Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2022; 22:e210169
https://doi.org/10.1590/pboci.2022.065
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Effect of Different Preparations of Fluoride Gel on Salivary pH of Albino Rats

Sadia Shakeel[1], Muhammad Sharjeel Ilyas[2], Ayesha Fahim[1], Amna Ahsan[2], Hina Majid[2], Mariam Ashraf[2], Naseem Akhter[3], Mohammad Khursheed Alam[4]

[1]Department of Oral Biology, University College of Dentistry, University of Lahore, Lahore, Pakistan.
[2]Department of Oral Biology, Post Graduate Medical Institute, Lahore, Pakistan.
[3]The Children's Hospital and Institute of Child Health, Lahore, Pakistan.
[4]Orthodontic division, Preventive dentistry department, College of Dentistry, Jouf University, Kingdom of Saudi Arabia.

Correspondence: Mohammad Khursheed Alam, Orthodontic division, Preventive dentistry department, College of Dentistry, Jouf University, Kingdom of Saudi Arabia. **E-mail:** dralam@gmail.com

Academic Editor: Alessandro Leite Cavalcanti

Received: 10 September 2021 / **Review:** 27 November 2021 / **Accepted:** 12 December 2021

How to cite: Shakeel S, Ilyas MS, Fahim A, Ahsan A, Majid H, Ashraf M, et al. Effect of different preparations of fluoride gel on salivary ph of albino rats. Pesqui Bras Odontopediatria Clín Integr. 2022; 22:e210169. https://doi.org/10.1590/pboci.2022.065

ABSTRACT

Objective: To evaluate the effect of different preparations of fluoride gels on the salivary pH of albino rats. **Material and Methods:** This experimental study consisted of 40 Albino rats randomly divided into four equal groups. Group A was the control group and received no intervention. Experimental group B received a topical application of 0.2% sodium fluoride gel. Experimental group C received topical application of stannous fluoride gel 0.4%. Experimental group D received topical application of APF gel (1.23% acidulated phosphate fluoride gel). The different preparations of the gels were applied once daily for 4 minutes on the occlusal surface of the right maxillary molars for 14 days. Salivary pH values were recorded immediately after the application of gels with the help of pH paper on day 1 and day 14. **Results:** There was a significant difference in the pH level of groups B, C and D after 14 days of fluoride application ($p < 0.05$). The non-parametric Kruskal Wallis test was applied for the comparison between the groups. **Conclusion:** This study concluded that all the fluoride gels after administration caused the acidic pH of saliva with the most acidic effect produced by APF gel.

Keywords: Acidulated Phosphate Fluoride; Dental Caries; Saliva; Salivary Glands; Sodium Fluoride.

Introduction

Fluoride is widely considered the cornerstone of modern dentistry. Because of its cariostatic properties, fluoride has been increasingly added to preventive dentistry in the form of toothpaste, gels, and mouth rinses [1]. The presence of fluoride in an acidic environment reduces the dissolution of calcium hydroxyapatite. The main action is inhibition of demineralization of enamel and retarding growth of the biotypes [2]. When bacteria metabolize sugars, they decrease the pH in saliva through the production of lactic acid. A fall in pH below the critical level of hydroxyapatite (pH 5.5) leads to demineralization of enamel and results in caries [3]. Because the fluoride ion coating is only partial, if the pH falls below level 5.5, the uncoated parts of the crystal will undergo dissolution on certain parts of the tooth. When the pH rises above the critical level of 5.5, the increased level of fluoride ion leads to remineralization, because it absorbs itself into the enamel and forms fluorohydroxyapatite [4]. Fluoride gels are easy to handle due to their high viscosity [5].

One fluoride gels frequently used in clinics is 0.2% sodium fluoride gel. It is a topical gel containing 900 ppm fluoride with calcium phosphate. It delivers calcium phosphate on the tooth surface to cause demineralization of enamel caries [6]. Another fluoride gel used in clinics is 1.23% acidulated phosphate fluoride gel (APF). APF gel is an acidic and highly concentrated gel and evidence-based clinical recommendations for its topical application on teeth are once a year to four times a year, depending upon the age and severity of the disease. The gel consists of 1.23% fluoride, which is equal to 12.3 mg of fluoride for every 1 mL [7]. American Dental Association recommended clinical use of 1.23% acidulated phosphate fluoride after reviewing the 71 trials, where the efficacy of various topical fluoride caries-preventive agents was reviewed [8]. However, it has been reported in many studies that APF gel after professional application causes accidental ingestion of fluoride by children and adults and is well retained by oral mucosa [9]. Stannous fluoride gel 0.4% has also been accepted as clinically effective as per US FDA [10].

Fluoride gels can be applied at clinics and homes under supervision. In clinics, a gel is applied with the help of mouth trays. The tray loaded with the gels is used to place over the teeth and the patient is asked to close the jaws as instructed. The loaded tray is placed in the mouth for a minimum of 4 minutes. After that tray is removed, the patient expectorates thoroughly and avoids drinking or eating for 30 minutes [11]. Fluoride can also be applied at home at bedtime. The patient uses custom-made trays for the upper and lower arch and applies a thin layer of gel on trays. The trays are then seated on upper and lower teeth for 5 minutes and then trays are removed, and the mouth is thoroughly expectorated. It can be applied for 14 days or more depending upon the severity of caries, whereas, in clinics, the gel is applied only once [11].

The oral cavity is kept moist by a film of fluid called saliva that coats the teeth and mucosa. The normal pH of saliva in humans is 6.7-7.6 [12] whereas the normal pH of rat saliva is (8.35-9.15) [13]. The most important function of this complex fluid is to maintain the wellbeing of the mouth [14]. It contains various factors required for host protection hence it can be an important biomarker for the diagnosis of several diseases [15]. The viscoelastic nature of saliva along with the salivary proteins helps in aiding digestion, increases antimicrobial action, and facilitates taste and lubrication [16]. The salivary proteins, namely the proline-rich proteins, mucins, histatins, cystatins, and statherins, attract calcium ions, promote remineralization, and protect the tooth surface [17].

Material and Methods

Experimental Animals

A total of 40 Albino rats of either gender, weighing (200-250gms) were obtained from animal house PGMI, Lahore. Diseased rats were excluded from the study. They were individually kept in a climate-controlled environment and were provided with food and water *ad libitum*. All the work was carried out according to the National Institutes of Health guidelines [18]. In addition, the study was conducted in accordance with the APA guidelines of non-human studies.

Experimental Design

A randomized controlled trial was conducted at the experimental research laboratory of Post Graduate Medical Institute (PGMI), Lahore, Pakistan. Animals were divided into four groups (Table 1) and each group was placed in separate cages which were labeled by tags. After the acclimatization of a period of one week, the experimental procedure was started.

Table 1. Details of animal groups.

Groups	Number of Animals	Assigned Names
A	10	Control
B	10	Experimental sodium fluoride gel 0.2% applied
C	10	Experimental stannous fluoride gel 0.4% applied
D	10	Experimental APF gel 1.23% applied

Application of Gel

The therapeutic reagents used in this study were 0.2% sodium fluoride gel, 0.4% stannous fluoride gel, and 1.23% APF gel. Animals of group A did not receive any gel, while animals all the experimental groups were sedated with an intramuscular injection of Ketamine-xylazine (10mg/Kg) before application of the gel. The different preparations of gel were applied with the cotton wool stick to the occlusal surface of the right maxillary molars for 4 minutes and then rinsed with water and immediately wiped away with dry cotton. This procedure was repeated for 14 days to the animals of groups B, C, and D (Table 1).

Analytical Assays

The saliva pH was measured immediately after the application of gels with a pH paper (Johnson Test Papers Ltd., West Midlands, United Kingdom) on day1 and day14. Readings were recorded according to the universal color scale.

Statistical Analysis

Statistical analyses were performed using SPSS 24 software (IBM Corp., Armonk, NY, USA). The normality of the data was tested with Shapiro-Wilk tests. The non-parametric Kruskal Wallis test was used for the comparison of the different pH values. Dunn-Bonferroni post hoc analysis was performed for pair-wise analysis in groups. The significance level was set at a p-value < 0.05.

Ethical approval

This study was approved by the Ethical Review Committee of (PGMI/LGH), Lahore, Pakistan (ERB/IRB/No. 5081/PGMI).

Results

The pH values in rat saliva varied considerably among all groups on day 1 and day 14 (Tables 2 to 5). The collected data showed significant deviation from normality. Therefore, the continuous variable was compared by the non-parametric Kruskal Wallis test. The pH value decreased significantly ($p<0.005$) among all groups on day 1 (Table 2) and day 14 (Table 4). Dunn-Bonferroni post hoc analysis was performed to compare pH values between and within groups on day 1 and day 14. Comparison between control Group A and experimental group C showed a statistically significant difference ($p<0.05$) at day 1 (Table 3) and ($p<0.005$) at day 14 (Table 5). Also, there was a statistically significant difference between control group A and experimental group D at day 1 ($p<0.005$) (Table 3) and at day 14 ($p<0.005$) (Table 5).

Table 2. Analysis of pH values at day 1 using the Kruskal Wallis test.

Groups	N	Mean Rank	p-value
A	10	18.00	0.001*
B	10	13.00	
C	10	6.50	
D	10	4.50	

*Statistically Significant.

Table 3. Pair-wise comparison of pH values at day 1.

Groups	Test Statistics	Std. Error	p-value
A and B	5.000	3.610	0.996
A and C	11.500	3.610	0.009*
A and D	13.500	3.610	<0.001
B and C	6.500	3.610	0.431
B and D	8.500	3.610	0.111
C and D	2.00	3.610	1.00

*Statistically Significant.

Table 4. Analysis of pH values at day 14 using the Kruskal Wallis test.

Groups	N	Mean Rank	p-value
A	10	18.00	<0.001*
B	10	13.00	
C	10	5.50	
D	10	5.50	

*Statistically Significant.

Table 5. Pair-wise comparison of pH values at day 14.

Groups	Test Statistics	Std. Error	p-value
A and B	5.000	3.642	1.000
A and C	12.500	3.642	0.004*
A and D	12.500	3.642	0.004*
B and C	7.500	3.642	0.237
B and D	7.500	3.642	0.237
C and D	0.000	3.642	1.000

*Statistically Significant.

Discussion

In the present study, 0.2% sodium fluoride gel, stannous fluoride gel 0.4%, and APF gel 1.23% were applied topically on the occlusal surface of right maxillary molars of albino rats for 14 days. This study investigated the comparison of salivary pH changes of the albino rats as a result of fluoride applications. It is

evident from this study that topical use of fluoride gel preparations results in critically low PH levels in the saliva of albino rats. An acidic environment in the oral cavity facilitates dental caries formation and progression [19]. In the present study, the control group demonstrated the pH of saliva in albino rats was (8.5-9.0) when recorded after 1 and 14 days, whereas the pH after application of different preparations of fluoride gels on day 1 and 14 turned acidic and APF gel causing most acidic salivary pH of albino rats (pH=4.0), this level is even lower than the critical level pH value which is 5.5 in humans. Bajraktarova-Valjakova et al. mentioned the hazards of acidulated phosphate fluoride gels and suggested discontinuing its home use due to high acidic content [20]. The use of beverages is also prohibited due to their high sugar content and acidogenic nature. These qualities make children prone to dental caries and in the present study, the same pattern was exhibited in salivary pH [21].

An electron microscopic study was conducted to evaluate the effects of APF gel on the oral mucosa of rabbits; the basal layer of oral mucosa showed signs of apoptosis with disrupted intercellular junctions and condensation of chromatin material [22]. Stannous fluoride is well known for its anti-cariogenic properties, but it also produces stains on gingivae and oral mucosa, so its use has been discouraged [23]; one of the findings in the present study is a significant decrease of pH in rat saliva after using stannous fluoride gel. The published articles also speak very high about the use of fluoride gel applications and find it equally beneficial to prevent dental caries [24]. Therefore, recommendations are made in a systemic review to use different fluoride applications for the non-restorative treatment of dental caries [24,25].

The data scarcely talk about the effect of fluoride gels on salivary pH, which makes it difficult for a clinician to use these preparations or go for an alternative way to treat caries.

Conclusion

The decreased pH of saliva will cause persistent bad breath, sensitivity to hot or cold food or beverages, and the tooth will be prone to cavities. This will lead to an increased incidence of caries and periodontal disease.

Authors' Contributions

SS	https://orcid.org/0000-0002-3301-288X	Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft and Writing - Review and Editing.
MSI	https://orcid.org/0000-0002-5552-0236	Conceptualization, Formal Analysis, Investigation, Writing - Original Draft and Writing - Review and Editing.
AF	https://orcid.org/0000-0003-2764-882X	Conceptualization, Formal Analysis, Writing - Original Draft and Writing - Review and Editing.
AA	https://orcid.org/0000-0003-3726-0407	Conceptualization, Validation, Writing - Review and Editing and Visualization.
HM	https://orcid.org/0000-0002-9939-4489	Conceptualization, Validation, Writing - Review and Editing and Visualization.
MA	https://orcid.org/0000-0001-9072-9871	Conceptualization, Validation, Writing - Review and Editing and Visualization.
NA	https://orcid.org/0000-0003-1585-3279	Conceptualization, Validation, Writing - Review and Editing and Visualization.
MKA	https://orcid.org/0000-0001-7131-1752	Conceptualization, Writing - Original Draft, Writing - Review and Editing and Supervision.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

References

[1] Bansal A, Ingle NA, Kaur N, Ingle E. Recent advancements in fluoride: A systematic review. J Int Soc Prev Community Dent 2015; 5(5):341-6. https://doi.org/10.4103/2231-0762.165927

[2] Yin IX, Yu OY, Zhao IS, Mei ML, Li QL, Tang J, et al. Inhibition of dentine caries using fluoride solution with silver nanoparticles: An in vitro study. J Dent 2020; 103:103512. https://doi.org/10.1016/j.jdent.2020.103512

[3] Uma E, Theng KS, Yi LLH, Yun LH, Varghese E, Soe HHK. Comparison of salivary pH changes after consumption of two sweetened Malaysian local drinks among individuals with low caries experience: a pilot study. Malays J Med Sci 2018; 25(4):100-11. https://doi.org/10.21315/mjms2018.25.4.10

[4] Kao Y-H, Igarashi N, Abduweli Uyghurturk D, Li Z, Zhang Y, Ohshima H, et al. Fluoride alters signaling pathways associated with the initiation of dentin mineralization in enamel fluorosis susceptible mice. Biol Trace Elem Res 2021; 199(8):3021-34. https://doi.org/10.1007/s12011-020-02434-y

[5] Duangthip D, Chen KJ, Gao SS, Lo ECM, Chu CH. Managing early childhood caries with atraumatic restorative treatment and topical silver and fluoride agents. Int J Environ Res Public Health 2017; 14(10):1204. https://doi.org/10.3390/ijerph14101204

[6] Lussi A, Buzalaf MAR, Duangthip D, Anttonen V, Ganss C, João-Souza SH, et al. The use of fluoride for the prevention of dental erosion and erosive tooth wear in children and adolescents. Eur Arch Paediatr Dent 2019; 20(6):517-27. https://doi.org/10.1007/s40368-019-00420-0

[7] Baik A, Alamoudi N, El-Housseiny A, Altuwirqi A. Fluoride varnishes for preventing occlusal dental caries: a review. Dent J 2021; 9(6):64. https://doi.org/10.3390/dj9060064

[8] Slayton RL, Urquhart O, Araujo MW, Fontana M, Guzmán-Armstrong S, Nascimento MM, et al. Evidence-based clinical practice guideline on nonrestorative treatments for carious lesions: a report from the American Dental Association. J Am Dent Assoc 2018; 149(10):837-49. e19. https://doi.org/10.1016/j.adaj.2018.07.002

[9] Warreth A, Abuhijleh E, Almaghribi MA, Mahwal G, Ashawish A. Tooth surface loss: a review of literature. Saudi Dent J 2020; 32(2):53-60. https://doi.org/10.1016/j.sdentj.2019.09.004

[10] Fiorillo L, Cervino G, Herford AS, Laino L, Cicciù M. Stannous fluoride effects on enamel: a systematic review. Biomimetics 2020; 5(3):41. https://doi.org/10.3390/biomimetics5030041

[11] Sudhanthar S, Lapinski J, Turner J, Gold J, Sigal Y, Thakur K, et al. Improving oral health through dental fluoride varnish application in a primary care paediatric practice. BMJ Open Quality 2019; 8(2):e000589. https://doi.org/10.1136/bmjoq-2018-000589

[12] Bel'skaya LV, Kosenok VK, Sarf EA. Chronophysiological features of the normal mineral composition of human saliva. Arch Oral Biol 2017; 82:286-92. https://doi.org/10.1016/j.archoralbio.2017.06.024

[13] Lan X, Chan JYK, Pu JJ, Qiao W, Pang S, Yang W-f, et al. Saliva electrolyte analysis and xerostomia-related quality of life in nasopharyngeal carcinoma patients following intensity-modulated radiation therapy. Radiother Oncol 2020; 150:97-103. https://doi.org/10.1016/j.radonc.2020.06.016

[14] Raghavan R, Shajahan P, Gibi M. An insight into the science behind saliva and its crucial role in oral health. J Dent Panacea 2021; 3(2):52-7. https://doi.org/10.18231/j.jdp.2021.013

[15] Ngamchuea K, Chaisiwamongkhol K, Batchelor-McAuley C, Compton RG. Chemical analysis in saliva and the search for salivary biomarkers - a tutorial review. The Analyst 2017; 143(1):81-99. https://doi.org/10.1039/c7an01571b

[16] Kim B, Lee SS, Yoo TH, Kim JM. Viscoelastic particle focusing in human biofluids. Electrophoresis 2021; 42(21-22):2238-45. https://doi.org/10.1002/elps.202000280

[17] Devarajan H, Somasundaram S. Salivary proteins and its effects on dental caries – a review. Drug Invent Today 2019; 11(6):1406-11.

[18] Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br J Pharmacol 2020; 40(9):1769-77. https://doi.org/10.1177/0271678X20943823

[19] Chen X, Xing H, Zhou Z, Hao Y, Zhang X, Qi F, et al. Nanozymes go oral: nanocatalytic medicine facilitates dental health. J Mater Chem 2021; 9(6):1491-502. https://doi.org/10.1039/D0TB02763D

[20] Bajraktarova-Valjakova E, Grozdanov A, Guguvcevski L, Korunoska-Stevkovska V, Kapusevska B, et al. Acid etching as surface treatment method for luting of glass-ceramic restorations, part 1: acids, application protocol and etching effectiveness. Open Access Maced J Med Sci 2018; 6(3):568-73. https://doi.org/10.3889/oamjms.2018.147

[21] Su H, Yang R, Deng Q, Qian W, Yu J. Deciduous dental caries status and associated risk factors among preschool children in Xuhui District of Shanghai, China. BMC Oral Health 2018; 18(1):111. https://doi.org/10.1186/s12903-018-0565-8

[22] Shakeel S, Majid H, Ashraf M, Ilyas MS. Histological effect of sodium fluoride and stannous fluoride gels on buccal mucosa of albino rat. Pak Postgrad Med J 2019; 30(01):12-6.

[23] Johannsen A, Emilson CG, Johannsen G, Konradsson K, Lingström P, Ramberg P. Effects of stabilized stannous fluoride dentifrice on dental calculus, dental plaque, gingivitis, halitosis and stain: a systematic review. Heliyon 2019; 5(12):e02850. https://doi.org/10.1016/j.heliyon.2019.e02850

[24] Gupta A, Sharda S, Nishant, Shafiq N, Kumar A, Goyal A. Topical fluoride-antibacterial agent combined therapy versus topical fluoride monotherapy in preventing dental caries: a systematic review and meta-analysis. Eur Arch Paediatr Dent 2020; 21(6):629-46. https://doi.org/10.1007/s40368-020-00561-7

[25] Urquhart O, Tampi MP, Pilcher L, Slayton RL, Araujo MWB, Fontana M, et al. Nonrestorative treatments for caries: systematic review and network meta-analysis. J Dent Res 2019; 98(1):14-26. https://doi.org/10.1177/0022034518800014