# Comparative Clinical Trial On The Effect Of Propolis Extract And Sodium Fluoride Mouth Rinse On Salivary *Streptococcus Mutans* Count

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**Abstract:**The objective of this study was to evaluate and compare the effect of sodium fluoride and Bee Propolis mouth rinses on salivary *S. mutans* count. Dental caries was recorded according to the criteria of WHO, 1997, using (dmf) index for primary dentition. **Subjects:** A sample composed of 60 children (30 boys and 30 girls) aged from 4-6 years with mean age 4.7 where all children had only primary dentition to avoid extreme variables. The children were divided into two groups each of 30 children. Group I: used sodium fluoride mouthwash 0.025%. Group II: used propolis mouthwash (10% ethanolic extract) under their parent's supervision twice daily for one month. **Methods:** Saliva samples were collected from the children before using the mouthwash, after one week, after 2 weeks and after one month of use. The amount of *S. mutans* in the oral cavity of the tested children was evaluated using selective media for *S. mutans* TYCSB.The results showed the presence of a statistically significant difference between the effect of both types on mean *S. mutans* count in girls and also a statistically significant difference between the effect of them on the change in *S. mutans* count in both sexes during the follow-up period.
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**Key words:** Streptococcus mutans, mouthwash, Propolis

## 1.Introduction:

Dental caries is a transmissible infectious disease in which *Streptococcus mutans* (*S. mutans*) are generally considered to be the main etiological agent in which a group of indispensable factors, the host, bacteria, fermentable carbohydrates and time are necessary for the disease to occur. Caries prevention is better than cure, it is regarded as a measure designed to prevent the disease and its clinical symptoms. From these preventive strategies, professional tooth cleaning and many types of mouth rinses have been evaluated for their plaque- reducing effectiveness and ability to reduce *S.* mutans.Selwitz et al., (2007).

The most commonly used mouth rinses are chlorhexidine, essential oils, triclosan and sodium fluoride. When preschool children use fluoride containing mouth rinses, they may swallow some of the rinsing solution, which may lead to fluorosis of the permanent dentition; this would be minimized by using the low sodium fluoride concentration (0.025%), rather than using the high concentration (0.05%). So the 0.025% sodium fluoride mouthrinse would be an acceptable daily rinsing concentration for preschool children ,Clarkson and McLoughlin( 2000) ;Poulsen( 2009).

Topical fluoride promotes remineralization and inhibits demineralization of enamel during caries process. Other effect of fluoride includes the inhibition of glycolosis, and a reduction in the production of extra-cellular polysaccharide. Fluoride also has an effect on the cariogenic potential of *S. mutans* and of course fluoride is bactericidal at high concentration, Camile (2009**).**

There are many other natural compounds like xylitol, green tea, mint and propolis had been applied as mouth rinses and can be used to protect against dental caries. Propolis is known as bee glue and bee propolis, it is a brownish resinous substance collected by bees, mainly from plants around their hives, used to reinforce the combs and to keep the hive's environment aseptic**,** Wander, (1995).

Propolis has medical and dental uses especially anesthetic, anti-bacterial, antibiotic, anti-inflammatory, anti-fungal, anti-viral and antioxidant properties, Parolia et al., (2009).

Topical application of Propolis mouthwash twice daily reduce the incidence of dental caries**,** it has a great potential against the bacteria related with dental caries such as *S.mutans* **.** Ting and Silver (2004) suggested that using a mouthwash of propolis ethanolic extract reduce plaque formation, reduce

bacteria in the mouth, relieve dental pain and gum inflammation i.e. Gingivitis & periodontitis.

So the aim of this study was to evaluate and compare the effect of sodium fluoride and Bee Propolis mouthrinses on salivary streptococcus mutans count in a group of Egyptian children.

## 2. Materials and Methods:

### 2.1. Materials

#### 2.1.1. Subjects:

This study was carried on 60 children from Outpatient clinic of Pediatric Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University. All children were examined clinically for dental caries using sterile diagnostic set including: plane mirror, sickle explorers number 23 and tweezers.

The children were divided into two groups:

**Group I**: Consisted of 30 children used fresh sodium fluoride mouth wash(0.025%) and

**Group II:** Consisted of 30 children used propolis mouthwash (10% ethanolic extract), children in both groups used mouthwash under their parent's supervision twice daily for one month.

#### 2.1.2. Samples:

Saliva samples were collected from each child by asking him to spit in a labeled sterile falcon tube. Each subject was refrained from tooth brushing in the morning and from eating or drinking at least 2 hours before sampling time.

#### 2.1.3. Microbiological Media:

TYCSB (Tryptone – yeast – cystine – sucrose – bacitracin) which is a selective media for the isolation of *Streptococcus mutans***.**

### 2.2. Methods

#### 2.2.1. Dental Caries evaluation:

Dental Caries was recorded according to the criteria of ,WHO (1997), using (dmf) index for primary dentition

#### 2.2.2. Microbiological analysis:

The amount of *Streptococcus mutans* in the oral cavity of the tested children was evaluated using selective media for *Streptococcus mutans* TYCSB (Tryptone – yeast – cystine – sucrose – bacitracin) which is a selective media for the isolation of *Streptococcus mutans***.** Saliva samples were transferred in an ice box to the laboratory of the Microbiology of the National Research Centre. Each saliva sample was stirred on the vortex for 30 seconds. This was followed by serial dilutions 1:10, 1:100 and 1:1000.For each dilution 20-30 micro liters of saliva were taken by the micropipette and cultured on the previously prepared media plates using a bent sterile glass rod. The plates were then incubated in 37ºC for 48 hours.

*Streptococcus mutans* was identified by its characteristic colony morphology. The colonies were raised, convex, opaque with rough margins and granular frosted appearance and glistening bubble often accumulated on the top of the colony. After 48 hours the plates were removed from the incubator, the most countable plates were selected, i.e. having 30-200 CFU/ml.

The number of colonies per milliliter (CFU/ml) was determined by the following equation: Number of colonies/ml (CFU/ml) = number of colonies counted × Inverse of dilution × inverse the cultured volume (ml).

#### 2.2.3. Statistical analysis:

Statistical analysis was carried out using SAS program**,** SAS (1988). *S. mutans* count was transformed to log count before analysis using the dilutions which were used in microbiological analysis. One way analysis of variance (Procedure ANOVA of SAS) followed by Duncan's multiple range test were used to test the correlation between dmft of the two groups and their salivary *S. mutans* count.

## 3. Results

During our semi-longitudinal study, the results showed that there was a statistically significant difference between the effect of two types on mean *S. mutans* count in girls and such significance was not detected in boys. This is shown in fig. (1&2) and table (1).

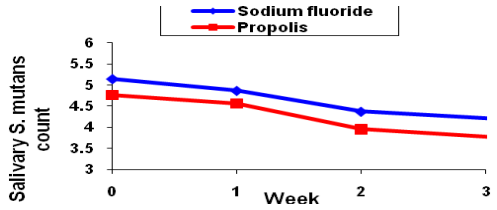


**Fig. 1.** Salivary *S. mutans* count in both groups during the follow-up period in girls.

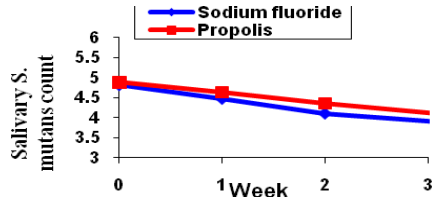


**Fig. 2.** Salivary *S. mutans* count in both groups during the follow-up period in boys.

**Table 1.** Mean and Standard Deviation (SD) of both mouth rinses on *S. mutans* count in girls and boys in three intervals.

| Group | Group I | | Group II | | |
|---|---|---|---|---|---|
| **Girls** | Mean | ±S.D. | Mean | ±S.D. | P-value |
| Before | 5.149 | 0.280 | 4.765 | 0.415 | 0.010 |
| 1 week | 4.869 | 0.211 | 4.568 | 0.464 | 0.043 |
| 2 weeks | 4.373 | 0.452 | 3.955 | 0.555 | 0.042 |
| 1 month | 4.055 | 0.436 | 3.590 | 0.530 | 0.020 |
| **Boys** | Mean | ±S.D. | Mean | ±S.D. | P-value |
| Before | 4.814 | 0.374 | 4.880 | 0.632 | 0.716 |
| 1 week | 4.472 | 0.465 | 4.633 | 0.580 | 0.386 |
| 2 weeks | 4.104 | 0.617 | 4.355 | 0.633 | 0.257 |
| 1 month | 3.727 | 0.629 | 3.883 | 0.651 | 0.488 |

From table (2) and fig. (3&4), it is clear that there is a statistical significant effect of each mouth rinse on mean change in *S. mutans* count in girls and boys during the follow-up period, while there was no statistically significant difference between the two groups.

Regarding sodium fluoride mouth rinse, the only statistical significant correlation with dmft was seen after one month of use, which was higher with dmft >6 than with propolis mouth rinse.

**Table 2.** Changes in *S. mutans* count using both mouth rinses on in girls and boys

| Group | Group I | | | Group II | | | P2 value |
|---|---|---|---|---|---|---|---|
| **Girls** | Mean | ±S.D. | P1 value | Mean | ±S.D. | P1 value | |
| Before - 1 week | -0.281 | 0.165 | 0.001* | -0.197 | 0.125 | 0.001* | 0.146 |
| 1 week - 2 weeks | -0.495 | 0.441 | 0.002* | -0.613 | 0.491 | 0.001* | 0.518 |
| 2 weeks - 1month | -0.318 | 0.322 | 0.004* | -0.365 | 0.185 | 0.001* | 0.642 |
| Before - 1 month | -1.094 | 0.495 | 0.001* | -1.175 | 0.561 | 0.001* | 0.695 |
| **Boys** | Mean | ±S.D. | P1 value | Mean | ±S.D. | P1 value | P2 value |
| Before - 1 week | -0.342 | 0.228 | 0.001* | -0.247 | 0.286 | 0.003* | 0.299 |
| 1 week - 2 weeks | -0.368 | 0.244 | 0.001* | -0.277 | 0.299 | 0.002* | 0.344 |
| 2 weeks - 1month | -0.377 | 0.286 | 0.001* | -0.472 | 0.390 | 0.001* | 0.427 |
| Before - 1 month | -1.087 | 0.382 | 0.001* | -0.997 | 0.692 | 0.001* | 0.643 |

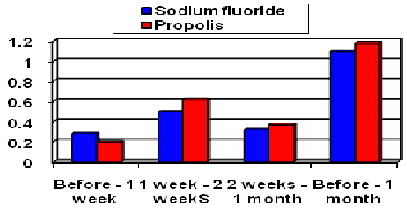


**Fig. 3.** Changes in salivary *S. mutans* count in both groups during the follow-up period in girls.

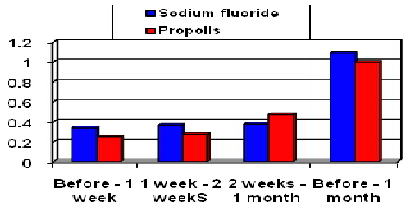


**Fig. 4.** Changes in salivary *S. mutans* count in both groups during the follow-up period in boys.

Regarding sodium fluoride mouth rinse, the only statistical significant correlation with dmft was seen after one month of use, which was higher with dmft >6 while with propolis mouth rinse there was a statistically significant correlation with dmft during the follow-up period which was higher with dmft > 6 except after one month it was statistically insignificant as shown in table (3).This table also showed that the effect of mouthrinse on mean *S. mutans* count by time within each dmft group is statistically insignificant between the two groups.

Also a statistically significant correlation was shown between the effect of sodium fluoride mouth rinse and dmft in girls which was higher with dmft 0>2 and dmft >6 than dmft 3>6, while shown between the effect of sodium fluoride mouth rinse and dmft in girls which was higher with dmft 0>2 and dmft >6 than dmft 3>6, while propolis mouth rinse had a statistical significant effect in boys and in total number which was higher with dmft >6 than dmft 0>2 and 3>6 as shown in table (4) and fig.(5).

It was seen also in this table that the effect of mouth rinse on total changes in *S. mutans* count during the follow-up period within each dmft group

is statistically insignificant except at dmft >6 in boys and in total count, it was statistically significant (P value 0.01) and propolis had a higher effect than sodium fluoride mouth rinse.

**Table 3.** The effect of mouthrinses on *S. mutans* count during the follow-up period within each dmft group.

| | Group | Group I | | | Group II | | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **dmft** | Mean | ±S.D. | **dt** | Mean | ±S.D. | **dt** | P value |
| **Before** | 0 - 2 | 99138 | 84856 | **a** | 77911 | 79026 | **b** | 0.480 |
| | 3 - 6 | 136444 | 147166 | **a** | 103257 | 125034 | **b** | 0.641 |
| | >6 | 159500 | 79064 | **a** | 336800 | 139324 | **a** | 0.013* |
| **1 week** | 0 - 2 | 58923 | 57151 | **a** | 55244 | 59484 | **b** | 0.864 |
| | 3 - 6 | 53111 | 36785 | **a** | 47771 | 44425 | **b** | 0.796 |
| | >6 | 86800 | 61915 | **a** | 172000 | 81290 | **a** | 0.055 |
| **2 weeks** | 0 - 2 | 34797 | 43014 | **a** | 28444 | 34715 | **ab** | 0.653 |
| | 3 - 6 | 21511 | 25838 | **a** | 23737 | 32268 | **b** | 0.880 |
| | >6 | 41600 | 36817 | **a** | 58800 | 42464 | **a** | 0.455 |
| **1 month** | 0 - 2 | 17418 | 25949 | **ab** | 13700 | 21707 | **a** | 0.668 |
| | 3 - 6 | 5156 | 5718 | **b** | 7074 | 12811 | **a** | 0.693 |
| | >6 | 28500 | 26939 | **a** | 26480 | 41400 | **a** | 0.916 |

**Table 4.** Shows the effect of each group on total changes in *S. mutans* count (from 0 time to 1 month) within each dmft group.

| | Group | Group I | | | Group II | | | |
|---|---|---|---|---|---|---|---|---|
| **Sex** | dmft | Mean | ±S.D. | **dt** | Mean | ±S.D. | **dt** | P value |
| **Boys** | 0 - 2 | -93329 | 77189 | **a** | -57886 | 53828 | **b** | 0.320 |
| | 3 - 6 | -46640 | 21381 | **a** | -102656 | 147822 | **b** | 0.426 |
| | >6 | -74800 | 54416 | **a** | -337900 | 176462 | **a** | 0.053* |
| **Girls** | 0 - 2 | -55600 | 7462 | **a** | -68236 | 76228 | **a** | 0.752 |
| | 3 - 6 | -237100 | 173831 | **b** | -80000 | 66185 | **a** | 0.304 |
| | >6 | -164720 | 28485 | **a** | --- | --- | **a** | --- |
| **Total** | 0 - 2 | -81720 | 65685 | **a** | -64211 | 66840 | **b** | 0.474 |
| | 3 - 6 | -131289 | 147093 | **a** | -96183 | 124176 | **b** | 0.621 |
| | >6 | -131000 | 58953 | **a** | -310320 | 164795 | **a** | 0.015* |

---= No valid number is available for statistical analysis.
dt= Duncan's Multiple Range Test for the effect of dmft group.
*Means with the same letter within each column and time are not significantly different at p≥0.05.
*a has higher significance than b.

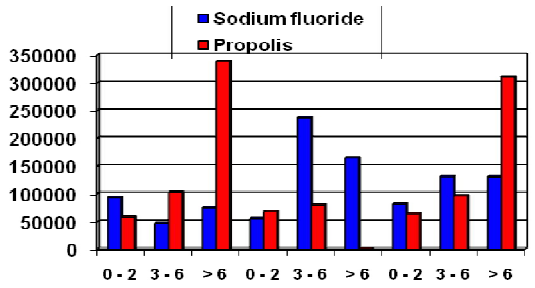


**Fig. 5.** Changes in salivary *S. mutans* count in different dmft groups during the follow-up period.

## 4. Discussion:

Dental caries is among the group of chronic diseases that are largely preventable by avoiding their risk factors, and much more improvement is expected from health interventions for the control of caries if performed appropriately**,** Fejerskov, (2004).

Prevention of dental caries in children is one of the hallmarks of contemporary pediatric dental practice. While there are multiple components of preventive dental programs developed by dentists for their child patients, perhaps none is as important and effective as the appropriate use of fluoride **,**Adair( 2006).

The use of mouth rinses to deliver chemotherapeutic agents is well accepted by the public, both by self administration and under supervision, mainly in school fluoride rinsing programs,Zero( 2006**).**

The present study was conducted to compare and evaluate the anticariogenic effect of two mouthrinses, sodium fluoride and propolis extract on the salivary *Streptococcus mutans* count in a group of Egyptian children.

The children's age were decided as they can easily rinse their mouth without swallowing the mouthwash to avoid any side effects as fluorosis, This agree with Adair(2006) who suggested that mouth rinses should be recommended only for those children who have demonstrated mastery of their swallowing reflex.

The results showed that the mean of the effect of using both mouth rinses on the log *S. mutans* count and on the change in *S. mutans* count in girls was statistically significant while the mean of the effect of using both mouthrinses on the *S. mutans* count in boys was statistically insignificant and on the change in *S. mutans* count in boys was statistically significant. This may be due to the importance of esthetics for the girls and their mothers and their high overall treatment response, also the degree of compliance of female patients is more than that of males. This result agreed with Nibras et al., (2007) who suggested that better oral health awareness and access to dental treatment is reflected more among girls than boys.

Also the results showed that the effect of using sodium fluoride mouthrinse was statistically significant after one month of use and had higher effect on children with higher caries index dmft > 6, this agreed with Jenkins et al.,(1994) who attributed the efficacy to its immediate bactericidal action during the time of application followed by a prolonged bacteriostatic action due to adsorption at the tooth surface, it also agreed with Clarkson and McLoughlin( 2000) who demonstrated that other effects of fluoride is the inhibition of glycolosis and reduction of extracellular polysaccharides production. Fluoride also have an effect on the cariogenic potential of *S. mutans*. In addition to fluoride's topical action on the inhibition of demineralization and promoting remineralization of enamel.

While the effect of using propolis mouthrinse was statistically significant during the first two weeks of use and had higher effect on children with higher caries index dmft >6 which agreed with Nishino(1996)who suggested that compounds from propolis strongly inhibited the growth, acid production and synthesis of insoluble glucan of *S. mutans* and also agreed with Duailibe (2007) who proposed that a significant reduction in the number of colonies is due to the effect of the propolis extract on bacterial growth.

Propolis mouth rinse had a significant effect on boys and on total count with dmft > 6 which agree with Koo et al., (2002) who hypothesized that propolis at low concentration can inhibit the growth of cariogenic bacteria and the activity of the glucosyl transferase enzyme which is associated with the pathogenesis of dental caries while it had an insignificant effect on girls which disagreed with Masaru et al., (2001) as they examined the antibacterial and the antiglucosyltransferase activity of propolis against *S. mutans* , and found that the growth of *S. mutans* was inhibited and the glucan synthesis was reduced.

While sodium fluoride had a significant effect on girls with dmft>6 and an insignificant effect on boys and on total count which agreed with Skold, (2005) who suggested that currently, in view of the widespread use of fluoridated toothpastes, the mouthwashes should only be used by individuals with high caries risk, and also agreed with Adair(2006) as he suggested that little additional benefit should be expected from fluoride mouthrinses in low-caries-risk children who are already using a fluoridated dentifrice.

Also in this study the results showed that the mean of the effect of both mouthrinses on the change in salivary *S. mutans* was statistically significant during the follow-up period, this agreed with the results of Kulkarni and Damle (2003) who revealed that sodium fluoride along with its antimicrobial property has remineralization potential and also agreed with Simone et al., (2006) who had found that their in vitro study indicated that at least two pathways by which propolis extracts exert cariostatic effect, first is the reduction of acid production of *S. mutans* and secondly the inhibition of the proton translocating F-ATP ase activities which is one of the mechanisms that *S. mutans* have developed to alleviate their influence of acidification.

It was conducted that the mean of the effect of both mouth rinses on *S. mutans* count during the follow-up period was statistically significant in girls and also the effect of each mouth rinse separately on the change in *S. mutans* count is statistically significant in total count which agreed with Ten Cate(1999) who have conducted that trace amounts of fluoride are effective in shifting the balance from demineralization to remineralization. This is attributed to the fluoride-enhanced precipitation of calcium phosphates, and the formation of fluorhydroxyapatite in the dental tissues. Also agreed with Sharma et al., (2004) who revealed that sodium fluoride inhibit carbohydrate utilization of oral

microorganisms by blocking enzymes involved in the bacterial glycolytic pathway.

Also agreed with Denise et al., (2004) who found that crude ethanolic extracts of propolis showed inhibitory doses against the glucosyltransferase of *S. mutans* and disagreed with Simone et al., (2006) ;William et al., (2006)whom suggested that these compounds may represent a virtually non-toxic means promising for controlling the acidogenic organisms associated with dental caries.

**References**


1. Adair SM. (2006): Evidence-based use of fluoride in contemporary pediatric dental practice. Pediatr. Dent; 28:133-142.
2. Camile SF.(2009): Mouthwashes.Aust Prescr; 32:162–4.
3. Clarkson J and McLoughlin J.( 2000):Role of fluoride in oral health promotion .Int Dent J; 50, 119–128.
4. Denise PL, Ademar AF, Ana Cristina MP, Jairo KB and Augusto CS. (2004): Comparative evaluation of in-Vitro effects of brazilian green Propolis and baccharis dracunculifolia extracts on cariogenic factors of *Streptococcus mutans*. Biol Pharm Bull; 27(11): 1834—1839.
5. Duailibe SD, Goncalves AG and Fernando JM. ( 2007):Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity. Antimicrobial agents and Chemotherapy; 46(5): 1302–1309.
6. Fejerskov O. (2004): Changing paradigms in concepts of dental caries: Consequences for oral health care . Caries Res; 38:182-191.
7. Jenkins S, Addy M and Newcombe RG.( 1994):Dose response of chlorhexidine against plaque and comparison with triclosan.J Clin Periodontol; 21: 250 – 255.
8. Koo H, Pedro LR, Jaime AC, Yong KP and William HB.( 2002):Effects of compounds found in propolis on *Streptococcus mutans* growth and on glucosyltransferase activity .Antimicrobial and Chemotherap; 46(5): 1302–1309.
9. Kulkarni VA and Damle SG.( 2003):Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the mutans streptococci count in saliva: An in vivo study .J Indian Soc Pedo Prev Dent; 21 (3):98-104.
10. Masaru S, Shuu F, Motohiko N, Ryozo Y, Chikako T, Hiroshi T, Hideo Y, Hiroyuki S and Kiyoshi O. (2001): Effect of propolis and propolis-containing toothpaste on the formation of dental plaque in vitro. Br Lib; 20(1): 5-10.
11. Nibras AM, Anne NÅ and Nils S.( 2007):
12. Dental caries prevalence and risk factors among 12-year old schoolchildren from Baghdad, Iraq: a post-war survey. Int Dent J; 57:36-44
13. Nishino M.( 1996):Anti-dental caries compounds in Brazilian propolis.Honeybee Sci.;17:151-154,
14. Parolia A, Manuel ST, Kundabala M and Mandakini M.( 2010):Propolis and its potential uses in oral health. Int J of Med and Med Sci;2(7): 210-215,
15. Poulsen S.( 2009):Fluoride-containing gels, mouth rinses and varnishes: An update of evidence of efficacy.European Archives of Paediatric Dentistry;10 (3).
16. Selwitz RH, Ismail AI and Pitts NB.( 2007):Dental caries.Lancet ; 369: 51-59.
17. Sharma U, Jain RL, Pathak A.( 2004):A Clinical Assessment Of The Effectiveness Of Mouthwashes In Comparison To ToothBrushing In Children.J Indian Sot Pedo Prev Dent.; 22 (2): 38 – 44.
18. Simone D, Pedro L, Rosalen R , Mitsue F, Hayacibara M, Jaime A, Cury B,
19. Sköld UM.( 2005):On caries prevalence and school-based fluoride programes in Swedish adolescents.
20. Swed Dent J; Suppl (178):11-75.
21. Statistical Analysis Systems (SAS) (1988):STAT/User's Guide, Release 6.03 ed., SAS Institute, Cary NC, USA,.
22. Ten Cate JM.( 1999):Current concepts on the theories of the mechanism of action of fluoride.Acta Odontol Scand; 57(6):325-329.
23. Ting PT and Silver S.( 2004)Allergic contact dermatitis to propolis .J Drugs Dermatol; 3(6):685-686.
24. Wander P.( 1995):Taking the sting out of dentistry.Dental Practice ; 25:3-8.
25. William HB, Marquis RE, Vera LG, Adilson S, Masaharu I and Hyun K. (2006):The influence of a novel propolis on mutans streptococci biofilms and caries development in rats.Biol Pharm Bull; 27(11) :1834—1839.
26. World Health Organization Oral health surveys (1997):Basic methods, 4a-ed.Geneva, WHO, 1-6.
27. Zero DT.( 2006):Dentifrices, mouthwashes, and remineralization /caries arrestment strategies.BMC Oral Health; Suppl,6 (1):9-17.