

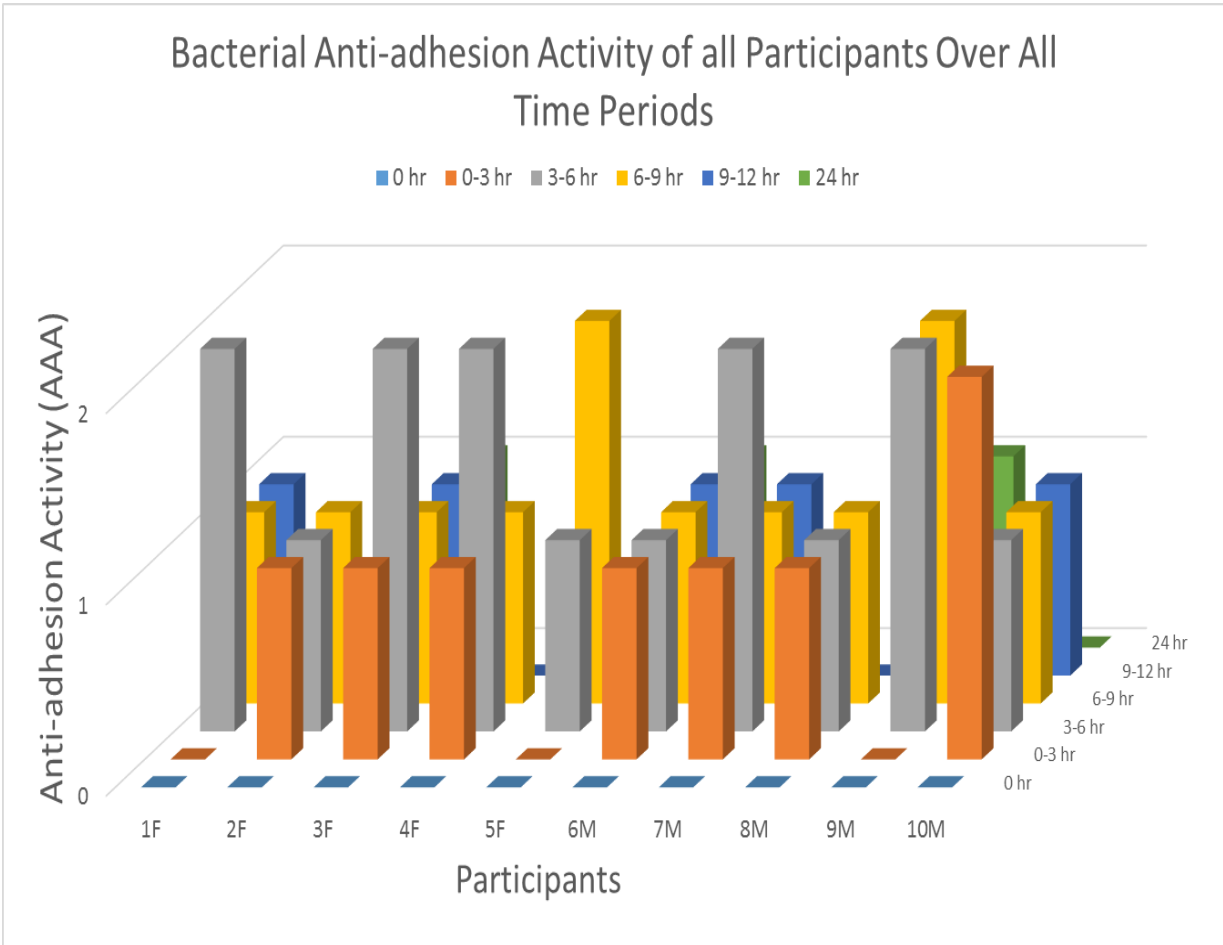


Figure 2

Percent Total Possible Bacterial Anti-adhesion Activity at Each Time Period

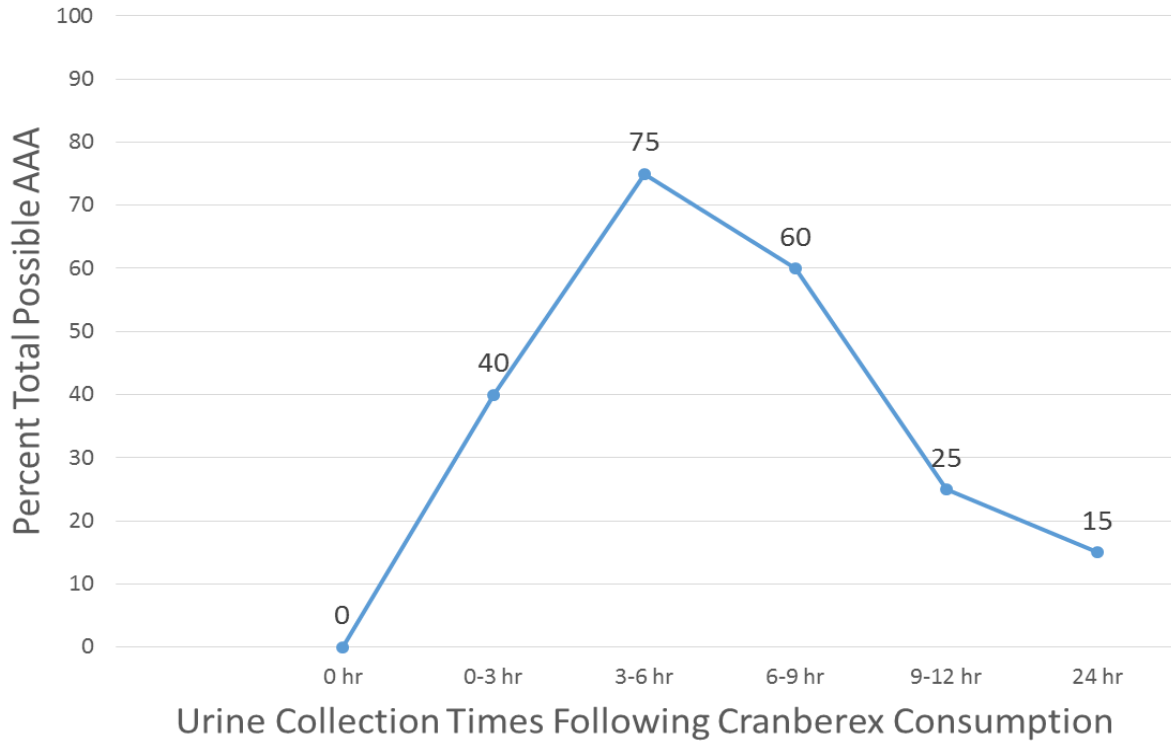
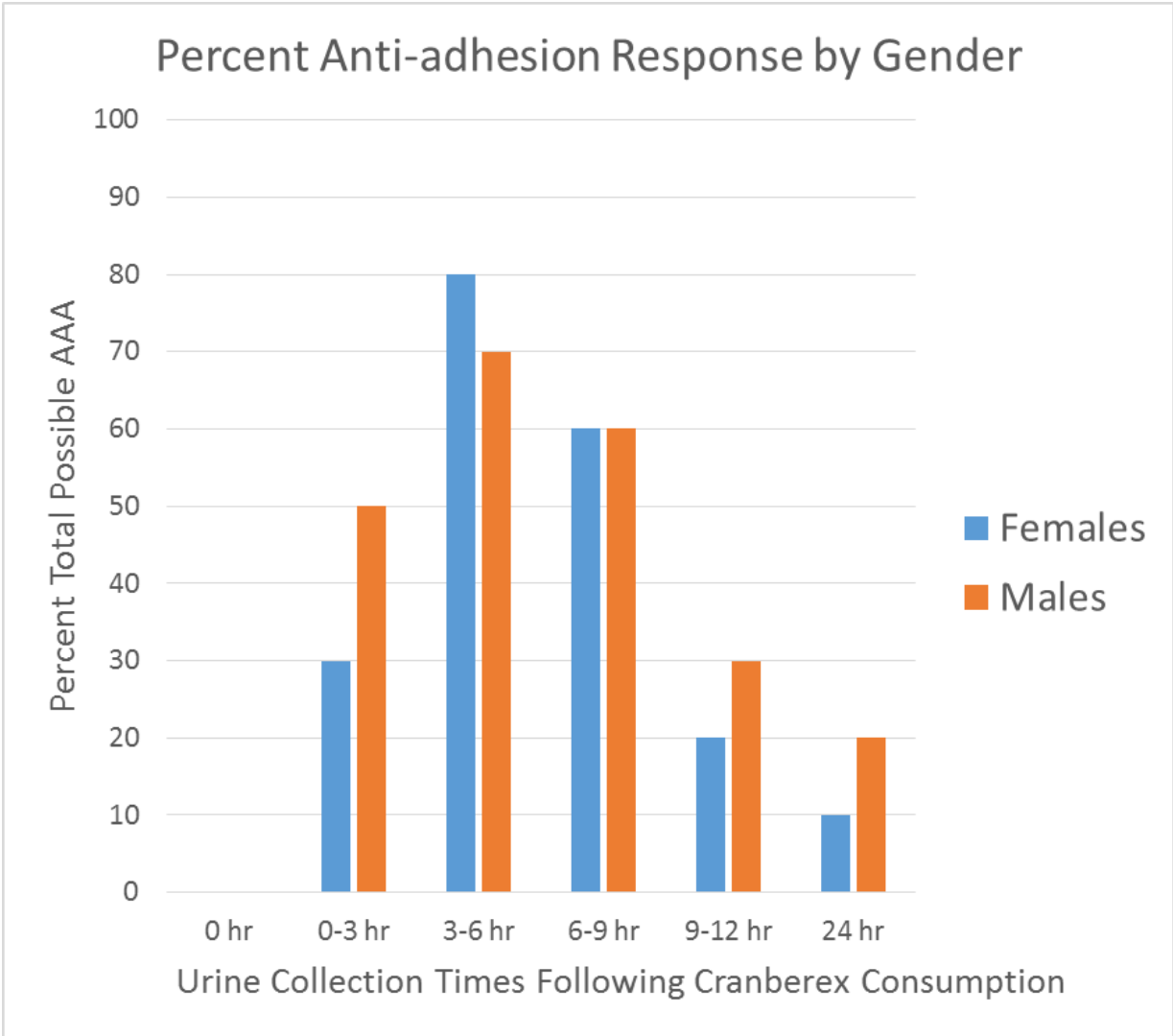


Figure 3



Raw Data Set

Cranberex

	<u>0 hr</u>	<u>0-3 hr</u>	<u>3-6 hr</u>	<u>6-9 hr</u>	<u>9-12 hr</u>	<u>24 hr</u>
1F	0	0	2	1	1	0
2F	0	1	1	1	0	0
3F	0	1	2	1	1	1
4F	0	1	2	1	0	0
5F	0	0	1	2	0	0
6M	0	1	1	1	1	1
7M	0	1	2	1	1	0
8M	0	1	1	1	0	0
9M	0	0	2	2	0	1
10M	0	2	1	1	1	0

F = Female

M = Male