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In Vivo Study of The Effect of Two Dental Preparations Containing Basil And Eucalyptus Essential Oils on The Salivary Levels of Streptococcus Mutans And Lactobacillus

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Abstract: *The prevention of dental caries searches for possibilities to control the pathogenic microbial flora by using natural products with prolonged discharged of the active ingredients and good penetration of the biofilm. The objective is to establish the effect of two dental preparations containing basil and eucalyptus oils on the salivary levels of Str. Mutans and Lactobacillus in in-vivo study. The study includes 90 clinically healthy individuals, randomly distributed in three groups – control, H3-treated and L3-treated. The applications are done on days 1,3,5,7 and 9. The effect on the microbial levels is analyzed with CRT bacteria test with incubator. The time control periods are 0, 10 and 30 days. The use of dental preparations H3 and L3 shows reduction of the cases of high salivary levels of Str. Mutans and Lactobacillus, with lowest levels registered on day 10. Both groups treated with the preparations show increase of the salivary levels of Str. Mutans and Lactobacillus on the 30th day, however, they remain statistically significantly lower, compared to the initial levels (p<0,05). The dental preparations containing basil and eucalyptus essential oils reduce the salivary levels of Str. Mutans and Lactobacillus, in in-vivo study, with markedly stronger effect of the L3 preparation.*

Keywords: *cariogenic microorganisms, essential oils, antimicrobial action*

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I. Introduction

It is well-known fact, that the cariogenic microorganisms, the fermentable carbohydrates, the resistance of the hard dental tissues, the parameters of the saliva and the duration of its effect, play major role in the etiology of the dental caries [1,2]. Main pathological factors are acidogenic and acidoduric microorganisms with strong cariogenic potential, such as *Str. Mutans* and *Lactobacillus* with their synergic action [3,4]. *Lactobactllus,* which are considered to be responsible for the worsening and the progression of the caries process, especially in individuals using F- and chlorhexidine, are proven to be resistant to them [5,6,7]. The essential oils are established to have antimicrobial action against microorganisms resistant to antibiotics and chemotherapeutics [6,7,8,9]. Studies show, that pure essential oils have stronger antimicrobial action, than mixtures of their main components, as this suggests that the secondary components are critical for the synergic action, although there is antagonistic and complementary effect [8,10]. It is proven, that regardless of whether in combination, independently or as mixtures of purified components [11,12], the essential oils have effect on various biochemical processes in the microorganisms, with different interactive antimicrobial effects [8,13,14]. A number of studies report, that in mixtures of 2 or 3 essential oils, the antimicrobial activity changes, as different results have been obtained in in-vitro and in-vivo studies [8,15]. The lower in-vivo activity is explained with the inhibiting action of the immune system of the body.

II. Materials And Methods

Clinical study has been conducted following approval of the Scientific Ethics Committee of Medical University Plovdiv. All participants are informed in detail about the nature of the study, and have given their written consent to participate in it. Object of the study are 2 dental essential-oil preparations, designated as H3 – with hydrophilic vehicle and L3 – with hydrophobic vehicle, with the following composition: H3 – 40% basil oil, 40% eucalyptus oil, 20% polyethylene glycols (PEG_{4000}); L3 – 46% basil oil, 46% eucalyptus oil, 8% SiO_2 (Aerosil 200).

2.1 Patients

The study includes 90 clinically healthy individuals, aged between 20 and 40 years, with at least 20 natural teeth, of which at least 5 obturated, with normal oral hygiene, diet and lifestyle. Patients with information for general diseases and allegories, taking medications for the period of the study and at least one month before that, with clinical signs of inflammatory processes in the parodontium and the oral cavity, were not included. The participants were equally distributed randomly in 3 groups, as follows: Group 1 – controls; Group 2 – treated with H3 dental preparation; Group 3 – treated with L3 dental preparation.Before the beginning of the study (day 0), the initial microbial levels of all participants were registered, and professional hygiene was performed for removal of tartar and plaque. The participants were instructed to maintain personal oral hygiene using brush and toothpaste without active ingredients, morning and evening for 2 minutes, in usual way.

In Groups 2 and 3, the respective dental preparations were applied in thin even layer on all teeth using a small brush, on days 1, 3, 5, 7 and 9. The surfaces of the teeth were preliminary cleaned with hydrogen peroxide, than washed and dried. The isolation of the operating field was done with lignin rolls for the duration of the procedure, and 15 minutes after the applications. The participants were instructed not to eat and drink within 2 hours after the procedures.

2.2 Microbiological test

The salivary levels of *Streptococcus mutans* and *Lactobacillus* are measured with CRT bacteria (Ivoclar Vivadent) with incubator, as the control time periods are on day 0 (before the beginning of the study), day 10 (after the completion of the application), and day 30 (20 days after the last application).

The saliva samples are taken at least 2 hours after the oral-hygienic procedures. The collection of saliva is done within 5 minutes in sterile containers. It is stained in uniform layers on the food environments, and the containers are placed in incubator for 48 hours. The growth zones of the microorganisms are measured according to the test scales and photographed.

2.3Statistical analysis

The comparison of the subject proportions among the studied groups for any fixed time moment (observation day) was accomplished by using the Fisher's exact test. For each studied group the McNemar test was applied to distinguish any change in the subject proportions between two observation days. The used significance level was 5% ($p<0.05$). Calculations were made with MS Excel 2016 and software environment R (www.r-project.org).

III. Results

The results from the study of the salivary levels of *Lactobacillus,* in the tested time periods of the three controlled groups are shown in (Fig. 1), (Fig. 2) and (Fig. 3).

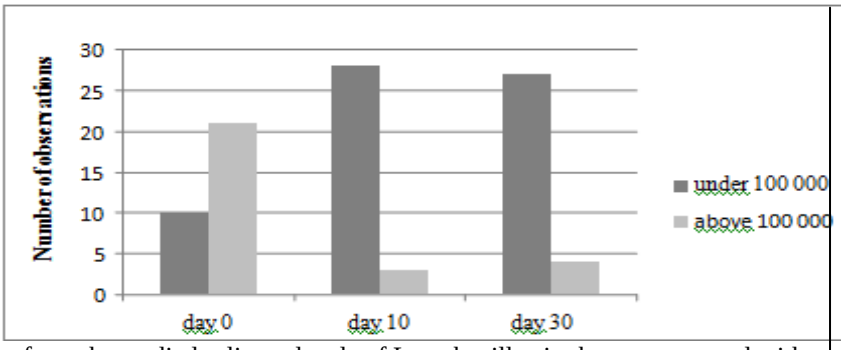

Figure 1. Results from the studied salivary levels of Lactobacillus in the group treated with product L3 in time

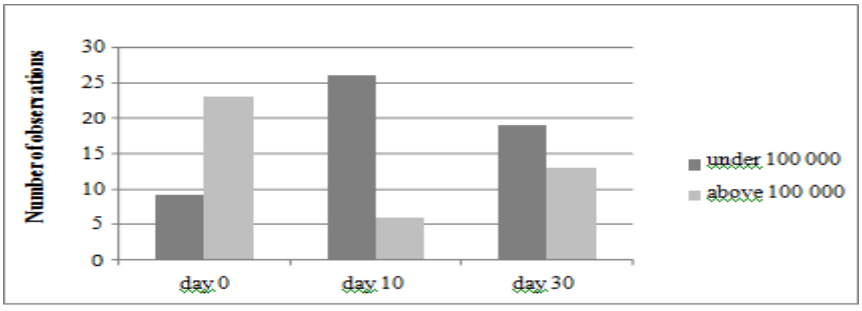

Figure 2. Results from the studied salivary levels of Lactobacillus in the control group in time

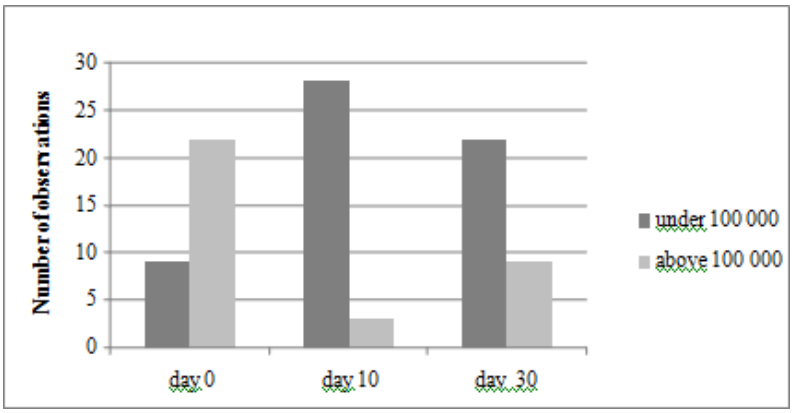

Figure 3. Results from the studied salivary levels of Lactobacillus in the group treated with product H3 in time

The comparison of the results between the three groups on day 0, shows that the values are commensurately close, which is confirmed also by the lack of statistically significant difference between them (p>0,05), which shows equality and equal distribution in the groups. The analysis of the results on the dynamics of the effect on salivary levels of *Lactobacillus* by groups shows:

1. The group treated with L3 – There is a dramatic reduction of the cases with high microbial levels, and an increase of the cases with under 100 000 U, which is mostly expressed between day 0 and day 10 (p-value=7,63.10^{-6}; p<0,05), remains unchanged between day 10 and 1 month (p-value=1,000; p>0,05), and remains significant compared to the initial level (p-value=1,53.10^{-5}; p<0,05).
2. The group treated with H3 – There is the same tendency of reduction of the number of cases with values above 100 000 U between day 0 and day 10 (p-value=3,81.10^{-6}; p<0,05). Despite the apparent increase of the cases with high microbial levels on month 1, there is not relevant difference (p-value=0,070; p>0,05), and the tendency remains significant (p-value=2,44.10^{-4}; p<0,05), compared to day 0.
3. The control group – There is apparent decrease of the cases with microbial levels above 100 000 U on day 10 (p-value=1,53.10^{-5}; p<0,05), however, unlike the treated groups, on month 1 there is again increase of these cases, as the difference is statistically significant (p-value=0,016; p<0,05).

(Fig. 4), (Fig. 5) and (Fig. 6) shows the results on the dynamics of the effect on salivary levels of *Str. mutans* by groups shows, in time.

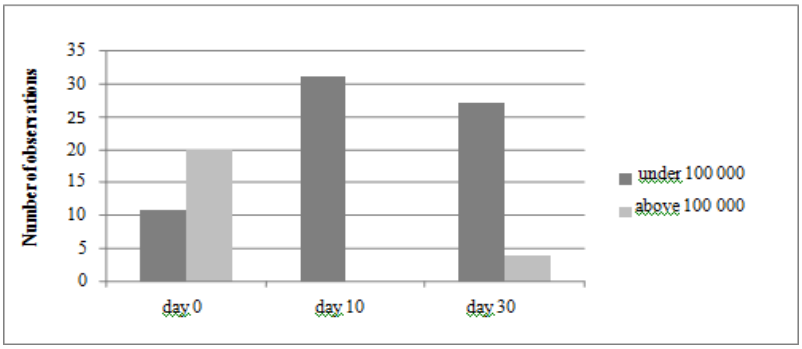

Figure 4. Results from the studied salivary levels of Str. mutans in the group treated with product L3 in time

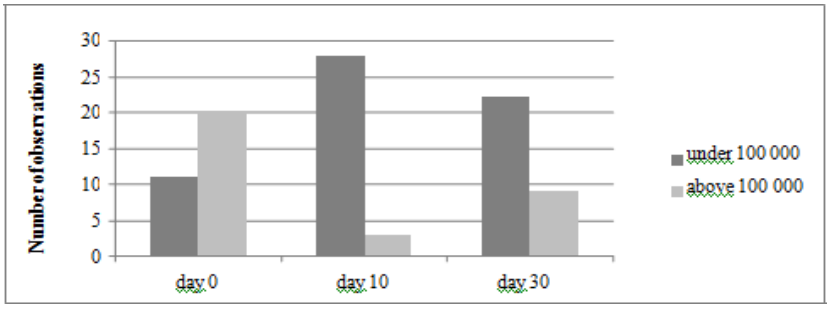

Figure 5. Results from the studied salivary levels of Str. mutans in the control group in time

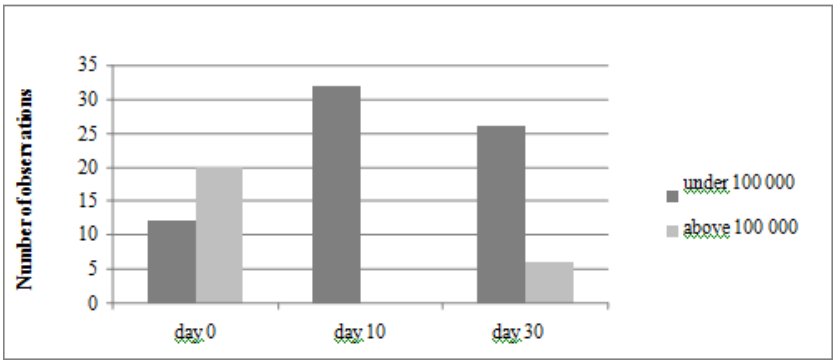

Figure 6. Results from the studied salivary levels of Str. mutans in the group treated with product H3 in time

The comparative analysis of the results on day 0, shows that the values in the three groups are almost identical (p>0,05), which shows equal distribution in the groups, and prevailing of the cases with microbial levels over 100 000 U. The analysis of the results concerning the salivary levels of *Str. mutans* by groups shows:

1. The group treated with L3 – There is a strong tendency towards reduction of the microbial levels, with maximum on day 10 (p-value=1,91.10^{-6}; p<0,05), with significant decrease on month 1 (p-value=0,125; p>0,05), but remains statistically significant compared to the initial level (p-value=2,44.10^{-4}; p<0,05).
2. The treated with H3 – there is significant increase of the cases with low microbial levels, which is most drastic on day 10 (p-value=1,91.10^{-6}; p<0,05), after which decreases on month 1 (p-value=0,031; p<0,05), but remains statistically significant compared to day 0 (p-value=1,22.10^{-4}; p<0,05).
3. The control group – there is reduction of the cases with high microbial levels on day 10 (p-value=1,53.10^{-5}; p<0,05). On month 1, however, there is apparent simultaneous increase of the cases of high microbial levels and reduction of the cases with low levels.

IV. Descussion

Streptococcus mutans and Lactobacillus are known to be associated with the formation of white carious spots [16], which are predictors of caries activity. During local changes, such as drop in the pH values and increased accumulation of biofilm, there is increased colonization of Streptococcus mutans, which create suitable acid environment for development of Lactobacillus. It is proven, that there is correlation between the presence of Str.muans and Lactobacillus in the saliva and in the dental plaque [17,18]. The last years, there is increased interest in the use of microbiological tests in relation with the prognosis and management of the dental caries [1]. The microbial levels of Streptococcus mutans and Lactobacillus in this study are tested with CRT bacteria, which allow their semi-quantitative determination in the saliva, and are known as "chair-side tests". The microbiological tests results in Karayasheva et al [17], show that 40% of the caries-active male and only 7.7% of the caries-active female with normal oral hygiene, show values of Streptococcus mutans over 100 000 U, i.e. high caries risk. The researchers believe that this is due to the better oral-hygiene care of women, compared to men. The study of the microbial levels on day 0 showed that there was predomination of the individuals with values over 100 000 U for both Streptococcus mutans, and Lactobacillus, which indicates that there is potentially high risk of development and progression of the carious process. The comparison of the results on day 0 and day 10, established reduction of the microbial levels in the treated and in the control group, which proves the importance of the mechanical causal therapy. Similar effect is observed also in a number of clinical tests of toothpastes and mouthwashes [19,20]. On month 1 is observed increase of the microbial levels, which is significantly higher in the control group, than in the treated groups. The obtained results prove the antimicrobial action of the preparations in in-vivo conditions, and maintenance of the results in time. These data is another prove, that the control on the biofilm only with mechanical means is insufficient for its management as a risk factor, and there is necessity of application of antimicrobial agents and regular prophylactic check-ups for individual control and management of the carious process.

We may conclude, that there is a permanent reduction of the microbial levels of Streptococcus mutans and Lactobacillus in the treated groups, as it is most apparent in the group treated with L3. The results are to be expected, considering its better adhesion to the surface of the teeth, and the prolonged discharge of the active ingredients during immediate contact with the biofilm. They also confirm the proved in number of studies properties of the essential oils, to inhibit the plaque formation, as they demonstrate not only antimicrobial effect, provided mostly by the aromatic agents, but also deeper penetration in the biofilm, compared to other agents, such as stannous fluoride and triclosan [5,8]. It is assumed, that the antimicrobial action of the essential oils is due to a large degree to the oxidized terpenoids, and to some hydrocarbonates [8,15], whose interaction could result in indifferent, antagonistic, complimentary or synergic effect [15,21]. The two tested preparations contain

basil oil, whose antimicrobial action is associated with the presence of eugenol and linalool, as synergic effect is observed against acidogenic microorganisms, especially in conditions with low pH levels in the oral cavity [22]. The results from the application of product H3 show, that it could be used as caries-protective agent, in cases of high salivary levels of *Streptococcus mutans* and *Lactobacillus*. The use of gel forms with capacity to hold to the tooth surfaces, slow wash off by the saliva and protracted discharge of the active ingredients, provide continuous antimicrobial effect in immediate proximity to the risk zones.

V. Conclusion

The use of dental preparations, with possible adhesion to the dental surfaces, slow washing by the saliva and prolonged release of the active ingredients, provides decreasing on the salivary levels of Streptococcus Mutans and Lactobacillus.

VI. Future Scope

The prevention and the non-operative treatment of the dental caries is risk patients with the use of antimicrobial agents for clinical topical application is part of the modern strategy for medical model and modern caries management.

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