# Effect of Green Tea Varnish on the Salivary Parameters of Children Exposed to Second Hand Tobacco Smoke

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**Abstract:**
**Importance: Children are the most vulnerable group exposed to SHS and green tea has been explored for its beneficial effects on improvement of salivary parameters and prevention of oral diseases.**
**Objective: The aim of this study wasto evaluate the effect of green tea varnish on the salivary parameters among primary school children aged 10-120 exposed to second hand tobacco smoke.**
**Design: The study was conducted using an intervention study (one group pretest-posttest design).**
**Participant: Stimulated saliva was collected from the 60 children of grade 6 students exposed to SHS at the baseline and after one, three and six months after green tea varnish application. Salivary flow rate, pH, buffering capacity and IgA increased significantly after one and three months of follow up.**
**Results: There were statistical significance differences between baseline and one and three months. values. Concerning TAC, the median value increased after one month. This increase was statistically significant (p = 0.00). Streptococcus mutans count decreased significantly after one and three months. Lactobacilli count decreased significantly after one, three and six months.**
**Conclusion: Green tea varnish has a beneficial effect on salivary parameters of children who were exposed to SHS and succeeded to overcome the parameters variations between children included in the intervention study.**
**Key words: Saliva, Green tea, Varnish**

## Introduction:

Second hand tobacco smoke is correlated with the increase of childhood dental caries as a result of salivary glands impairment and change in oral bacteria. Children who live in homes with parents who smoke have more decayed, missed or filled teeth due to caries compared with children living with parents who never smoked (González-Valero et al., 2018). Also, there is a significant relation between SHS and periodontal diseases, which are pathological conditions of supporting structures of the teeth; gingiva, alveolar bone and periodontal ligaments (Karsiyaka Hendek et al., 2019). Also, it is well known that head and neck cancers are more prevalent in adults exposed to SHS during their childhood (Mariano et al, 2021). Second hand tobacco smoke damage the protective effect of human saliva by decreasing the salivary flow rate, lowering the salivary pH and buffering capacity, decreasing the level of salivary immunoglobulins, and increase the proliferation of bacteria, which results in subsequently an increased risk of oral diseases (Rezaei & Sariri, 2011). Saliva of children exposed to SHS contains cotinine which is the active metabolite of nicotine. The half-life of cotinine (16-19 hours) is longer than that of nicotine (1-4 hours), so, measurement of cotinine level is a suitable and reliable screening tool for determination of exposure to SHS (Erdemir et al., 2010). Green tea has been investigated in 2010 for its beneficial effects on prevention of diseases, and this is because it has a protective effect from bacteria and viruses (Chacko et al., 2010). Green tea polyphenols, especially catechin, theanine and amino acid with their antioxidant properties have the ability to improve the salivary parameters and consequently protect oral diseases such as dental caries, gingivitis, periodontitis, halitosis, oral inflammations and oral cancers (Masoumi et al., 2016).

**The aim of this study was to** evaluate the impact of green tea varnish on the salivary parameters among governmental primary school children aged 10-12 years exposed to second hand tobacco smoke.

## Subject and Methods:

The approval of the Ethics Committee of the High Institute of Public Health for conducting the research was taken.
The study was conducted using an intervention study (one group pretest-posttest design), among children aged 10-12 years who exposed to SHS. 60 children were chosen randomly from governmental primary school children in Alexandria. The following school children were excluded from the study:Smokers, Children not exposed to smoking, systemically unhealthy children, Children taking additional fluoride prophylaxis, other than the use of fluoridated tooth paste, Children with history of antibiotic therapy within the previous 3 months and Children taking antihistaminic. A written consent was obtained from the guardians of the study participants after explanation of the purpose and benefits of the study. Using Sample Size Calculators, based on the effect size of the mean difference of streptococcus mutans count which was 0.4 (Thomas et al., 2017), a 5% alpha error, a 0.80 power and a standard deviation of the change in the outcome of 1. The minimum required sample was 49 and was rounded to 60 students.

A chart was used to collect the required data of salivary parameters from the participating children. The examined salivary parameters included salivary flow rate, pH, buffering capacity, total protein, IgA, TAC, cotinine level. Collection of salivary samples were done by giving instructions to children not to eat or drink and not to brush their teeth or use any mouth washes for two hours prior to the saliva collection. Saliva samples were collected during the school hours between 9 and 11 am. The whole saliva was stimulated by chewing free sugar gum for 30 seconds and saliva collected during first 10 seconds was discarded. Saliva was then collected by asking the children to expectorate into sterile graduated glass jar for 5 minutes (Yamuna Priya & Muthu Prathibha, 2017). Flow rate was calculated immediately by the researcher through dividing the total volume of saliva collected (ml) by the period of collection (5 min)(Shitsuka et al., 2018). The children were asked to continue expectoration till the saliva reaches 5 ml. The collected saliva for each child was divided into 2 sterile cyrotube, using a sterile disposable syringe. (4 ml of the saliva for biochemical analysis and 1 ml for microbiology). The cyrotubes containing saliva were placed in ice boxes.All salivary samples were identified by a code number that was identical to the code used on salivary assessment chart.

***Green tea varnish preparation and application:***

To prepare 5% green tea varnish, 100 mg of the epigallocatechin-3-gallate (EGCG) powder was added to 2 ml of ethyl cellulose and ethanol in a 1:1 ratio. After 1 hour of sonication for homogenization, the varnish was refrigerated at 2 to 5°C in a dark container till use, the green tea varnish was prepared according to a study which confirmed its concentration as that for fluoride varnish (Duraphat 5% fluoride)(Daneshyar et al., 2018). Green tea varnish was fabricated in Pharmacognosy Department, Faculty of Pharmacy, Alexandria University. The application of the varnish was done once after baseline assessment of salivary parameters, the assessment was repeated after 1, 3 and 6 months. Participated children were asked to maintain their regular oral hygiene practice during the six months of the study period. Dental prophylaxis was performed with a bristle brush, the teeth were dried and the varnish was applied on all the surfaces of the teeth with the aid of a brush, then the researcher waited for five minutes for the evaporation of the solvent and finally the children were instructed not to eat or drink for half an hour, and not to brush the teeth for 12 hours(da Silva et al., 2012).

***Statistical analysis:***

The collected data were revised, coded and analyzed using SPSS version 20 software (Kirkipatric & Feeney, 2013). Significance of the obtained results was judged at 5% level. The given graphs were constructed using Microsoft Excel software. Categorical variables were summarized by frequency and percent.All variables were tested for normal distribution using the Shapiro–Wilk test.Quantitative data were described by median as measures of central tendency, and IQR as measures of dispersion. Non parametric Friedman ANOVA test was used to identify the significant difference among the four assessment periods When the Friedman test yielded a significant result, the Wilcoxon signed ranks post hoc test was used for pairwise comparisons.

**Results:**

Table (1) shows the distribution of children by their characteristics. It appears from the table that the age of the studied children ranged between 10 and 12 years with a mean of 11.2±0.026 years. More than three quarters of children (78.3%) were aged 10 to less than 11 years. Children aged 11-12 constituted 21.7% of the sample. Regarding gender, females constituted more than half (55%) of the sample.Regarding the number of cigarettes, the primary school children were exposed to at their homes/day.Children who were exposed to less than 10 cigarettes constituted 31.7%, those who were exposed to 10-20 cigarettes were 15.0% and about half (53.3%) of the children were daily exposed to more than 20 cigarettes at their homes.

**Table (1): Distribution of the children included in the intervention study**

| **Characteristics** | **No. (n= 60)** | **%** |
|---|---|---|
| **Age** | | |
| 10- | 47 | 78.3 |
| 11-12 | 13 | 21.7 |
| **Mean±SD:** | **11.2±0.02** | |
| **Gender** | | |
| Male | 27 | 45 |
| Female | 33 | 55 |
| **No. of cigarettes** | | |
| < 10 | 19 | 31.7 |
| 10-20 | 9 | 15.0 |
| > 20 | 32 | 53.3 |

Table (2) showsthe median value of the stimulated flow rate was 2.30,0.40-3.30 at baseline before the application of green tea varnish. It increased after one month to be 3.30, 2.30-4.30, and then there was a slight decrease after three months of follow up (2.60, 2.20-4.00). After six months of follow up, the flow rate decreased (2.30, 0.60 -4.00) to be similar to the baseline. There was a statistical significance difference between baseline versus one- and three-months' values (p = 0.000), while there was no statistical difference between the

stimulated flow rate at baseline and after six months of follow up (p =0.99). Regarding pH, the median value of the salivary pH was 6.38, 4.50-7.75 at baseline, then increased after one month of green tea varnish application (7.60, 6.80-7.94). It slightly decreased after three months (7.50, 5.90-7.93), and then decreased again after six months (6.70, 4.50-7.97) to be nearly the same as the baseline value. The difference between baseline and after one and three months was statistically significant (p = 0.000). There was no statistically significance difference between the baseline and after six months of follow up (p = 0.68). Concerning the buffering capacity, the median value at baseline was 1.20, 0.40-2.00, then increased to 1.79, 1.00-2.50 after one month, then began to decrease after three months (1.50, 0.80-2.50) to became nearly the same as the baseline after six months of follow up (1.25, 0.60-2.00). There was a statistically significant increase between baseline and both after one and three months of follow up (p = 0.001), while there was no statistically significant difference between the buffering capacity of saliva at the baseline and after six months of follow up (p = 0.68). There was no statistically significant change regarding the median value salivary total protein between the baseline (1.20, 0.30-1.90) and the three follow up periods (1.15, 0.20-1.70), (1.16, 0.20-1.90) and (1.20, 0.20-1.90) respectively (p= 0.085). As regards IgA, the median value at the baseline was 882.5, 52.0-1146.5, then increased after one month (998.5, 872.0-1881.5), as well as after three months (992.0, 655.0-1829.0). It then decreased after six months (900.5, 89.1-1103.0) to be nearly as the baseline. There was a statistically significant difference between the baseline IgA medians and after one and three months (p = 0.00), but there was no statistically significant difference between baseline and after six months of follow up (p = 0.12).

Concerning TAC, the median value at baseline was 0.23, 0.00-0.67, then it increased significantly after one month (0.83, 0.11-2.39) (p = 0.00). TAC decreased at three (0.30, 0.07-1.40) and six months (0.33, 0.05-0.77) andthere was no statistically significance difference between the baseline level of TAC and after three (p = 0.15) and six months (p = 0.89) of follow up. Regarding cotinine level at the baseline, it was 30.00, 20.50-66.50. Salivary cotinine decreased after one, three and six months to be 23.50, 12.00-38.00, 24.00, 15.00-31.50 and 25.25, 20.00-37.50 respectively, but this difference was not statistically significant (p = 0.079).

**Table (2): Median values of biochemical salivary parameters at the baseline and throughout the follow up periods**

| Biochemical parameters | **Baseline** Median (IQR) | **Month 1** Median (IQR) | **Month 3** Median (IQR) | **Month 6** Median (IQR) | **Sig** |
|---|---|---|---|---|---|
| **Flow rate (ml/min)** | 2.30 (0.40 -3.30) | 3.30 (2.30 -4.30) | 2.60 (2.20 -4.00) | 2.30 (0.60 -4.00) | 0.001* |
| **Sig** | | 0.000+ | 0.000+ | 0.99 | |
| **pH** | 6.38 (4.50-7.75) | 7.60 (6.80-7.94) | 7.50 (5.90-7.93) | 6.70 (4.50-7.97) | 0.001* |
| **Sig** | | 0.000+ | 0.000+ | 0.68 | |
| **Buffering capacity (mg/dl)** | 1.20 (0.40-2.00) | 1.79 (1.00-2.50) | 1.50 (0.80-2.50) | 1.25 (0.60-2.00) | 0.001* |
| **Sig** | | 0.001+ | 0.001+ | 0.68 | |
| **Protein (g/dl)** | 1.20 (0.30-1.90) | 1.15 (0.20-1.70) | 1.16 (0.20-1.90) | 1.20 (0.20-1.90) | 0.085 |
| **IgA (ng/ml)** | 882.5 (52.0-1146.5) | 998.5 (872.0-1881.5) | 992.0 (655.0-1829.0) | 900.5 (89.1-1103.0) | 0.001* |
| **Sig** | | 0.000+ | 0.000+ | 0.12 | |
| **TAC (nmol/µl)** | 0.23 (0.00-0.67) | 0.83 (0.11-2.39) | 0.30 (0.07-1.40) | 0.33 (0.05-0.77) | 0.001* |
| **Sig** | | 0.000+ | 0.15 | 0.89 | |
| **Cotinine (ng/ml)** | 30.00 (20.5-66.5) | 23.50 (12.0-38.0) | 24.00 (15.0-31.5) | 25.25 (20.0-37.5) | 0.079 |

*Significance between baselines versus three follow up periods $p \leq 0.05$ + significance with baseline $p \leq 0.05$

+ Significance with baseline Table (3) shows that streptococcus mutans count median value was $95.0x10^{5,}$ 0.1-2000.0 at the baseline, then it dramatically decreased after one month ($0.10x10^{5,}$ 0.0x105-600 $x10^5$) and after three months ($0.3x10^{5,}$ 0.0 $x10^5$-700 $x10^5$) of follow up after green tea varnish application. This decrease was statistically significant (p = 0.000). After six months of follow up, the salivary streptococcus counts ($101.5x10^{5,}$ $0.1x10^5$-$1850.0x10^5$) increased again to be nearly the same as the baseline value(p = 0.25). Concerning lactobacilli, the median value of the bacterial count was $1.1x10^5$, 0.001 $x10^5$-12.33 $x10^5$ at baseline, then it dramatically decreased after one month ($0.005x10^5$, $0.0x10^5$-$0.03x10^5$) and three months follow up ($0.007x10^5$, $0.0x10^5$-$0.06x10^5$) and this decrease was statistically significant (p = 0.000). The salivary lactobacilli count increased slightly after six months ($0.4x10^5$, $0.001x10^5$-$8.32x10^5$), but was still lower than the baseline with a significant difference (p = 0.02).

**Table (3): Median values of microbiological salivary parameters at the baseline and throughout the follow up periods**

| Microbiological parameters | **Baseline** Median (IQR) | **Month 1** Median (IQR) | **Month 3** Median (IQR) | **Month 6** Median (IQR) | **Sig** |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **Streptococcus mutans x10$^5$ (CFU/ml)** | 95.0 (0.1-2000.0) | 0.1 (0.0 -600.0) | 0.3 (0.0-700.0) | 101.5 (0.1-1850.0) | 0.000* |
| **Sig** | | 0.000$^+$ | 0.000$^+$ | 0.25 | |
| **Lactobacilli x 10$^5$ (CFU/ml)** | 1.1 (0.001-12.33) | 0.005 (0.0-0.03) | 0.007 (0.0-0.06) | 0.4 (0.001-8.32) | 0.000* |
| **Sig** | | 0.000$^+$ | 0.000$^+$ | 0.02$^+$ | |

*Significance between baseline versus three follow up periods $p \leq 0.05$
$^+$ Significance with baseline

Table (4) shows the children who were exposed to more than 20 cigarettes daily at home showed lower salivary median value of flow rate (1.90, 0.40-3.00) than those who were exposed to less than 10 (2.30 ,1.30-3.30) and from 10 to 20 cigarettes (2.30, 1.60-3.10) at the baseline. The difference was statistically significant (p = 0.01). Then, the flow rate increased after one month (3.30, 2.30-4.00, 3.30, 2.50-4.30 and 3.30, 2.30-4.00), as well as after three months of follow up (3.30, 2.30-4.00, 2.60, 2.30-3.90) and it was 2.60, 2.20-4.00 for the three groups respectively, and there was no statistically significant difference (p > 0.05). The flow rate then decreased after six months of follow up and the difference between the three groups was statistically significant (p =0.01).

Children who were exposed to more than 20 cigarettes daily at home showed lower median value of salivary pH (6.00, 4.50-6.35) than those exposed to less than 10 (6.90, 4.90-7.75) and between 10 and 20 cigarettes (6.55, 4.90-7.37) at the baseline. The difference was statistically significant (p = 0.02). The median value of salivary pH increased after one month (7.77, 7.30-7.93, 7.68, 7.10-7.94 and 7.50, 6.80-7.94), and after three months of follow up (7.50, 6.50-7.83, 7.68, 5.90-7.93 and 7.50, 6.50-7.89) for the three groups respectively, and there were no statistically significant differences between the three groups (p > 0.05). After six months of follow up, the median pH values decreased again for those exposed to less than 10 (6.75, 5.00-7.75), and between 10 and 20 cigarettes (6.61, 5.00-7.34), although, the median pH value (6.42, 4.50-7.27) for children exposed to more than 20 cigarettes decreased after six months follow up but was still more than its value at baseline. There was no statistically significant difference between the three groups (p >0.05).

Regarding the buffering capacity, there were no statistically significant differences (p >0.05) between the median values of children exposed to less than 10, between 10 and 20 and more than 20 cigarettes (1.40, 0.70-1.80, 1.20, 0.90-1.70 and 1.20, 0.40-2.00 respectively) at the baseline. After one month of follow up, the median values of salivary buffering capacity increased (1.90, 1.00-2.50, 1.90, 1.30-2.00 and 1.50, 1.00-2.50) for three groups respectively. The median values of salivary buffering capacity decreased after three months of follow up (1.60, 1.00-2.30, 1.40, 0.90-1.60 and 1.50, 0.80-2.50), and after six months of follow up (1.30, 0.80-1.90, 1.20, 0.70-1.50 and 1.20, 0.60-2.00) for three groups respectively to be almost the same as the baseline values(p >0.05).

Children exposed to more than 20 cigarettes daily at home showed lower median value of salivary total protein (1.20, 0.30-1.60) than those exposed to less than 10 (1.20, 0.90-1.90) and between 10 and 20 cigarettes (1.20, 0.60-1.90) at baseline. The difference was statistically significant (p = 0.04). After one month of follow up, salivary total protein median values slightly decreased for children exposed to less than 10 and between 10 and 20 cigarettes (1.10, 0.20 -1.70 and 1.00, 0.20 - 1.70 respectively), to be not statistically different from children exposed to more than 20 cigarettes (1.30, 0.20-1.70) (p = 0.72). The median values of salivary total protein almost remained the same at three months of follow up after green tea varnish application for the children of the three groups (1.30, 0.30-1.90, 1.10, 0.30-1.70 and 1.10, 0.20-1.90 respectively). After six months, the median values of salivary total protein slightly increased for the three groups (1.50, 0.60 - 1.80, 1.20, 0.60 - 1.90 and 1.20, 0.50 - 1.60 respectively) (p >0.05).

Regarding IgA, children exposed to less than 10 cigarettes showed nearly the same median (908.5, 125.0-1881.5) as children exposed to between 10 and 20 (908.5, 169.0-1429.0) and higher median than those exposed to more than 20 cigarettes (861.0, 52.0-1091.5). There was no statistically significant difference between the three groups at the baseline (p = 0.37). There was a slight increase in the median values of salivary IgA for the three groups after one month (999.5, 925.0-1376.0, 1039.0, 843.5-1846.5 and 990.5, 155.5-1187.0 respectively) and three months of follow up (992.0, 872.0-1103.5, 993.5, 909.5-1043.5 and 992.0, 172.0-1103.5 respectively). After six months of follow up, there was a relapse in salivary IgA median values (938.5, 189.1-1043.5, 900.0, 199.1-1429.0 and 839.5, 91.5-1091.5) to be nearly the same as baseline values for the three groups respectively. There were no statistically significant differences between the three groups throughout the three follow up periods after green tea varnish application (p >0.05).

As regards, salivary TAC median values for children exposed to between 10 and 20 cigarettes (0.23, 0.1-0.54) and those exposed to more than 20 cigarettes (0.21, 0.05-0.47), they were lower than those exposed to less than 10 cigarettes daily at home (0.38, 0.13-0.76) at the baseline. The difference was statistically significant (p = 0.01). The TAC median values increased after one month follow up for the children in the three groups (0.94, 0.40-2.39, 0.99, 0.19-1.39 and 0.74, 0.05-1.38 respectively). There was a gradual decrease in the median values after three months of follow up (0.32, 0.16-1.17, 0.36, 0.15-1.40 and 0.33, 0.07-1.19) and after six months of follow up (0.30, 0.15-0.77, 0.23, 0.11-0.54 and 0.18, 0.05-0.53) for the three groups respectively to return to baseline readings. There were no statistically significant differences between the three groups throughout the three follow up periods after green tea varnish application (p >0.05).

Children exposed to more than 20 cigarettes daily at home showed higher salivary cotinine median value (33.75, 22.00-66.50) than those exposed to less than 10 (24.50, 15.50-37.50) and those exposed to between 10 and 20 cigarettes (28.50, 10.50-36.50) at baseline. There was statistically significant difference between the three groups (p = 0.003). The salivary cotinine median values almost remained with the same values for the children exposed to less than 10 and between 10 and 20 cigarettes at the three follow up periods after green tea varnish. But for children exposed to more than 20 cigarettes, the salivary cotinine median values were less than the baseline median after one, three and six months of follow up (24.50, 20.00-35.50, (24.50, 20.00-31.50 and 27.50, 23.00-67.50 respectively). There were no statistically significant differences between the three groups after one and three months (p >0.05), but after six months of follow up there were statistically significant differences between children exposed to more than 20 cigarettes and those exposed to less than 10 and between 10 and 20 cigarettes (p = 0.01).

**Table (4): Median values of biochemical salivary parameters by number of cigarettes at baselines and throughout the follow up periods**

| Biochemical parameters | <10 cigarettes (n=19) Median(IQR) | 10-20cigarettes (n=9) Median(IQR) | >20cigarettes (n=32) Median(IQR) | Sig. |
|---|---|---|---|---|
| **Flow rate (ml/min)** | | | | |
| *Baseline* | 2.30 (1.30 - 3.30)[a] | 2.30 (1.60 - 3.10)[a] | 1.90 ( 0.40 - 3.00)[b] | 0.01* |
| *1-Month* | 3.30 (2.30 - 4.00) | 3.30 (2.50 - 4.30) | 3.30 ( 2.30 - 4.00) | 0.51 |
| *3-Months* | 3.30 (2.30 - 4.00) | 2.60 (2.30 - 3.90) | 2.60 ( 2.20 - 4.00) | 0.13 |
| *6-Months* | 2.60 (2.20 - 3.50)[a] | 2.30 (1.50 - 3.30)[a] | 2.30 ( 0.60 - 3.00)[b] | 0.01* |
| **pH** | | | | |
| *Baseline* | 6.90 (4.90 - 7.75)[a] | 6.55 ( 4.90 - 7.37)[a] | 6.00 ( 4.50 – 6.35)[b] | 0.02* |
| *1-Month* | 7.77 (7.30 - 7.93) | 7.68 ( 7.10 - 7.94) | 7.50 ( 6.80 - 7.94) | 0.05 |
| *3-Months* | 7.50 (6.50 - 7.83) | 7.68 ( 5.90 - 7.93) | 7.50 ( 6.50 - 7.89) | 0.82 |
| *6-Months* | 6.75 (5.00 - 7.7) | 6.61 ( 5.00 - 7.34) | 6.42 ( 4.50 - 7.27) | 0.57 |
| **Buffering capacity (mg/dl)** | | | | |
| *Baseline* | 1.40 (0.70 - 1.80) | 1.20 ( 0.90 - 1.70) | 1.20 ( 0.40 - 2.00) | 0.25 |
| *1-Month* | 1.90 (1.00 - 2.50) | 1.90 ( 1.30 - 2.00) | 1.50 ( 1.00 - 2.50) | 0.24 |
| *3-Months* | 1.60 (1.00 - 2.30) | 1.40 ( 0.90 - 1.60) | 1.50 ( 0.80 - 2.50) | 0.23 |
| *6-Months* | 1.30 (0.80 - 1.90) | 1.20 ( 0.70 - 1.50) | 1.20 ( 0.60 - 2.00) | 0.70 |
| **Protein (g/dl)** | | | | |
| *Baseline* | 1.20 (0.90 - 1.90)[a] | 1.20 ( 0.60 - 1.90)[a] | 1.20 (0.30 - 1.60)[b] | 0.04* |
| *1-Month* | 1.10 (0.20 -1.70) | 1.00 ( 0.20 - 1.70) | 1.30 (0.20 - 1.70) | 0.72 |
| *3-Months* | 1.30 (0.30 - 1.90) | 1.10 ( 0.30 - 1.70) | 1.10 (0.20 - 1.90) | 0.19 |
| *6-Months* | 1.50 (0.60 - 1.80 ) | 1.20 ( 0.60 - 1.90) | 1.20 (0.50 - 1.60) | 0.66 |
| **IgA (ng/ml)** | | | | |
| *Baseline* | 908.5 ( 125.0 - 1881.5) | 908.5 (169.0 - 1429.0) | 861.0 (52.0 - 1091.5 ) | 0.37 |
| *1-Month* | 999.5 ( 925.0 - 1376.0) | 1039.0 (843.5 - 1846.5) | 990.5 (155.5 - 1187.0 ) | 0.86 |
| *3-Months* | 992.0 ( 872.0 - 1103.5) | 993.5 (909.5 - 1043.5) | 922.0 (172.0 - 1103.5 ) | 0.21 |
| *6-Months* | 938.5 ( 189.1 - 1043.5) | 900.0 (199.1 - 1429.0) | 839.5 (91.5 - 1091.5 ) | 0.32 |
| **TAC (nmol/µl)** | | | | |
| *Baseline* | 0.38 ( 0.13 - 0.76 )[a] | 0.23 ( 0.10 - 0.54)[b] | 0.21 ( 0.05 - 0.47)[b] | 0.01* |
| *1-Month* | 0.94 ( 0.40 - 2.39) | 0.99 ( 0.19 - 1.39 ) | 0.74 ( 0.05 - 1.38 ) | 0.15 |
| *3-Months* | 0.32 ( 0.16 - 1.17) | 0.36 ( 0.15 - 1.40 ) | 0.33 ( 0.07 - 1.19 ) | 0.19 |
| *6-Months* | 0.30 ( 0.15 - 0.77) | 0.23 ( 0.11 - 0.54 ) | 0.18 ( 0.05 - 0.53 ) | 0.47 |
| **Cotinine (ng/ml)** | | | | |
| *Baseline* | 24.50 (15.50 - 37.50)[a] | 28.50 (10.50 - 36.50 )[a] | 33.75 (22.00 - 66.50)[b] | 0.003* |
| *1-Month* | 22.50 (12.00 - 38.00) | 25.50 (14.00 - 30.50 ) | 24.50 (20.00 - 35.50) | 0.30 |
| *3-Months* | 22.50 (15.00 - 31.50) | 23.00 (13.00 - 31.00 ) | 24.50 (20.00 - 31.50) | 0.25 |
| *6-Months* | 24.50 (12.00 - 33.00)[a] | 24.75 (13.00 - 36.50 )[a] | 27.50 (23.00 - 67.50)[b] | 0.01* |

$X_{KW}$ Kruskal-Wallis test, *Significant results≤.05
Superscripts with different letters are statistically significant by pair-wise comparison

Table (5) shows that children exposed to less than 10 cigarettes showed lower bacterial count median value (10.0x105, 0.01x$10^5$ - 600.0x$10^5$) than those exposed to 10 to 20 cigarettes (20.0x$10^5$, 0.02x$10^5$ -1850.0x$10^5$) and those exposed to more than 20

cigarettes (75.0x105, $0.01x10^5$ -$2000x10^5$) at baseline. The differences were statistically significant (p = 0.001). After one month of follow up, there was a decrease in the median values of salivary streptococcus mutans count ($0.08x10^5$, $0.0x10^5$ -$590.0x10^5$, $0.1x10^5$, $0.0x10^5$ -$600x10^5$ and $0.3x10^5$, $0.01x10^5$ -$680.0x10^5$), as well as, after three months of follow up ($0.09 x10^5$, $0.0 x10^5$ $200.0 x10^5$, $0.3 x10^5$, $0.01 x10^5$ -$500.0 x10^5$ and $0.4x10^5$, $0.0x10^5$-$700.0x10^5$) for children of the three groups respectively and the difference between the different groups was not statistically significant (p >0.05). After six months of follow up, there was an increase in the median values of bacterial count of the three groups ($8.0x10^5$, $0.01x10^5$-$490.0 x10^5$, $16.0x10^5$, $0.01x10^5$ -$990.0x10^5$ and $108.5 x10^5$, $0.04 x10^5$-$1450.0x10^5$ respectively) and the differences were statistically significant (p = 0.002).

Concerning lactobacilli, children exposed to less than 10 cigarettes daily showed lower median values of bacterial count ($0.20x10^5$, $0.001x10^5$-$1.0x10^5$) than children exposed to between 10 to 20 ($0.9x10^5$, $0.003x10^5$-$12.3x10^5$) and those exposed to more than 20 cigarettes ($1.1x10^5$, $0.01x10^5$-$97.8x10^5$) at baseline. The difference was statistically significant (p =0.001). There was a dramatic decrease in the salivary lactobacilli count median values of the three groups after one month ($0.00x10^5$, $0.0x10^5$-$0.03x10^5$, $0.00x10^5$, $0.0x10^5$-$0.01x10^5$ and $0.006x10^5$ $0.0x10^5$-$0.02x10^5$ respectively), three months ($0.002x10^5$, $0.0x10^5$-$0.05x10^5$, $0.006x10^5$, $0.0x10^5$-$0.05x10^5$ and $0.01x10^5$, $0.0x10^5$-$0.06x10^5$ respectively) and six months of follow up ($0.03x10^5$, $0.001x10^5$-$6.2x10^5$, $0.3x10^5$, $0.001x10^5$-$7.3x10^5$ and $0.6x10^5$, $0.001x10^5$-$8.3x10^5$ respectively). There were no statistically significant differences between children exposed to different number of cigarettes daily at home at the three follow up periods after green tea varnish application (p >0.05).

**Table (5): Median values of microbiological salivary parameters by number of cigarettes at the baseline and throughout the follow up periods**

| *Microbiological parameters* | *<10cigarettes (n=19) Median (IQR)* | *10-20cigarettes (n=9) Median (IQR)* | *>20cigarettes (n=32) Median (IQR)* | *Sig.* |
|---|---|---|---|---|
| **Streptococcus mutans $x10^5$ (CFU/ml)** | | | | |
| *Baseline* | 10.0(0.01-600.0) [a] | 20.0(0.02-1850.0) [b] | 75.0(0.01-2000.0) [c] | 0.001* |
| *1-Month* | 0.08(0.0-590.0) | 0.1(0.0-600) | 0.3(0.01-680.0) | 0.370 |
| *3-Months* | 0.09(0.0-200.0) | 0.3(0.01-500.0) | 0.4(0.0-700.0) | 0.908 |
| *6-Months* | 8.0(0.01-490.0)[a] | 16.0(0.01-990.0) [b] | 108.5(0.04-1450.0) [c] | 0.002* |
| **Lactobacilli x $10^5$ (CFU/ml)** | | | | |
| *Baseline* | 0.2(0.001-1.0) [a] | 0.9(0.003-12.3) [b] | 1.1(0.01-97.8) [b] | 0.001* |
| *1-Month* | 0.00(0.0-0.03) | 0.0(0.0-0.01) | 0.006(0.0-0.02) | 0.447 |
| *3-Months* | 0.002(0.0-0.05) | 0.006(0.0-0.05) | 0.01(0.0-0.06) | 0.373 |
| *6-Months* | 0.03(0.001-6.2) | 0.3(0.001-7.3) | 0.6(0.001-8.3) | 0.322 |

$X_{KW}$ Kruskal-Wallis test, *Significant results≤.05
Superscripts with different letters are statistically significant by pair-wise comparison

**Discussion:**

Median values of stimulated salivary flow rate, pH and buffering capacity of the results of this study at the baseline were less than the normal salivary levels (2-5 ml/min, 6.4-7.4 and 0.9-1.3 mg/dl respectively) (Makawi et al., 2017), (Punj, 2018)then there was a significant increase in the salivary flow rate, pH and buffering capacity after one and three months (p <0.05) to become similar to the normal level.

The increase in salivary flow rate lead to the increase in salivary pH and buffering capacity (Buzalaf et al., 2012). Significant increase in salivary flow rate may be due to the vasodilatation effect of catechins on the capillary network which supply the salivary glands (Jang et al., 2013). This vasodilatation can produce a 20-fold increase in the blood flow of the salivary glands, which ensures the production of large volumes of saliva over a long period of time (Shalal, 2017).

These results were in agreement with many studies, such as a study conducted in Canada, showed that there was a significant increase in the salivary flow after drinking 4 cups of green tea daily for one month (Gibbard et al., 2011). Another study conducted in Iran, showed significant increase in the salivary flow rate and pH among adolescents after rinsing with green tea (Masoumi et al., 2016). A study conducted in Turkey among adolescents, revealed that there were significant increases in salivary pH and buffering capacity after drinking green tea (Demir et al., 2017), which means that the effect of green tea is systemically and topically. In an Indian study conducted among school children, there were significant increases in salivary pH and buffering capacity after daily rinsing with green tea for one month (Kamalaksharappa et al., 2018).A study done in India, examined the effect of green tea mouth rinse on salivary pH and buffering capacity for children between 8–12 years and the results were statistically significant (p<0.05) (Talreja et al., 2018).

In the current study, the median values of salivary total protein and IgA at the baseline were less than the normal salivary levels (0.9-1.5 g/dl and 1000-1700 ng/dl respectively) (Preethi et al., 2010, El-Gebaly et al., 2012). The salivary total protein mostly did not change through the follow up periods, even though, the salivary IgA significantly increased after one and three months follow ups to be almost

equal to the normal salivary level. The explanation is that the concentration of some proteins increased as IgA, while the concentration of others decreased as alpha amylase. In general, salivary proteins have both protective and harmful properties; some proteins as IgA facilitate the clearance of oral streptococci adsorbed to tooth surfaces, in contrast, alpha-amylase promotes the adherence of these bacteria and digests starch to dietary maltose, which is a source of acid production, so inhibition of salivary amylase reduced the incidence of caries. The interaction and binding between green tea and amylase reduced the enzymatic activity of alpha-amylase resulted in decrease of salivary bacteria. (Hara et al., 2012).

In Taiwan 2014, a study was conducted to examine the effect of green tea on saliva of adolescents. It was found that IgA significantly increased after 30 min of drinking green tea (Lin et al., 2014). This result is in coincidence with the current study results which found significant increase in means of IgA and TAC between baseline and after one and three months ($p<0.05$).

In the current study, the salivary cotinine was much higher than the normal salivary level (12ng/ml) (Jarvis et al., 2008) and the salivary TAC was less than the normal level (1.6-2.1 nmol/µL) (Abdellatif et al., 2011) at the baseline. The increase in salivary TAC after one month and the decrease in salivary cotinine after green tea varnish at the three follow up periods were still not as the normal level. This result was in agreement with the study conducted in Taiwan 2014, concerning examination of the effect of green tea among adolescents, it was found that salivary TAC level significantly increased after drinking green tea (Lin et al., 2014). Green tea catechins is a powerful antioxidant molecule that has the ability to protect cells against damage. Nicotine is thought to cause toxic effects through oxidative stress, the ability of green tea catechins to neutralize the cytotoxic effects induced by nicotine on oral epithelial cells and gingival fibroblasts and restrict the damage of DNA, RNA, oxidize protein, oxidize lipids and cell suicide (Desjardins & Grenier, 2012). Green tea catechins have antioxidative, anti-inflammatory, antiarteriosclerotic and antibacterial effects (Singhal et al., 2017).

Median values of streptococcus mutans and lactobacilli count at the baseline of the present study were much higher than the normal non pathological count in the oral flora which should be $< 1x10^5$ CFU/ml for streptococcus mutans and $< 0.1x10^5$ CFU/ml for lactobacilli (Guo & Shi, 2013). There were strong significant decreases in the median values of salivary streptococcus mutans counts after one and three months and the significant decrease of lactobacilli count remained for the six months of follow up after intervention to be within the normal salivary values. Study results were in agreement with a study conducted in India, where it was found that green tea mouth rinse produced significant reduction in salivary streptococcus mutans count among Indian children aged 7-12 years (Goyal et al., 2017). The explanation of the effect of green tea is that green tea catechins can prevent proliferation of salivary bacteria and interfere with the bacterial bonding to tooth enamel by changing bacterial phenotype, or inhibiting the glycosyltransferase bacterial enzyme (Hajiahmadi et al., 2019). Also, another study conducted in India to examine the effectiveness of green tea toothpaste on salivary streptococcus mutans and lactobacilli counts among adolescents, and it was found that salivary bacteria decreased significantly after using green tea toothpaste for one month (Prabakar et al., 2018). Another study conducted in Iran, to compare the efficacy of green tea mouth rinse and green tea gel on salivary streptococcus mutans and lactobacilli counts among 12-18 old teenagers. It was found that after one month, both vehicles had the same significant decrease of salivary streptococcus mutans and lactobacilli after intervention (Ahmadi et al., 2019).

Salivary parameters; flow rate, pH, buffering capacity, IgA, streptococcus mutans and lactobacilli had significant difference between baseline and after three months of green tea varnish application, also the effectiveness on lactobacilli continued till after six months follow up (De Luca et al., 2017). Varnish as a vehicle stick to the tooth and has more durability so provides longer exposure to the tooth surface than gel, toothpaste or mouth rinse. Also, topical varnish slowdown the release of its active substances so its effectiveness lasts after three or six months(Baik et al., 2021).This may be explained by the fact that green tea catechins are absorbed by the salivary glands then secreted into the oral cavity for prolonged time (Sulistiyani et al., 2021).

A comparison between chemical varnish (fluoride varnish) and natural herbal varnish showed that the two types gave the same effect on salivary parameters. Green tea maintained the alkalinity of saliva, and this may be due to its polyphenols and catechins content. The catechins present in green tea represent a marked effect on pH of saliva. Green tea catechins prevents acid production and preserves pH within the normal range (6.4–7.4), which is not favorable for Streptococcus mutans growth. This is in agreement with researches which proved that green tea possesses anticariogenic and antibacterial properties (Kamalaksharappa et al., 2018; Talreja et al., 2018).

Salivary flow rate, pH, and TAC in the current study, were significantly higher, while salivary total protein, cotinine, streptococcus mutans and lactobacilli were significantly lower in children exposed to less than 10 and between 10 and twenty cigarettes daily than those children exposed to more than 20 cigarettes daily at their homes. These significant differences were not present after green tea varnish application, but were found after six months of follow up for salivary cotinine and streptococcus mutans. There is an association between the TAC and SHS dose, and this could be related to the fact that exposure to SHS affect salivary antioxidant defense system. (Azadbakht et al., 2016). Also, SHS causes injury to ductal secretory unit of salivary glands as a result of tobacco related toxic products, and this damage may increase with the increased dose of SHS and subsequently, affect salivary parameters (Khan et al., 2018).

The study results coincide with a study conducted in Turkey which assessed salivaryflow rate and pH in relation to SHS intensity (<10, 10-20 and >20 cigarettes/day). It was found that salivary flow rate, pH, and buffering capacity were significantly negatively correlated with the intensity of SHS ($p<0.05$) which is similar to the results in this study (Avşar et al., 2008). Also, another study conducted in Turkey, to evaluate the relationship between number of cigarettes the children were exposed to at their homes and salivary protein and cotinine level, found that the dose-response relationship between the salivary **c**otinin**e** level of the SHS

children and the number of cigarettes smoked daily by parents was found to be significantly different between each of the three categories in the SHS group (< 10, 10-20 and > 20 cigarettes/day), which is in agreement with current results (Avşar et al., 2009). A study conducted in Iran (2016), found that the most important salivary antioxidants; uric acid and ascorbic acid were significantly affected by SHS causing oxidative stress, implicated in many disorders. So it was supposed that uric acid could be used as salivary markers of exposure SHS (Azadbakht et al., 2016). Results of this study are in consistent with the results of a study done among 5-10 years' children in India, which found that there was significant positive correlation between number of cigarettes and streptococcus mutans and lactobacillus colonies (Menon & Bhat, 2019). This may be explained by the fact that nicotine promotes the biofilm formation and metabolism of salivary bacteria, this biofilm is more structurally formed with longer bacterial chain length and more orientated cell arrangement. And with the increase in the number of cigarettes the children are exposed to, the dose of nicotine will increase and the bacterial count will increase consequently (Wu et al., 2019).

**Conclusion:**

- Green tea varnish has an obvious effect on improving biochemical as well as microbiological salivary parameters.
- The effect of green tea varnish continues for three months, and disappears after six months of follow up.
- Green tea varnish can overcome the effect of high number of cigarettes the children exposed on the quality of salivary parameters among the children included in the study, and this beneficial effect continued for six months for some parameters.

**Recommendations:**

- Utilization of green tea in different forms of dental preventive products as toothpaste, mouthwash and gel.
- Use of green tea varnish as a preventive measure in dental clinic.
- Education of patients about the beneficial effect of green tea.
- Evaluate the effect of green tea varnish on salivary parameters in different age groups.

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