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**"Study on the influence of using a toothpaste with cranberry extract on dental plaque and on the count of Mutans Streptococci"**

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**Protocol**

Study to test product vs standard on the same subjects

Tested product: Tablet toothpaste with Exocyan® Cran cranberry extract

Standard: commercially available Crest® toothpaste in tube

20 healthy volunteers

3 brushings per day

6 weeks with Toothpaste Tablets/ 15 days period of "wash out" / 6 weeks with the standard

The volunteers were selected among dental students. Criteria for inclusion were: no caries, no orthodontic treatment, no antibiotic within the last month, no general pathology. No specific request on diet change or control was done, the volunteers keeping their usual diet.

This study was conducted between 2010 and 2011, starting on November 16<sup>th</sup> 2010 (first inclusion) and ending on April 4<sup>th</sup> 2011.

**At day 0 and day + 6 weeks:**

- Determination of plaque index

The index used is Turesky's, after coloration on all teeth.

0: no plaque on the tooth surface

1: isolated spots on top of the tooth

2: thin continuous strip of plaque, less than 1 mm on the top of the tooth

3: plaque strip of more than 1 mm thick and covering less than one third of the tooth.

4: plaque covering at least one third of the tooth, but less than two thirds of the crown of the tooth.

5: plaque covering two-thirds or more of the crown of the tooth.

- Plaque removal

Using a sterile Pasteur pipette

Dilute in 2 ml of sterile distilled water: measure absorbance at 650nm

50 µl cultured for research and count of Mutans Streptococci on *Mitis salivarius* agar and blood agar

## **Results**

Recruitment of volunteers:

Among the first 20 volunteers recruited, one was quickly ruled out due to taking antibiotics that may disturb the oral flora; He was replaced very quickly.

Later, two other volunteers were excluded: a dental cavity was diagnosed in one and the other used a toothpaste other than that tested during a weekend.

In total, we have 18 volunteers who followed the protocol perfectly.

Plaque scores (Table 1):

The plaque scores were collected at the same hours for each volunteer, wherever possible. A 4 hours wait after brushing was observed in each case.

The mean plaque scores at Day 0 are very close, indicating that the washout period was sufficient.

The results show a difference between the two toothpastes tested. 66.6% of volunteers showed a decrease of the plaque index after the use of Tablet toothpaste, whereas only 38.8% of volunteers showed a decrease of the plaque index after the use of Crest Fluoride Toothpaste.

After 6 weeks of use of tablets toothpaste, the average plaque index is lower than that of Day 0, the mean percentage of plaque index being decreased by 17% (mean plaque score from 1.72 to 1.47). Differentials obtained are higher in volunteers with higher plaque scores on Day 0 (> 2): mean percentage of plaque index being decreased by 34.7% (mean plaque score from 2.41 to 1.79) for those volunteers.

Comparatively, the average plaque index increased by 3.4% when using the standard for 6 weeks (mean plaque score from 1.76 to 1.82).

Mutans Streptococci count in plaque (Table 2):

Given the small amount of plaque retrieved, it was not possible to measure the OD as planned in the protocol.

The amounts of plaque collected could not thus be perfectly calibrated and counting can only be analyzed in terms of presence (or absence) of Mutans Streptococci.

We have not evidenced disappearance of Mutans Streptococci in dental plaque, whatever the toothpaste tested. 50% of volunteers using the Tablet toothpaste experienced a decrease of total Mutans Streptococci count while the other 50% saw an increase of the same count.

Plaque index	Tablet toothpaste		delta D-42 – D-0	Toothpaste tube		delta D-42 – D-0
	Day 0	D +42		Day 0	D +42	
ANN POG	1,21	1.51	+0.30	1.32	1.50	+0.18
AST RIC	2.05	1.41	0.64	1.87	1.87	0
AUR SEL	0,78	0.69	0.09	1.32	1.62	+0.30
BEN FAB	2.12	2.26	+0.14	2.07	1.78	0.29
BRI PAI	1.52	1.06	0.46	1.45	1.82	+0.37
CAM LAU	1.72	1.64	0.08	2.23	2.23	0
CLA RAN	1,64	1.48	0.16	1.34	2.05	+0.71
DAR NGU	2.85	1.89	0.96	2.00	1.76	0.24
EST CRE	1.51	1.53	+0.02	1.52	1.55	+0.03
EDO CHO	1,39	1.56	+0.17	1.98	1.95	0.03
LAE SEL	1.05	0.98	0.07	1.50	1.71	+0.27
LOU CHA	1.07	1.62	+0.55	1.75	1.89	+0.14
JUL COT	1.7	1.2	0.50	1.80	1.53	0.27
LEI RIN	2.14	1.25	0.89	1.43	1.35	0.08
MAR DUC	2.53	1.75	0.96	1.82	2.21	+0.39
MAR LAT	1.06	1.06	0	1.56	1.29	0.27
MAR LEG	1.8	1.42	0.38	1.85	1.89	+0.04
THI DAN	2.8	2.2	0.60	2.8	2.78	0.02
Average plaque	<b>1.72</b>	<b>1.47</b>		<b>1.76</b>	<b>1.82</b>	
Std deviation	<b>0.58</b>	<b>0.39</b>		<b>0.37</b>	<b>0.34</b>	

Table 1: Plaque indexes at Day 0 and after 42 days for each toothpaste

Delta = Plaque index at 42 days minus plaque index at Day 0

Count of <i>Mutans streptococci</i> Log CFU/ml	Tablet toothpaste		Toothpaste tube	
	Day 0	D + 42 days	Day 0	D + 42 days
ANN POG	3.49	5.32	5.54	6.23
AST RIC	4.95	5.42	5.0	5.30
AUR SEL	4.50	5.35	4.45	3.90
BEN FAB	5.92	4.89	4.75	4.0
BRI PAI	4.73	4.89	3.96	5.42
CAM LAU	5.58	5.01	4.15	5.10
CLA RAN	2.51	1.48	2.00	5.12
DAR NGU	4.66	4.90	5.28	4.00
EST CRE	No count possible (too many colonies)	5.15	5.27	4.00
EDO CHO	4.52	5.15	5.16	5.74
LAE SEL	5.1	3.85	5.18	No culture
LOU CHA	5.63	5.50	4.26	5.03
JUL COT	5.11	5.09	5.47	4.75
LEI RIN	5.33	4.97	4.75	4.34
MAR DUC	5.20	5.40	4.38	5.56
MAR LAT	4.7	5.28	4.8	5.68
MAR LEG	5.03	3.30	3.30	5.61
THI DAN	No count possible (too many colonies)	6.3	5.20	4.83

Table 2: *Mutans Streptococci* count

Results are expressed in Log<sub>10</sub> of CFU number (Colony Forming Unit, that correspond to viable bacteria)/ml of plaque suspension