



Assessment of the Deleterious Effects of Therapeutic Antitussives on Enamel. Mapping the Chemical Profile of Over the Counter Cough Lozenges Using Analytical HPLC

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RS and MK designed the study. Author RR conducted the clinical part of the study. Author RS wrote the protocol and wrote the first draft of the manuscript. Authors PR and NM managed the analyses of the study. Author RR managed the literature searches. Authors MY and VJ performed the statistical analysis. Authors MK and RS wrote the final draft of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: 1) To evaluate and compare changes in the micro hardness of enamel after exposure to different cough lozenges.

2) To assess the pH of dissolved lozenge solutions, and

3) To analyze the number of components in cough lozenges using Analytical HPLC (High-performance liquid chromatography).

Study Design and Methods: Experimental Confirmatory Study.

Forty extracted human molars were selected for the study. The teeth were embedded in self-cured acrylic resin except a small rectangular area of 3.0 mm × 2.0 mm on the buccal surface. Micro hardness tests were carried out with a Vickers Diamond Indenter with a 50 gm load for 15 seconds. Based on the lozenges used, the samples were randomly divided into 4 groups (n=10);
Group 1: Control - No treatment
Group 2: Lozenge - A
Group 3: Lozenge - B
Group 4: Lozenge - C
Lozenges were dissolved in 20 ml of artificial saliva for 30 minutes. After complete dissolution, the samples were immersed in lozenges solution for 30 minutes at room temperature, four times a day for one week. After each exposure specimens were washed in deionized water for 20 seconds and immersed in artificial saliva until the next experimental step. At the end of 7 days, again the micro hardness of the enamel surfaces was measured with the Vickers Indenter at the same specifications.
Change in the pH of artificial saliva following dissolution of lozenges was assessed using Microprocessor pH meter.
Components of cough lozenges were separated and analyzed using Analytical HPLC.
Results: Paired t- test and ANOVA were used for statistical analysis. All experimental groups showed a significant decrease in micro hardness of tooth enamel. Analytical HPLC revealed the complex composition of these lozenges.
Conclusion: Increased consumption of cough lozenges reduces the micro hardness of teeth which may lead to erosion, sensitivity and caries susceptibility.

Keywords: Micro hardness; cough lozenges; dental erosion; analytical HPLC.

1. INTRODUCTION

Cold and cough are extremely common medical conditions. Though innocuous, they can be debilitating and affect the quality of life. Hence, a lot of over the counter (OTC) medications have flooded the market which claim to provide instant and long lasting relief [1].

Many patients report to dental clinics during cold and cough with dental pain or sensitivity. Cold associated with maxillary sinusitis can simulate tooth pain, especially in the upper premolars and molars. This can be attributed to the proximity of maxillary roots to the floor of the sinus [1].

Patients reporting with increased sensitivity should be investigated for consumption of cough lozenges. Consumption of acidic candies decreases the salivary pH to 4.5 along with softening of enamel, which has been shown by various in vivo and in vitro studies [2-5]. This suggests that (excessive) consumption of acidic candies can contribute to the development of dental erosion, especially in individuals with low salivary flow rates and low salivary buffer capacity.

Previous studies have analyzed the sucrose content of cough drops and identified a positive correlation with dental caries [6]. However,

literature search did not reveal any study evaluating the effect of cough lozenges on the properties of teeth; whether they reduce the micro-hardness of the tooth.

The working hypothesis states that OTC cough lozenges with three different chemical formulations affect the micro hardness of teeth.

The aim of the present study was

- 1) To evaluate and compare changes in the micro hardness of enamel after exposure to different cough lozenges.
- 2) To assess the pH of dissolved lozenge solutions, and
- 3) To analyze the number of components in cough lozenges using Analytical HPLC.

2. MATERIALS AND METHODS

Forty freshly extracted human molars were collected with informed consent, cleaned of soft tissues, stored in a solution of 1% chloramine-T at 4°C, and used within one month. The criteria for tooth selection included non-carious, unrestored teeth with no developmental defects and no visible evidence of abnormal enamel cracks.

The study protocol was approved by the Ethical committee of Army College of Dental Sciences, Secunderabad, Telangana.

2.1 Specimen Preparation

The teeth were embedded in self-cured acrylic resin, obtaining 2.0 cm x1.0 cm thick specimens. The exposed buccal surface of enamel was flattened using a diamond disk (Confident Mighty Lab Digi C-108 A) followed by water cooled polishing with silicon carbide papers no. 1200 to 4000. A test area, 3 mm x 2 mm in dimension was marked for micro hardness (Fig. 1).

The samples were randomly divided into 4 groups with 10 samples in each group (n=10) based on the lozenges used (Table 1):

Group 1: Control (Artificial saliva, MP Sai Biomed, Bombay)

Group 2: Lozenge – A (Vicks cough drops, Procter & Gamble, Cincinnati, Germany)

Group 3: Lozenge - B (Koflet- H lozenges, The Himalaya Drug Company, India)

Group 4: Lozenge – C (Strepsils lozenges, Reckitt Benckiser Healthcare (UK) Ltd)

according to manufacturer's guidelines. The specimens were placed on the platform of the tester and stabilized. 10X objective lens was focused on the selected area to indent. Indentations were made at the rate of 50 grams load for 15 seconds. The indentation formed was viewed and the average micro hardness of the specimen was determined from two indentations to avoid discrepancy. The procedure was repeated for all the forty specimens.

Patients generally keep the lozenge in the buccal vestibule till it dissolves. Prior to starting the experiment, the authors dissolved different lozenges in different quantities of saliva and concluded that the average dissolution time was 30 minutes and amount of saliva required, 20 ml. Hence, Lozenges were dissolved in 20 ml fresh artificial saliva for 30 minutes. After complete dissolution, the samples were immersed in the lozenges solution for 30 minutes at room temperature (Fig. 1).

This procedure was carried out four times a day over a span of one week to simulate clinical usage.

2.2 Baseline Evaluation

Baseline Vickers Micro Hardness Number (VHN) were recorded using Vickers Micro Hardness Tester (Buehler, USA). The tests were performed

After each exposure, samples were washed in deionized water for 20 seconds and immersed in fresh artificial saliva to simulate the oral remineralization phase until the next experimental step.

Table 1. Details of cough lozenges used in the study

Materials employed	Composition (According to Manufacture)
Lozenge-A	4.75 mg – Menthol (Pudina)
Lozenge-B	126 mg - Honey 7.31 mg - Chebulic Myrobalan (Haritaki) 0.97 mg - Siamese Ginger (Kulanjana) 0.74 mg – Catechu (Khadira) 2.10 mg - Clove oil (Lavanga) 2.40 mg - Combination of Indian Long Pepper, Black Pepper and Ginger (Trikatu)
Lozenge-C	1.2 mg - 2,4 Dichlorobenzyl alcohol 0.6 mg - Amylmetacresol
Control	2 gm - Methyl-p-hydroxy benzoate 10 gm - Sodium carboxymethylcellulose 0.625 gm - KCl 0.059 gm - MgCl ₂ .6H ₂ O 0.166 gm - CaCl ₂ .2H ₂ O 0.804 gm - K ₂ HPO ₄ 0.326 gm - KH ₂ PO ₄ 1 lt - Distilled water

2.3 Final Evaluation

At the end of 7 days, the samples were rinsed with distilled water and blotted dry. Again the micro hardness of the enamel surfaces was measured with the Vickers indenter as described previously. The values were compared with the initial baseline values to check for change in the micro hardness of enamel after exposure to different cough lozenges (Fig. 1).

The changes in surface micro hardness (SMH) were calculated as change in VHN from the baseline. Comparison of before and after treatment values was done using the paired-t test. Comparison within groups was performed using ANOVA.

2.4 Evaluation of pH

Change in the pH of artificial saliva following dissolution of lozenges was quantitatively assessed using Microprocessor pH meter (Global Electronic, Hyderabad, India) (Fig. 1). Analysis of

different components of cough lozenges using Analytical HPLC: The components of lozenges- A, B and C were separated and subjected to Analytical HPLC (Shimpack Make Shimadzu, 4.6 x 250 mm x 5 μ m, C-18, Birla Institute of Technology and Science (BITS) Pilani, Hyderabad) (Fig. 1).

2.5 Solution Preparation

Cough lozenges were dissolved in 20:80 (v/v) Water:Methanol solution. 200 μ l of the 20:80 solution was further diluted with 800 μ l of methanol.

10 μ l of this solution was filtered using 0.22 μ syringe filters, and injected into the column under specified chromatographic conditions (Table 2).

The analyte peaks were identified by comparison with those of respective standard (methanol) for their retention time and the chromatogram was recorded.



Fig. 1. Procedure (A) Sample preparation, 3 mm x 2 mm enamel window. (B) Cough lozenges immersed in 20 ml of artificial saliva, (C) Exposure of samples to dissolved lozenge mixture (D) Vicker's micro hardness test (E) Evaluation of pH (F) Analytical HPLC

Table 2. Method parameters

HPLC	
Column specification	Shimpack Make Shimadzu, 4.6 × 250 mm × 5 µm, C-18
Column temperature	40°C
Flow rate	1.0 ml/min
Detector	SPD – M20A Diode Array Detector
Injection volume	10 µl
Wavelength	201 nm
Acquisition time	20 min
Pump-A	30% (Distilled water)
Pump-B	70% (Methanol)

3. RESULTS

pH values of control and test solutions were analyzed (Table 3).

Statistical analysis showed that all the experimental groups exposed for a period of 1 week showed significant reduction in the micro hardness of enamel. The mean, standard deviations, standard error mean and statistical differences for each group are presented in Tables 4 and 5.

Results of analytical HPLC are depicted in Fig. 2.

4. DISCUSSION

Teeth are subjected to various physical, chemical and mechanical insults in the course of a single day, yet are structurally and functionally vital [7].

Tooth enamel is the hardest substance in the human body and has a unique combination of hardness and fracture toughness that protects teeth from demineralization [8].

VHN is obtained by dividing the load (in kg) applied to a pyramidal diamond of specific size divided by the projected area of the impression.

$VHN = L/A$, where A= the projected area of the impression in mm^2 and L = the load in kg.

It is especially used to measure hardness of hard and brittle substances such as tooth dentin and enamel. VHN is simple, precise and the minute changes (in the square shaped indent) obtained post treatment can be easily detected [9]. The VHN of enamel is in the range of 270 to 360 [9]. We used VHN as a measure of the decision in the micro hardness of enamel after exposure to cough lozenges solution.

Cough drops have two categories of ingredients. The major portion is made up of sugar, corn syrup, acids, colors, and flavors. Strepsils (honey and lemon) flavored lozenges contain two active ingredients, amylmetacresol and dichlorobenzyl alcohol. Both of these are mild antiseptics that kill the bacteria associated with mouth and throat infections. Koflet-H contains honey (Madhu) and clove (*Syzygium aromaticum*) which are used to treat cough, due to their antitussive and anti-inflammatory properties and soothing effect on the respiratory tract. Vicks mainly contains menthol, which is typically isolated from the *Mentha arvensis* plant or distilled from peppermint oil. It has a cooling effect in the mouth that helps relieve irritation and also works as an expectorant.

The chemical composition of OTC cough lozenges varies. However, sensitive teeth are common to all of them. Three commonly used cough drops, with different chemical composition were dissolved in artificial saliva (pH-6.75) and the pH of the resultant mixture was recorded. The pH values were acidic.

Since acidogenic compounds occurring in cough lozenges consist of multi-component mixtures, their separation and determination is challenging.

Analytical HPLC of cough lozenges was carried out to confirm that they had one or two components as listed by the manufacturers to which the erosive effect could be attributed.

Table 3. pH values of control and test solutions analyzed using microprocessor pH meter

Groups	pH values
1) Control	6.75 ± .02
2) Lozenge-A	4.92 ± .02
3) Lozenge-B	4.75 ± .02
4) Lozenge-C	2.39 ± .02

Table 4. Comparison of VHN of enamel on day 1 (Pre- treatment) and day 7 (After- treatment) in four groups using paired t-test

Groups	Mean VHN value ± SD (Pre- treatment)	Mean VHN value ± SD (After- treatment)	t- test	p- value
1) Control	384.10 ± 22.02	387.70 ± 21.8	-3.443	0.007
2) Lozenge-A	380.90 ± 21.4	122.50 ± 21.6	22.600	0.000*
3) Lozenge-B	377.00 ± 23.8	117.14 ± 18.3	24.706	0.000*
4) Lozenge-C	327.90 ± 49.6	99.52 ± 3.8	14.379	0.000*

*The mean difference is significant at the 0.05 level

Table 5. Pairwise comparison of VHN of four groups on day 7 (After- treatment) using Bonferroni Post Hoc test

Groups (G)	Mean difference in VHN value ± Standard Error (After-treatment)	p – value (After – treatment) (p – value -0.05)
G1 x G2	265.20 ± 20.3	0.000*
G1 x G3	270.56 ± 22.3	0.000*
G1 x G4	288.18 ± 20.1	0.000*
G2 x G3	5.36 ± 22.5	1.000
G2 x G4	22.98 ± 21.9	0.042*
G3 x G4	17.62 ± 19	0.209

* The mean difference is significant at the 0.05 level

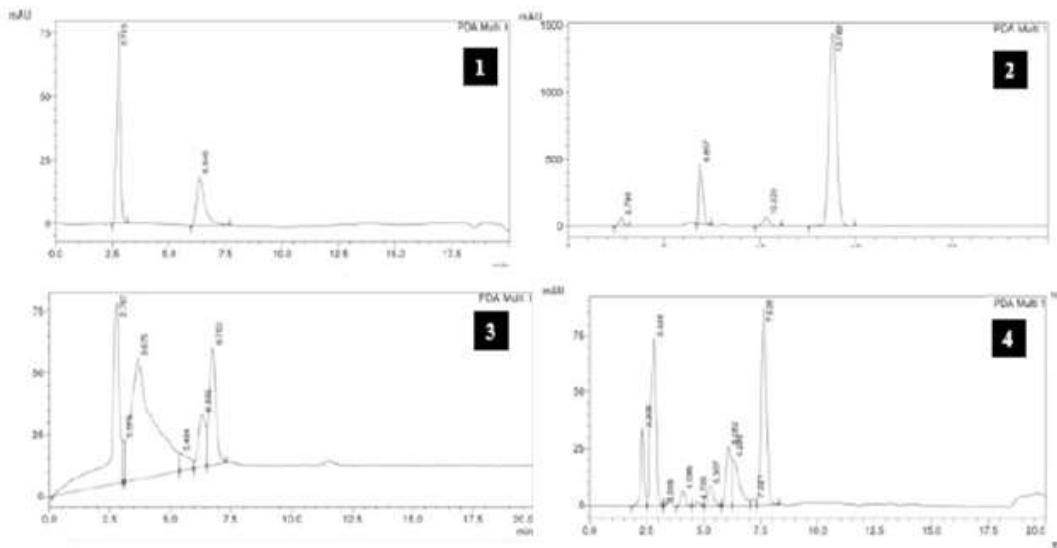


Fig. 2. (1) Standard chromatograph of Methanol (standard) (2) Standard chromatograph of Lozenge-A (3) Standard chromatograph of Lozenge-B (4) Standard chromatograph of Lozenge-C

HPLC utilizes the fact that different compounds have different migration rates at a particular column and mobile phase.

Analytical HPLC revealed multiple peaks for all the study samples. This points to the multicomponent and indeterminate composition of these lozenges.

However, ayurvedic formulations do not lend themselves to distinct chemical profiling making isolation challenging. The chief constituents of the ayurvedic mixtures, may themselves yield different peaks. Therefore, the erosive effect cannot be attributed to any single component using analytical HPLC.

There is significant reduction in enamel micro hardness under acidic challenge [2,10-13]. Acidic exposure for 5 days causes erosion [14]. In this study, there was a drastic dip in the hardness with exposure to acidic lozenges for a mere two hour window for one week. Though the samples were rinsed and stored in artificial saliva (pH-6.75) to simulate remineralization after exposing the teeth to cough lozenges, [15] this did not seem to be able to overcome the demineralization. This can probably be attributed to the presence of sodium carboxymethyl cellulose (CMC) which enhances the saliva's viscosity, lowering the salivary flow and forms complexes with calcium and/or phosphate ions making them unavailable for remineralization of the lesions [16].

In the oral cavity, remineralization is a more dynamic process and may minimize damage from lozenge consumption. This is due to the buffering capacity of saliva. It contains calcium, phosphate and fluoride ions which neutralize the acid after an acidogenic challenge and maintains enamel surface integrity [17,18].

The findings of the present study were established in laboratory conditions and can be furthered by studying a large population consuming cough lozenges regularly. The results of the present study, if validated by in vivo evaluation, can be shared with pharmaceutical companies to prompt the identification of the acidogenic components. If they are not important as antitussives, suitable alteration in the composition of the lozenges may be beneficial in preserving enamel. Alternatively the lozenges can carry usage guidelines.

5. CONCLUSION

Over the counter cough lozenges with varied chemical composition are acidogenic with detrimental effect on the micro hardness of enamel and may lead to erosion, sensitivity and caries susceptibility.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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