

## HISTOLOGICAL EFFECT OF SODIUM FLUORIDE AND STANNOUS FLUORIDE GELS ON BUCCAL MUCOSA OF ALBINO RAT

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### ABSTRACT

**Background:** Different concentrations of fluoride preparations are being used enormously in dental clinics to prevent dental caries and treat tooth sensitivity.

**Objective:** The objective of this study was to compare the histological effects of sodium fluoride gel and stannous fluoride gel on buccal mucosa of albino rats.

**Methods:** This experimental study comprised of 30 Albino rats of average weight 200-250gms which were randomly divided into 3 groups. Out of total 30 rats Control group A was assigned 10 albino rats and rest of the 20 rats were included in experimental groups B and C containing 10 rats each. Animals of group B were topically treated with 0.2% sodium fluoride gel at a dose of 0.5mg/Kg as per body weight on buccal mucosa. The rest of the 10 animals of group C were given 0.4% stannous fluoride gel topically at a dose of 1mg/kg as per bodyweight. Both fluoride gels were applied once daily for 4 minutes with the help of cotton pellet on buccal mucosa for 14 days and were sacrificed on last day under deep anesthesia. Buccal mucosa of each group was taken out for biopsy. The specimens were treated with haematoxylin and eosin stain.

**Results:** There is a statistically significant difference in mean epithelial thickness (p-value <0.001) and number of apoptotic cells in buccal mucosa between control and experimental groups (p-value <0.001). Comparison between experimental groups has shown a significant difference in number of apoptotic cells (p-value 0.000).

**Conclusion:** Histological analysis showed that sodium fluoride gel has significant apoptotic effect on buccal mucosa as compared to stannous fluoride gel.

**Keywords:** Fluoride, Dental caries, sodium fluoride, stannous fluoride, buccal mucosa

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### INTRODUCTION

Dental caries is an infectious disease caused primarily by the complex interaction of cariogenic oral flora (biofilm) with fermentable dietary carbohydrates on the tooth surface over time<sup>1</sup>. A wide range of fluoride supplements in the form of tooth pastes, rinses, gels and tablets are used to treat tooth sensitivity and prevent dental caries<sup>2</sup>. These fluoride preparations help in preventing conditions of enamel fluorosis<sup>3</sup>. These agents are incorporated directly in whitening gels and applied on tooth surface to prevent sensitivity of dentine<sup>4</sup>. Wang et al., (2004) have shown that high fluoride concentration can cause damage to DNA leading to its

fragmentation and trigger apoptosis<sup>5</sup>. One of the fluoride gels frequently used in clinics is 0.2% sodium fluoride gel. It is available as a topical gel containing 900 ppm fluoride with calcium phosphate<sup>6</sup>. Stannous fluoride gel 0.4% has also been used in clinics. It contains 1000 ppm fluoride<sup>7</sup>. It has been reported in many studies that fluoride gels after professional application cause accidental ingestion of fluoride by children and adults and is well retained by oral mucosa<sup>8</sup>.

Sodium Fluoride gel can be applied at clinics with the help of mouth trays and stannous fluoride gel can be applied at home under supervision. Method of application of stannous fluoride gel is to use at home at bed time after tooth brushing twice a day or once a day for 14 days depending upon severity of tooth sensitivity<sup>9</sup>. Whereas in clinics the gel is applied only once. The application is repeated after every 6 months at clinics<sup>10</sup>.

Oral mucosa is the surface of oral cavity that is lined internally by mucous membrane. Oral mucosa comprises of 60% of lining mucosa that is found on alveolar mucosa, lips, inner lining of cheek, floor of

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mouth, vestibular fornix and soft palate<sup>11</sup>. The inner lining of cheek is histologically named as buccal mucosa. Histologically, buccal mucosa consists of stratified squamous epithelium. The epithelium is keratinized in albino rats where as in human it is non keratinized<sup>12</sup>.

Sodium fluoride is causing toxic effects on oral mucosal fibroblasts *in vitro* by inhibiting the proteins production<sup>13</sup>. One hour *in vitro* exposure of gingival explants to stannous fluoride or sodium fluoride concentration 0.1% can reduce cellular proteins and DNA synthesis markedly<sup>14</sup>. Apoptosis is a programmed cellular death that is characterized by controlled auto digestion, which means cell membrane integrity, thus cells die without disturbing their neighbours<sup>15</sup>. Histologically, apoptotic cells may be single or in clusters with shrinkage and dense purple pyknotic nuclei. Apoptotic bodies can be visible along with cell breakage<sup>16</sup>.

**RATIONALE**

Current study was designed to compare toxic effects of different gels on buccal mucosa of rats. This study will help dentist to prioritize the use of gels in treatment of patients.

**METHODS**

An experimental animal study was conducted at Experimental Research Laboratory of Post Graduate Medical Institute, Lahore to compare histological effects of sodium fluoride gel and stannous fluoride on buccal mucosa of 30 Albino rats after 14 days of application. The therapeutic reagents used in this study were 0.2% sodium fluoride gel and 0.4% stannous fluoride gel.

**PROCEDURE**

A total of 30 Albino rats of either sex, weighing (200-250gms) were obtained from animal house VRI, Lahore. They were individually kept in a climate controlled environment and were provided with food and water *ad libitum*. Animals of each group were placed in the respective cages which were labeled by tags. After the acclimatization of a period of one week, the experimental procedure was started (table 1).

**REAGENTS**

- Sodium fluoride gel 0.2% by the name of GC Tooth Mouse was obtained from GC Corporation Tokyo, Japan. It is a topical gel with Calcium phosphate and fluoride. It contained 2% sodium fluoride.

- Stannous fluoride gel 0.4% was obtained from Henry Schein Inc. in USA. It was available in mint flavor. Its ingredients were carbomer, citric acid, flavoring agent and glycerin.

Rats of all groups were sedated with intramuscular injection of Ketamine-xylazine (10mg/Kg) before application of gel. Buccal mucosa was exposed with the help of tweezer and the area was marked by Indian ink. The gel was applied with the cotton wool stick to the marked area for 4 minutes and then rinsed with water and immediately wiped away with dry cotton. Animals were sacrificed on 14<sup>th</sup> day and buccal mucosa from the marked area was dissected by punch biopsy technique. Slides were prepared and stained with H&E stain. Epithelial thickness was measured with the help of ocular micrometer. Apoptotic cells were counted under microscopic field of vision.

**Table 1:** Showing detail of animal groups.

Groups	Total No. of Animals	Assigned name and Reagent
A	10	Control(No application)
B	10	Experimental (0.2% sodium fluoride gel) topically applied on buccal mucosa for 14 days.
C	10	Experimental (0.4% stannous fluoride gel) topically applied on buccal mucosa for 14 days.

**RESULTS**

At day 14, there is a statistically significant difference in mean epithelial thickness between control and experimental groups (p-value 0.001), whereas there is

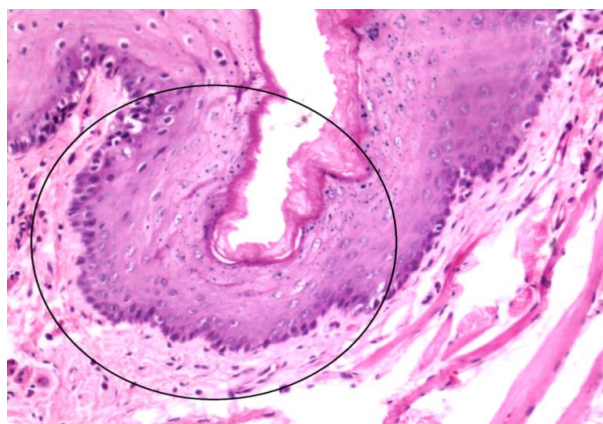
**Table 2:** Mean Epithelial Thickness and number of apoptotic cells of experimental and control groups.

Variables	Groups	N	Mean	S.D	p-value
Epithelial thickness (µm)	Group A	10	176	27.93	0.001
	Group B	10	17	2.74	
	Group C	10	41	13.42	
No. of Apoptotic cells	Group A	10	0	0	0.001
	Group B	10	13	2.45	
	Group C	10	11.8	2.49	

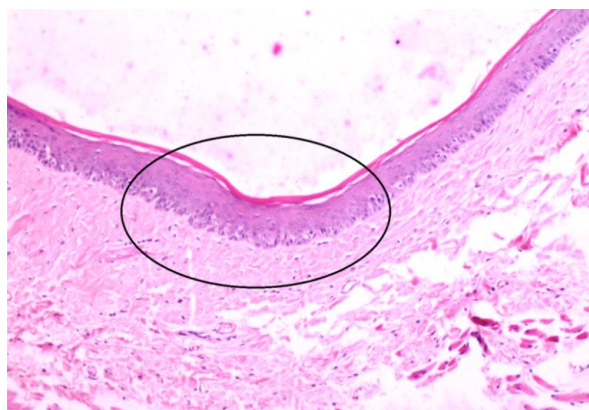
no statistically significant difference between group B and group C (p-value 0.176) (table 2, 3; fig. 1, 2, 3).

**Table 3:** Comparison of mean epithelial thickness and number of apoptotic cells of experimental and control groups.

Variables	Groups	p-value
Epithelial thickness (µm)	Group A-B	0.001
	Group A-C	0.001
	Group B-C	0.176
No. of Apoptotic cells	Group A-B	0.001
	Group A-C	0.001
	Group B-C	0.033



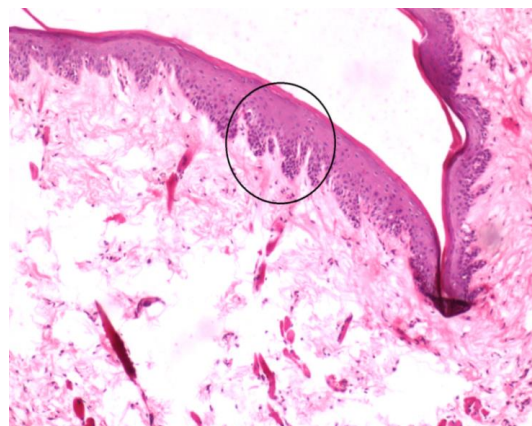
**Figure1:** Photomicrograph of histological section of buccal mucosa of control group on day 14 showing maximum epithelial thickness as compared to experimental groups. H and E stain.10X showing normal buccal mucosa.



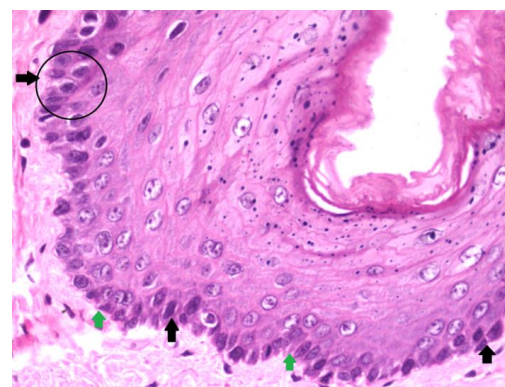
**Figure 2:** A photomicrograph of buccal mucosa of experimental group B at day 14 under 10X. H and E stain showing epithelial shrinkage.

There is a statistically significant difference of number of apoptotic cells in control group A as compared to experimental groups B and C (p-value

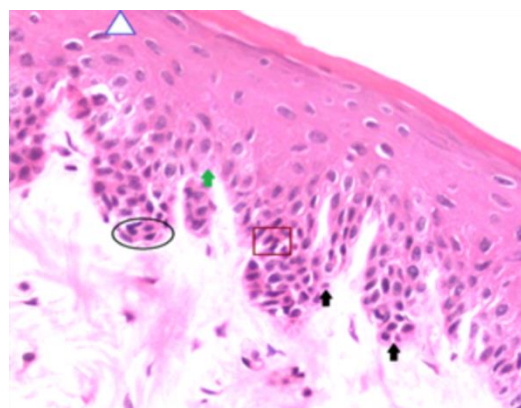
0.001) and also between experimental groups B and group C, (p-value 0.033) (table 2, 3 ; fig. 4, 5, 6).



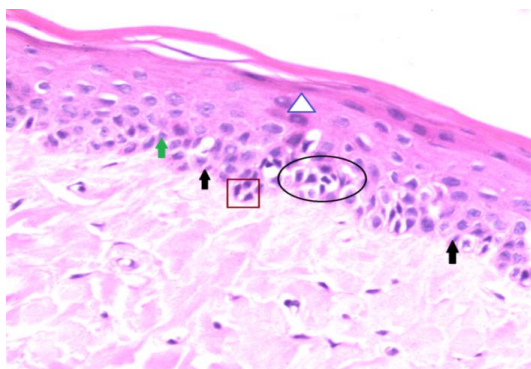
**Figure 3:** A photomicrograph of histological section of buccal mucosa of experimental group C at day 14 under H and E stain 10X (10x10=100) showing epithelial shrinkage.



**Figure 4:** Photomicrograph of Histological section of buccal mucosa of control group on 1 day. H and E stain. 40X (40x10=400) showing normal epithelium.



**Figure 5:** A photomicrograph of buccal mucosa of experimental group B at day 14 under 40X (40x10=400). H and E stain, Black circle shows apoptotic cells,



**Figure 6:** A photomicrograph of histological section of buccal mucosa of experimental group C at day 14 under H and E stain 40X (40x10=400). Black circle shows apoptotic cells.

The above mentioned results clearly depict that both gels produce toxicity in buccal mucosa but sodium fluoride gel is significantly toxic than stannous fluoride gel.

## DISCUSSION

In the present study 0.2% sodium fluoride gel and stannous fluoride gel 0.4% were applied topically on buccal mucosa of albino rats for 14 days. This study investigated the comparison of apoptotic changes on the buccal mucosa of albino rats as a result of fluoride applications. It is evident from this study that fluoride in excess quantity results in toxic effects on soft tissue of buccal mucosa which is also endorsed from the previous study conducted by Barbier et al., 2010, which depicted the toxicity of fluoride on hard tissues of teeth such as enamel and some soft tissues of lungs, brain and kidneys<sup>17</sup>.

Sodium fluoride treatment also gradually declines the expression of the anti-apoptotic protein Bcl-2. Sodium fluoride also produces apoptotic effects and causes the alteration of bcl2 family protein expression in osteoblastic cells<sup>18</sup>. These results help in understanding the mechanism by which NaF mediates cytotoxicity and apoptosis.

The analysis of essential quantitative parameters, mean epithelial thickness of buccal mucosa and number inflammatory cells, exhibited statistically significant results. This was comparable with a study conducted by Chia et al., 2008 in which epithelial thickness decreased with days in response to application of fluoride gel<sup>19</sup>. Similar results were shown by Hongmei et al., 2013 that when NaF at different concentrations of 0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mM were treated to chondrocytes, it was observed that their cell viability was decreased and the rate of apoptosis increased significantly with the

gradient concentration of NaF in a time- and dose-dependent manner<sup>20</sup>.

## CONCLUSION

This experiment has shown that sodium fluoride gel exhibits more apoptotic activity on buccal mucosa as compared to stannous fluoride gel. Dental clinicians should be aware of possible oral adverse effects of high dose of sodium fluoride gel that have so far been unrecognized and are used for variety of conditions, particularly dental caries and tooth sensitivity.

## ETHICAL APPROVAL:

The study was approved from Ethical Review Committee of Postgraduate Medical Institute, Lahore, Pakistan.

## AUTHORS' CONTRIBUTION:

**SS:** Study Design, Manuscript Writing, Data Collection

**HM:** Drafting

**MA:** Approval of the final version of Manuscript

**MSI:** Revision critically for important intellectual content.

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