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Original Article

Effect of carbonated drinks on wound healing of oral epithelium

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ABSTRACT

Background: Carbonated drinks are the second most consumed non-alcoholic beverages in the world after tea. The effects of these drinks on hard tissues and vital organs of the body have been proved beyond doubt. This study, however, explains the effect of these drinks on wound healing of oral epithelium.

Methods: Thirty-six male Wistar rats were considered for the study. A circular wound of 3.0 mm was created on the buccal mucosa of all animals and they were divided into two groups. Animals in group 1 were fed with chow pellet and water, while those in group 2 were fed with a commercially available carbonated drink instead of water. Six animals from each group were euthanized at 0, 7, and 21 days. Wound site was histologically assessed for differences in thickness and characteristics of the regenerating epithelium between two groups.

Results: There was a marked difference in the healing pattern between the two groups. Animals in group 1 showed a normal healing pattern at the end of day 21. In the group 2, the regenerated epithelium showed hyperplasia and hyperkeratosis along with acanthosis at the end of the experiment with a subsequent delayed inflammatory reaction at day 21.

Conclusion: Consumption of carbonated drinks can disrupt oral wound healing. The contents in carbonated drinks have a proinflammatory action on the soft tissue. Results suggest that epithelial changes seen in experimental group 2 could be a result of constant irritation by the acidic and fizzy nature of carbonated drinks.

1. Introduction

The oral mucosa is lined by non-keratinized stratified squamous epithelium supported by a loose connective tissue,[1] a barrier against exogenous substances and pathogens.[2] Soft drinks are the second most consumed beverages in the world. Worldwide average consumption of carbonated drinks increased from 9.5 gallons per person per year in 1997 to 11.4 gallons per person per year in 2010.[3] The oral cavity is quite often subjected to minor and major wounds on a daily basis. During healing of such wounds, the type of diet plays an imperative role.

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Table 1 – Detail of animal groups.

Groups	Experimental day	Number of animals	Remarks
Experimental group 1	Day 0	6	Food with water
	Day 7	6	
	Day 21	6	
Experimental group 2	Day 0	6	Food with carbonated drink
	Day 7	6	
	Day 21	6	

Previous studies proved the effect of carbonated drinks on kidney,[4] Liver,[5] teeth,[6] *etc.*, but not enough literature is available on oral mucosa. The present study emphasizes on the histological effects of carbonated drinks on wound healing of buccal epithelium of albino rats.

2. Materials and methods

An experimental animal study was conducted at the Post Graduate Medical Institute, Lahore to observe histological changes in soft tissue on post-wound days 7 and 21. A randomly selected carbonated drink was used for this experiment. The study protocol was approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore and Ethical Committee of PGMI, Lahore. Thirty-six healthy male adult albino Wistar rats, weighing between 180 and 250 g, were procured from NIH, Islamabad. Animals were tagged and were housed in cages with wire bar lids to hold the water bottle and feed, to prevent contamination with urine and feces. Bedding was placed directly into the cage to allow the absorption of urine. They were kept in a well-ventilated room at ambient temperature of 28.0 ± 2.0 °C and humidity (60 ± 10%) under 12-h light/dark cycles and well provided with food and water *ad libitum*. All animals were consistently checked for signs of pre- and post-procedure infections.

All animals used in this study were handled as per the international, natural, and institutional guidelines for the care and use of laboratory animals in biomedical research as promulgated by the National Research Council.[7] The rats were divided into two equal groups by using a random number generator (Table 1). Animals in groups 1 and 2, after induction of soft tissue wound, were given *ad libitum* access to water and carbonated drink, respectively.

On day 0, a uniform piece of tissue was removed from the left buccal mucosa of rats using a disposable punch biopsy tool of 3.0 mm circumference[8] after anesthetization with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) by an intraperitoneal injection.[9] The cut was made deep to the level of the dermis. The wound was left open for healing and the animal was returned to its cage to recover from anesthesia. None of the animals showed signs of post-procedural infection. On day 0, 7, and 21, six animals ($n = 6$) from each group were placed in a carbon dioxide plus chloroform chamber and euthanized under deep anesthesia.[7] The whole left cheek was dissected out and washed with saline for further treatment. The tissue was processed and Hematoxylin and Eosin stain was used for routine histological study of the buccal epithelium. Data were analyzed by using two-paired student's *t*-test for quantitative differences between experimental group 1 and experimental group 2 at the 5% level of significance. A *p*-value of equal to or less than 0.05 was considered statistically significant.

3. Results

In both groups, the normal (baseline) epithelium at day 0 was stratified squamous keratinized, with four basic layers. It consisted of a darkly stained layer of tall columnar cells known as stratum basale (basal layer), over which there were 7–8 strata of polyhedral cells forming the stratum spinosum (prickle layer). On top of these were 3–5 layers of flat cells forming stratum granulosum (granular layer). These layers were covered by a fine layer of anucleated corneocytes forming the stratum corneum (cornified layer). Rete ridges extending down toward the connective tissue had a usual conical shape. The epithelial thickness was measured to be 295 ± 5.00 μm. The lamina propria displayed fine network of collagen bundles with fibroblasts and blood vessels (Fig. 1).

On post-wound day 7, there was a prominent increase in the thickness of epithelium in both groups. In group 1, the stratified squamous epithelium of mean thickness of 153.33 ± 4.08 μm (Table 2) was present. The epithelium consisted of a single layer of large columnar cells constituting stratum basale; 4–5 layers of polyhedral cells forming stratum spinosum and 2–3 layers of flat cells with keratohyalin granules forming stratum granulosum were present on top (Fig. 2).

Table 2 – Comparison of thickness of epithelium (μm) between groups 1 and 2 on Day 7 and Day 21.

Experimental day	Experimental group 1 (in μm) (mean ± S.D.)	Experimental group 2 (in μm) (mean ± S.D.)	Number of animals in each group (N)	*p* value
Day 0	295 ± 5.00	295 ± 5.00	6	>0.00
Day 7	153.33 ± 4.08	56.67 ± 4.07	6	<0.001
Day 21	285.0 ± 4.47	401.67 ± 7.53	6	<0.001