A Comparative Evaluation Of The Changes In Plaque pH Before And After Eating Chocolates In Children.

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**Aim:** The aim of the study was to evaluate and compare the pH response of dental plaque before and after consumption of different types of chocolates in children. **Methodology:** This *in vivo* study was done on 300 (Three hundred) children which compared the acidogenicity of different commercially available Chocolate bars by evaluating the pH of plaque at different time intervals taken at baseline, immediately and 30 minutes after eating chocolates using a digital pH meter. **Results:** In all chocolates, the mean plaque pH showed a linear decrease with time and the decrease was evident highest in Dairy Milk Fruit & Nut followed by KitKat, Milkybar, 5 Star and Dairy Milk being the least. (Dairy Milk < 5 Star < Milkybar < KitKat < Dairy Milk Fruit & Nut). **Conclusion:** i) Plaque pH drop was seen in all the tested chocolates and was above the critical pH (5.7-5.5), ii) Cadbury Dairy Milk was found to be the least acidogenic among all the five chocolate bars, iii) Cadbury Dairy Milk Fruit & Nut was found to be the most acidogenic among all chocolates, iv) Filled chocolates (fruit & nuts and wafer) exhibited more acidogenicity than others.
INTRODUCTION:
Dental caries is a chronic multifactorial disease of microbial origin. Bacteria are responsible for dental caries and converts sucrose into by-products, one of which is acid. Glucose and fructose can also be converted to acid. The most common acid produced during this process is lactic acid which is responsible for pH drops in the plaque. The pH value at which enamel begins to dissolve (“critical pH”) is not known, although widely assumed. Different estimates of “critical pH” have ranged from 5.7 to 5.5 or lower. If the pH falls below the critical pH, demineralization of tooth enamel occurs, leading to carious lesions. Cariogenicity in studies have been related to plaque acidogenicity, which has been studied by measuring dental plaque pH. Modern diets of urban populations contain a wide range of processed foods, refined flours, foods consisting of mixtures of starch and sugars and foods with a soft consistency. The cariogenic potential of starch containing food products seems to be influenced by a large number of factors such as the processes used for manufacturing these products, the frequency of consumption, different tendencies for retention on the dentition, presence of caries-promoting or inhibitory ingredients, and the interactions with salivary amylase. It is obvious that it is impossible to standardize food consumption. Food preferences differ from person-to-person and from society-to-society. In general, people throughout the world, especially children, like to eat chocolates, sweets and snack foods. Foods that we consume may activate dental caries which has led to a great deal of research on diet. Chocolate is an accessible luxury that we treat ourselves for personal gratification. It has undergone a significant transformation since its origin, going from being a simple drink consumed by indigenous people to a specialty product. In the 18th century, Carolus Linnaeus, who was a Swedish botanist, changed the name of cocoa tree giving it the Greek name Theobroma Cacao, which is now the official botanical name that literally means "Food of the Gods". Chocolate is consumed all over the world, and the largest chocolate manufacturers are based in North America and Europe. Top consumers are the Swiss (8.8 kg/year/per capita), and bottom level consumers are the Chinese (100 g per year/per capita). The average chocolate consumption per capita in India is 100g to 200g per person and ranks sixth worldwide in the chocolate consumption. It is apparent that while the consumption of chocolate by children is widespread, it is also highly variable and probably increasing along with increasing snacking activity in general. A survey by the British consumer magazine in 2007 found that children were most likely to spend their pocket money on snacks, including sweets, chocolates, and crisps. According to a study by Naveed S et al in 2015, total 145 children of age 5-10 years were questioned about their chocolate consumption habit. Twelve (12) of them answered that they do not take chocolate due to its bitter taste, 93 children answered that they eat 1 to 3 chocolates daily, 25 children answered that they take 4 to 6 chocolates daily, 8 children answered that they take 7 to 9 chocolates daily, while 7 children answered that they take more than 10 chocolates daily. In a longitudinal study of 5-11 year old girls in the United States, it was found that chocolate was ranked highest, along with Skittles, cookies and ice cream, and preferences remained relatively stable as the children grew up. A longitudinal study of French children reported that chocolate was nominated by 84–86% of children as one of their ten favorite foods. Also, in Edinburgh and Liverpool, children nominated chocolate as among their favorite foods. The popularity of chocolate in India is also linked to its affordability. The chocolate market is dominated by global giants Mondelez and Nestle, which sell local favourites such as Cadbury Dairy Milk, Cadbury Dairy Milk Fruit & Nut, Cadbury 5 Star, Nestle KitKat and Nestle Milkybar and many more. In India most of the parents pamper their children by giving chocolates in tiffins thinking that their son/daughter will happily eat their school meals. Also the secret to their popularity is that they are inexpensive chocolate products and priced as low as Rs.5 for a bar to appeal to value-conscious consumers. Mintel says; the most popular chocolates in India are those sold in Rs.5 and Rs.10 packages. Comparing the acidogenic potential of different commercially available chocolates may help a
Pediatric dentist to give an authenticate advice to parents. Thus the aim of this in-vivo study was to evaluate and compare the pH response of dental plaque before and after consumption of different types of chocolates in children.

MATERIALS AND METHODS
This study was performed in the outpatient department of Paediatric and Preventive Dentistry, Career Postgraduate Institute of Dental Sciences and Hospital (CPGISD), Lucknow. The study design and protocol was analyzed and approved by the Institutional Ethics Committee of CPGIDS, Lucknow. The procedure to be performed was explained to the parents/guardians and a written consent in Hindi/English was obtained from them. Three hundred (300) children of both male and female were recruited in the study over a period of six months. The children included in the study were healthy children aged between 5-10 years and medically compromised children were excluded in the study.

METHODOLOGY:
Test groups:
Children were randomly assigned to the five groups of commercially available chocolate bars to be tested as shown below: (Figure 1)
- Group A: Dairy Milk (mfd. by Cadbury, India)
- Group B: Dairy Milk Fruit & Nut (mfd. by Cadbury, India)
- Group C: KitKat (mfd. by Nestlé, India)
- Group D: Milkybar (mfd. by Nestlé, India)
- Group E: 5 Star (mfd. by Cadbury, India)

Plaque Collection: (Figure 2)
At the onset, procedure was explained to the children and parents/guardians. Children participating in the study were asked to refrain from toothbrushing at least for 24 hours and from eating or drinking (apart from water) at least 2.5 hours prior to each visit. For each child on the test day, pooled plaque sample was removed from six buccal surfaces of maxillary and
mandibular posterior teeth representing all the quadrants of the mouth, using a sterile blunt explorer. Fosdick (1957)\textsuperscript{[10]} method was used for estimation of plaque pH. For each child three plaque samples were collected, at baseline (before consumption of the chocolate bar), immediately after consumption of the chocolate bar and at 30 minutes interval from the time of consumption of the chocolate bar. Each plaque sample was thoroughly mixed with 20 ml of distilled water, measured by a pipette into a disposable sample container until it dissolved and was measured for plaque pH.

**Figure 2: Plaque Collection**

*Determination of plaque pH:*
The plaque pH was measured by using portable standard digital pH meter with glass microelectrode (\textit{JSE Digital pH Meter}) (Figure 3). The head of pH bulb was completely immersed into the sample for each of the plaque samples. The values displayed digitally were recorded after the fluctuations in the reading stopped. After recording the baseline plaque pH, each child was provided with the respective chocolate bar (about 13 grams) and instructed to eat the chocolate for 5 minutes to standardize the consumption time. The child was monitored to consume the chocolate completely without sharing. The second plaque sample was collected and assessed for plaque pH immediately after the consumption of chocolate bar. The third plaque sample was collected at 30 minutes interval from the time of consumption of the chocolate bar and assessed for plaque pH. The electrode was cleaned with a stream of distilled water between each measurement. Calibration of pH meter (\textit{JSE Digital pH Meter}) was done using buffer solutions with pH 4.0 and 7.0, prepared using the buffer tablets (\textit{Thermo Fisher Scientific India Pvt. Ltd, Powai, Mumbai, India}), each time before the commencement of experiment. Similar procedure was followed in the estimation of the plaque pH for each of the test groups. Thus, the data obtained for each group was tabulated and statistically analyzed. Analysis was performed on SPSS software (Windows version 22.0).
OBSERVATIONS AND RESULTS
The present study evaluates and compares the change in plaque pH before and after eating chocolates in children. Total three hundred (300) healthy children aged between 5-10 years were recruited in the study. The children were randomized equally into 5 groups (i.e. 60 children per group) and treated (i.e. eating of chocolates) with five different chocolates namely Dairy Milk, Dairy Milk Fruit & Nut, KitKat, Milkybar and 5 Star. The outcome measure of the study was plaque pH assessed before and after eating chocolates (immediate and after 30 minutes) measured using the technique of Fosdick (1957). Each child was subjected to about 13 grams of chocolates.

Plaque pH
The pre (baseline) and post (immediate and after 30 minutes) plaque pH of five different groups (Dairy Milk, Dairy Milk Fruit & Nut, KitKat, Milkybar and 5 Star) is summarised in Table 1 and also depicted in Graph 1.

Table 1: Pre and post plaque pH of five groups over the periods.

<table>
<thead>
<tr>
<th>Chocolates</th>
<th>Baseline (n=60)</th>
<th>Immediate (n=60)</th>
<th>After 30 minutes (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Milk</td>
<td>6.40 ± 0.37</td>
<td>6.27 ± 0.35</td>
<td>6.09 ± 0.32</td>
</tr>
<tr>
<td>Dairy Milk Fruit &amp; Nut</td>
<td>6.87 ± 0.16</td>
<td>6.25 ± 0.25</td>
<td>5.82 ± 0.82</td>
</tr>
<tr>
<td>KitKat</td>
<td>6.74 ± 0.26</td>
<td>6.33 ± 0.24</td>
<td>5.93 ± 0.21</td>
</tr>
<tr>
<td>Milkybar</td>
<td>6.48 ± 0.34</td>
<td>6.18 ± 0.23</td>
<td>5.87 ± 0.28</td>
</tr>
<tr>
<td>5 Star</td>
<td>6.51 ± 0.30</td>
<td>6.22 ± 0.27</td>
<td>5.95 ± 0.24</td>
</tr>
</tbody>
</table>

The pre and post plaque pH of five groups is summarised in Mean ± SD.
The plaque pH in Dairy Milk at baseline, immediate and after 30 minutes ranged from 5.05-7.00, 5.00-6.95 and 5.00-6.90 respectively with mean (± SD) 6.40 ± 0.37, 6.27 ± 0.35 and 6.09 ± 0.32 respectively and median 6.40, 6.30 and 6.08 respectively. In Dairy Milk Fruit & Nut, it ranged from 6.50-7.10, 5.70-6.79 and 5.10-6.20 respectively with mean (± SD) 6.87 ± 0.16, 6.25 ± 0.25 and 5.82 ± 0.82 respectively and median 6.90, 6.22 and 5.90 respectively. In KitKat, it ranged from 6.21-7.23, 5.90-6.88 and 5.40-6.50 respectively with mean (± SD) 6.74 ± 0.26, 6.33 ± 0.24 and 5.93 ± 0.21 respectively and median 6.68, 6.35 and 5.93 respectively. In Milkybar, it ranged from 5.90-7.08, 5.80-6.70 and 5.20-6.90 respectively with mean (± SD) 6.48 ± 0.34, 6.18 ± 0.23 and 5.87 ± 0.28 respectively and median 6.50, 6.10 and 5.90 respectively. In 5 Star, it ranged from 6.00-7.23, 5.80-6.90 and 5.40-6.55 respectively with mean (± SD) 6.51 ± 0.30, 6.22 ± 0.27 and 5.95 ± 0.24 respectively and median 6.51, 6.12 and 5.93 respectively.

In all chocolates, the mean plaque pH showed linear decrease with time and the decrease was evident highest in Dairy Milk Fruit & Nut followed by KitKat, Milkybar, 5 Star and Dairy Milk being the least. (Dairy Milk < 5 Star < Milkybar < KitKat < Dairy Milk Fruit & Nut)

At final evaluation, the net mean decrease (i.e. mean change from baseline to after 30 minutes) in plaque pH of Dairy Milk Fruit & Nut was found to be the maximum (15.29%) followed by KitKat (11.95%), Milkybar (9.42%), 5 Star (8.59%) and Dairy Milk showed the minimum (4.79%) (Table 2 and Graph 2). Moreover, the net mean decrease in plaque pH of Dairy Milk Fruit & Nut was found 10.50, 3.34, 5.87 and 6.70% higher respectively as compared to Dairy Milk, KitKat, Milkybar and 5 Star.

Graph 1: Pre and post mean plaque pH of five groups over the periods.

The plaque pH in Dairy Milk at baseline, immediate and after 30 minutes ranged from 5.05-7.00, 5.00-6.95 and 5.00-6.90 respectively with mean (± SD) 6.40 ± 0.37, 6.27 ± 0.35 and 6.09 ± 0.32 respectively and median 6.40, 6.30 and 6.08 respectively. In Dairy Milk Fruit & Nut, it ranged from 6.50-7.10, 5.70-6.79 and 5.10-6.20 respectively with mean (± SD) 6.87 ± 0.16, 6.25 ± 0.25 and 5.82 ± 0.82 respectively and median 6.90, 6.22 and 5.90 respectively. In KitKat, it ranged from 6.21-7.23, 5.90-6.88 and 5.40-6.50 respectively with mean (± SD) 6.74 ± 0.26, 6.33 ± 0.24 and 5.93 ± 0.21 respectively and median 6.68, 6.35 and 5.93 respectively. In Milkybar, it ranged from 5.90-7.08, 5.80-6.70 and 5.20-6.90 respectively with mean (± SD) 6.48 ± 0.34, 6.18 ± 0.23 and 5.87 ± 0.28 respectively and median 6.50, 6.10 and 5.90 respectively. In 5 Star, it ranged from 6.00-7.23, 5.80-6.90 and 5.40-6.55 respectively with mean (± SD) 6.51 ± 0.30, 6.22 ± 0.27 and 5.95 ± 0.24 respectively and median 6.51, 6.12 and 5.93 respectively.

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Table 2: Net mean decrease in plaque pH (%) of five different chocolates from respective baseline to after 30 minutes.

<table>
<thead>
<tr>
<th>Chocolates</th>
<th>Net mean decreases in plaque pH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Milk</td>
<td>4.79</td>
</tr>
<tr>
<td>Dairy Milk Fruit &amp; Nut</td>
<td>15.29</td>
</tr>
<tr>
<td>KitKat</td>
<td>11.95</td>
</tr>
<tr>
<td>Milkybar</td>
<td>9.42</td>
</tr>
<tr>
<td>5 Star</td>
<td>8.59</td>
</tr>
</tbody>
</table>

Further, of total mean decrease in plaque pH, Dairy Milk Fruit & Nut showed maximum decrease (30.5%) followed by KitKat (23.9%), Milkybar (18.8%), 5 Star (17.2%) and Dairy Milk the minimum (9.6%) (Graph 3).
DISCUSSION
The outcome measure of this study was plaque pH assessed before (baseline) and after eating chocolates (immediate and after 30 minutes). According to Stephan curve, the pH starts returning to baseline within 40 minutes, therefore time interval chosen was baseline, immediate and after 30 minutes.\(^{[11]}\) Prior to consumption of test chocolate bars resting plaque pH was recorded to provide baseline values against which the rise and drop in pH could be evaluated. The overall baseline values measured were in the range of 6.40 to 6.87. The results tend to confirm previous reports of plaque pH testing showing a subject-to-subject variation in response to test chocolates as individuals in a population differ considerably in plaque pH due to variation in caries susceptibility.\(^{[12]}\) In the current study, the mean plaque pH showed linear decrease with time in all chocolates and the decrease was evident highest in Dairy Milk Fruit & Nut followed by KitKat, Milkybar, 5 Star and Dairy Milk the least after 30 minutes. (Dairy Milk < 5 Star < Milkybar < KitKat < Dairy Milk Fruit & Nut)

The probable reason of Dairy Milk Fruit & Nut being the most acidogenic could be due to the longer retention ability of the fillings (raisins and almonds) and also could be attributed to the sucrose concentration of the fruits which additively makes it more acidogenic. These results were in accordance with a study done by Hegde A et al in 2009\(^{[13]}\), who also found that the chocolate with fruits and nuts showed the maximum drop in pH from the 15-30 minutes period when compared with caramel and milk chocolate. However; the results of a study done by Nirmala SVSG in 2016\(^{[14]}\), showed a maximum drop of pH with wafer chocolate (5.58) than fruit and nuts chocolate (5.74) at 30 minutes when the filled chocolates (chocolates with fruits and nuts and wafer) were compared, suggesting that chocolate with wafer was more acidogenic than fruit and nuts chocolate.

In this study, Dairy milk showed significant fall in the pH but less than that of KitKat and Dairy Milk Fruit & Nut, this could be due to the fact that it contains less amount of cocoa solids (4.4%) as compared to other chocolates, thus masking the anticariogenic properties of cocoa\(^{[15]}\) and also it contains refined flour (maida) making its texture sticky followed by lesser clearance from the tooth structure enabling it to be more retentive and therefore possibly more acidogenic.

In this study, Milkybar had a drop in plaque pH but less than that of KitKat and Dairy Milk Fruit & Nut making it less acidogenic. As Milkybar contains high amount of milk solids (26.9%) in comparison to other chocolates, its relatively less acidogenicity can be supported by the fact that milk contains casein which is a milk phosphoprotein that stabilizes calcium and phosphate ions within the tooth, making it least susceptible to the process of demineralization.\(^{[14]}\)

5 Star (caramel filling) showed a second least drop in plaque pH as compared to other chocolates except Dairy Milk. This could be due to the reason that chocolate caramel bars although exhibits high initial retention rates
has a very rapid rate of clearance from the teeth.\textsuperscript{[16]} It also contains whey powder (cow\'s milk), which is composed mainly of lactose - a milk sugar. Based on studies, lactose-milk sugar has been shown to be less acidogenic than other sugars which could also be another probable reason for 5 Star to be relatively less acidogenic in the present study.\textsuperscript{[17]} In a contrast, a study done by Vasanthakumar AH \textit{et al} in 2016 \textsuperscript{[18]}, found caramel chocolate to be more acidogenic as it showed maximum decrease in plaque pH at 20 minutes after consumption when compared to the other types of chocolates. (milk, white, dark chocolate and sucrose solution) Dairy Milk showed the least drop in plaque pH making it to be least acidogenic among all other chocolate bars tested in the study. This could be attributed to its better oral clearance as it does not contain any additional constituents like dried fruits & nuts, caramel fillings and wafers which are more retentive in comparison to other chocolates. However, \textit{Pearce EI and Hampton ML in 1987} \textsuperscript{[19]}, being among the few to describe the tests products with their commercial names, found milk chocolate (Cadbury\’s Dairy Milk) to be of intermediate cariogenicity in-vitro. The American Academy of Pediatric Dentistry states that \"There is evidence that food containing milk casein, calcium, phosphorus and cocoa, all of which are found in chocolate, may be less likely to contribute to dental caries than sucrose alone or other snack foods.\"\textsuperscript{[20]} Overall the effect of test chocolates on plaque pH over the time, ANOVA showed significant ($p < 0.01$ or $p < 0.001$ respectively) effect of both groups (i.e. chocolates) and periods (i.e. time). Further, the interaction effect of both groups and periods (Group*Period) on plaque pH was also found to be significant. For each group (i.e. chocolates), comparing the difference in mean plaque pH between periods (i.e. intra group), Tukey test showed significant ($p < 0.05$ or $p < 0.001$) decrease in plaque pH at both immediate and after 30 minutes as compared to baseline in all groups. Furthermore, in all groups, it also decreased significantly ($p < 0.001$) at after 30 minutes as compared to immediate. The literature showed lesser number of studies pertaining to plaque pH with the chocolates tested in this study; therefore more comparisons couldn\'t be made.

**CONCLUSION**

Based on the results of the present study it was concluded that :

1. Plaque pH drop was seen in all the tested chocolates and was above the critical pH (5.7-5.5).
2. Cadbury Dairy Milk was found to be the least acidogenic among all the five chocolate bars.
3. Cadbury Dairy Milk Fruit & Nut was found to be the most acidogenic among all chocolates.
4. Filled chocolates (fruit & nuts and wafer) exhibited more acidogenicity than others.
5. Although chocolates are one of the commonly consumed snacks worldwide, it should be remembered that they are not entirely safe for teeth and frequent consumption by children of any such food containing fermentable carbohydrates should be avoided. However, if consumed sensibly with adequate oral hygiene measures consumption of these can be considered.
6. In addition to plaque pH evaluation, other factors like type of sugars and other components present in different chocolate brands, dissolubility in saliva and wettability to tooth surfaces need to be investigated to arrive at a definitive conclusion.

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