ORIGINAL ARTICLE

Histological Effect of Sodium Fluoride Gel on Buccal Mucosa of Albino Rats

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ABSTRACT

Aim: Fluoride gels are being used abundantly among dental practitioners to treat tooth sensitivity and white spot lesions.

Methods: This experimental study comprised of 30 Albino rats of average weight 200-250 grams which were divided into 3 groups. 10 out of 30 rats were randomly assigned into control group A and rest of the 20 rats 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 at day 1 for 4 minutes, with the help of cotton pellet. This group was sacrificed on the same day after half an hour of application of gel. The rest of the 10 animals of group C were given same dose of 0.2% sodium fluoride gel topically once daily for 4 minutes on buccal mucosa for 14 days and were sacrificed on 14th day under deep anesthesia.

Results: Histological analysis showed that sodium fluoride gel used in the study produced apoptotic effects on buccal mucosa of albino rats with the toxicity increasing in a time dependant manner.

Conclusion: Therefore there is a need not to use any type of fluoride gels in clinical practice as it may cause periodontal problems.

Keywords: Buccal mucosa, sodium fluoride, albino rat

INTRODUCTION

Dental caries is the most pronounced problem of today. Dental caries is a disease that involves the localized chemical dissolution of dental hard tissues due to acids produced by plaque bacteria[1]. Fluoride is a well known and effective prophylactic agent for caries. Its local or systemic application has therefore been recommended widely over the past decades[2]. However fluoride gels in its appropriate range is thought to be effective and safe[3]. Fluorides in excess quantity results in toxic effects on hard tissues of teeth such as enamel and some soft tissues of lungs, brain and kidneys[4]. Fluoride gels are being used widely to treat tooth sensitivity and to remineralize white spot lesion[5]. They also improve surface appearance[6]. Fluoride gels are easy to handle due to their high viscosity[7]. The fluoride gel that has been used in clinics is 0.2% sodium fluoride gel. The concentration of fluoride in this gel is 900 ppm and it is acidulated[8]. However it has been reported in many studies that fluoride gel after professional application causes oral retention and ingestion of fluoride by children and adults[9]

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Fluoride gels can be professionally applied at clinics and can be applied under supervision at home. In clinics, gel is applied with the help of mouth trays. Another method of application of fluoride gel is to use at home at bed time after tooth brushing twice a day or once a day depending upon severity of caries. The home made gel can be applied for 14 days or more depending upon severity of caries, whereas in clinics the gel is applied only once. The application is repeated after every 6 months at clinics[10].

Oral Mucosa on topical application can serve as long term fluoride reservoir[11]. Oral mucosa is the surface of oral cavity that communicates with external environment and is lined by mucous membrane[12]. On the basis of functional criteria, oral mucosa can be divided into masticatory mucosa, lining mucosa and specialized mucosa. Histologically, buccal mucosa of human consists of stratified squamous non-keratinized epithelium[13].

According to one study, sodium fluoride at a concentration of (150-250mg/kg) causes not only chronic but toxic effects such as apoptosis in several kinds of human gingival epithelial cells[14]. Apoptosis is a programmed cell death that occurs under physiological conditions[15]. Under light microscope, apoptotic cells can be single or in form of clusters. They are round, oval with dark eosinophilic cytoplasm and dense purple nuclei, cell shrinkage and pyknosis are visible. Apoptotic bodies can be visible along with cell breakage[16].

MATERIALS AND METHODS

An experimental animal study was conducted at Experimental Research Laboratory of Post Graduate Medical Institute Lahore to study histological effects of fluoride gels on buccal mucosa of 30 Albino rats after 1st day and 14 days of application. The therapeutic reagent used in this study was 0.2% sodium 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.* 10 animals of each group were placed in the respective cages which were labeled by tags. After acclimatization of a period of one week, experimental procedure was started.

Reagent: Sodium fluoride gel 0.2% by the name of GC Tooth Mouse was obtained from GC Corporation Tokyo, Japan. It is a topical cream with Calcium phosphate and fluoride was also obtained from Henry Schein Inc. in USA. It contained 2% sodium fluoride. It had optimized low pH for penetration.

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. Histological effects of fluoride gel on buccal mucosa of Albino rats were studied after sacrificing the animals of group A and B on 1st day and group C on 14th days (Table 1).

RESULTS

Qualitative analysis: After 1 day of intervention in experimental group B the cellular shrinkage was statistically significant (p-value 0.05) as well as statistically significant difference was observed at day 14 in experimental group C (p-value 0.005).

The Eosinopilia was statistically different **(p-value 0.005)** in group B and C when compared with control group A. Difference among groups according to severity of nuclear pyknosis were statistically significantly different at day 1 and day 14 **(p-value <0.001**). Intercellular bridge disruption severity was statistically significantly different at day 1 and day 14 **(p-value 0.008 and 0.002 respectively)** (Table 2)

Quantitative analysis: At day 1, Mean Epithelial thickness of group A was statistically significantly more than other groups **(p-value <0.001).** Epithelial thickness of Group B was more than group C and showed statistically significantly different results **(p-value 0.033).** When Apoptosis was observed in group B it was found more than group A and group C (p-value <0.001) (Table 3).

Table 1: Detail of animal groups.

Groups	No of Animals	Specifications.
A	10	CONTROL
B	10	Experimental (0.2% Sodium Fluoride gel applied on buccal mucosa for 01 day (0.5 mg/Kg/bw)
C	10	Experimental (0.2% Sodium Fluoride gel applied on buccal mucosa for 14 days (0.5 mg/Kg/bw)

Table 2: Comparison of qualitative variables between experimental and control groups.

Qualitative Variables	A (control)		B (Experimental at day one)		C	
	Absent	Present	Absent	Present	Absent	Present
Cellular shrinkage	10	-	4	6	-	10
Eosinophilia	10	-	2	8	2	8
Nuclear pyknosis	10	-	-	10	0	10
Intercellular Bridge Disruption	10	-	2	8	-	10

Table 3: Mean epithelial thickness and number of apoptotic cells of experimental and control groups.

		N	Mean	S.D	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Epithelial thickness	Group A	10	176	27.93	141.32	210.68
	Group B	10	41	13.42	24.34	57.66
	Group C	10	17	2.74	13.6	20.4
No. of Apoptotic cells	Group A	10	0	0	0	0
	Group B	10	13	2.45	9.96	16.04
	Group C	10	11.8	2.49	8.71	14.89

Fig. 1: Photomicrographs of histological section of buccal mucosa of control group (A) showing maximum epithelial thickness as compared to experimental groups (B & C). Mean epithelial thickness of experimental groups is less than control group. H and E stain under 10X (10x10=100)

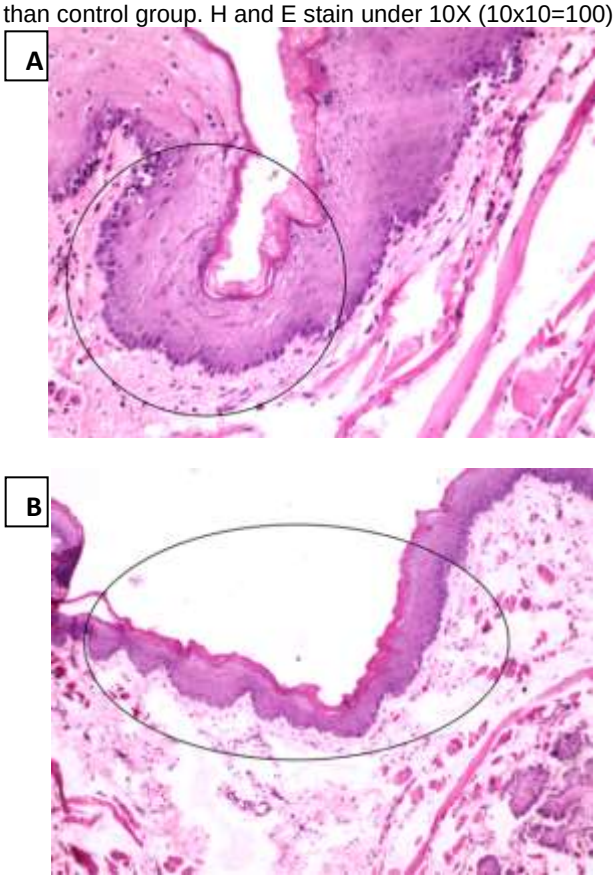


Fig. 2: Photomicrograph of histological section of buccal mucosa of control group H and E stain under 40X (40x10=400) showing normal epithelium. Black arrow shows normal size columnar cells of stratum basale without any shrinkage. Green arrow shows that cells are tightly packed with no intercellular bridge disruption, black circle shows normal nuclei without any pyknosis. Cells of all layers are normal without any signs of apoptosis.

Fig. 3: A photomicrograph of buccal mucosa of experimental group B at day 1 under 40 X (40x10=400). Black arrow shows cellular shrinkage, green arrow shows intercellular bridge disruption, maroon square shows apoptotic cells, black circle shows nuclear pyknosis. White and blue triangle shows eosinophilia.

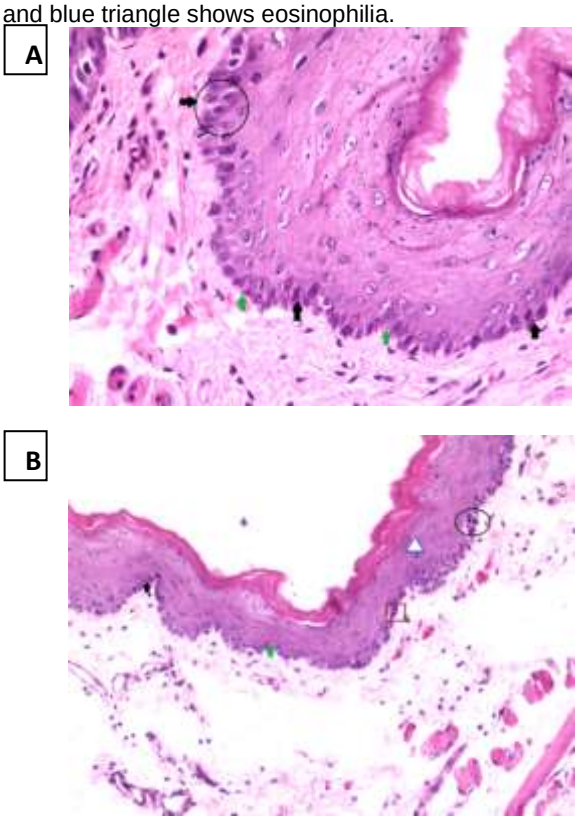
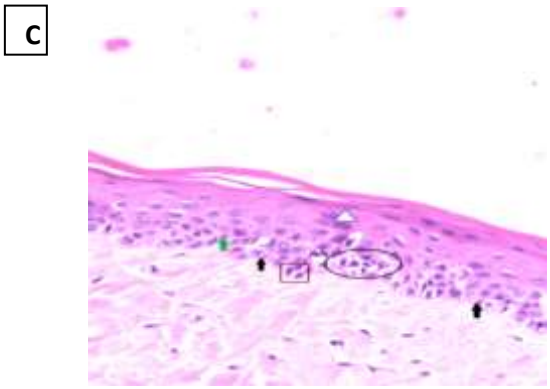


Fig. 4: A photomicrograph of buccal mucosa of experimental group C at day 14 under 40X (40x10=400). H and E stain, Black arrow shows marked cellular shrinkage, green arrow shows intercellular bridge disruption, maroon square shows apoptotic cells, black circle shows pyknotic nuclei.



DISCUSSION

In the present study sodium fluoride gel 0.2% was applied topically on buccal mucosa of albino rats.

This study investigated the histological changes seen as apoptosis on the buccal mucosa of albino rats as a result of fluoride applications. According to two reports fluoride gel in high concentration causes periodontal necrosis and esophageal wall thickening[17,18]. Fluoride gels at millimolar range can affect cellular functions such as enzyme activity. It also causes DNA damage and cell cycle changes[19]. Sodium fluoride also produces apoptotic effects and causes the alteration of bcl2 family protein expression in osteoblastic cells[20,21] Sodium fluoride treatment also gradually declines the expression of the anti-apoptotic protein Bcl-2. It causes cleavage of poly (ADP-ribose) polymerase and also increased activation of caspase-3. These results help in understanding the mechanism by which NaF mediates cytotoxicity and apoptosis[22].

The control (no gel application) group demonstrated the structural integrity of rat buccal epithelium (stratified squamous keratinized). Their epithelial layers were intact with highest mean epithelial thickness, no cellular shrinkage and intercellular connections showed healthy state of buccal epithelium. At day1, mean epithelial thickness of experimental group B was less than control group. This was due to increase in cell death and decrease in cell number. Their results showed that at day 1 there were signs of apoptosis initiation. At 14 day the mean epithelial thickness of group C was least as compared to experimental groups B and control group A. 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. Epithelial cells of control group when examined at day 1 and 14 under H and E stain reflected normal morphology without any cellular shrinkage. Overall cellular shrinkage in group C demonstrated an increase by day 14. This was the representative of late phase apoptosis. These results were same as with the work of Shinj et al., 2002 in which he used gastric irritants and cellular shrinkage was increased in time dependant and dose dependant manner.

After 1st day of intervention, eosinophilia was seen in all cases of group B. After 14 days of intervention, it was noted that eosinophilia increased due to exposure of buccal mucosa with the passage of time. In the light of nuclear pyknosis parameter it was found that cells of control group had intact nuclei. After application of gels on 1st day, all cases of group B reflected nuclear pyknosis but group C showed severe pyknosis after 14 days of intervention.

Intercellular bridge disruption was found to be absent in all cases of control group. On 1st day of application of gels, moderate intercellular bridge disruption was seen in group B. After 14 days of exposure to gels, cases of group C showed severe intercellular bridge disruption. The present results reported that intercellular bridge disruption increased in severity in a time dependant manner. This reason was that more cells were dying with apoptosis advancing to late phase losing their contacts and became shrunken and dark. The same results were reported by Chia et al., 2008. This study has revealed that so far no attention has been given for prevention of apoptosis induced by fluoride gel. Dental clinicians should be aware of possible oral adverse effects of high dose of fluoride gels that have so far been unrecognized and are used for variety of conditions, particularly dental caries and tooth sensitivity.

CONCLUSION

In summary, this study experimentally demonstrated that apoptosis was caused by sodium fluoride gel being used extensively. Therefore there is a need not to use any type of fluoride gels in clinical practice as it may cause periodontal problems. In cases where it is necessary to use fluoride gels as in sensitivity of teeth or post radiation oral cancer patients, alternatives should be find out to address these problems. If fluoride treatment is to be given then rubber dam should be used to protect the oral mucosa. This contribution can tremendously enhance the quality of life.

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