# Assessment of the Effects of Several Products of Dental Use After Topical Administration to Different Mice Strain

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*Products frequently used in dental practice most often come into contact with tissues and cells and frequently produce their injury. The aim of the present study was to assess the effects of different products of dental use on cutaneous physiological parameters after topical application to hairless and colored mice. In order to conduct the present study, we used as materials the following solutions: Chlorhexidine digluconate 2%, Chlumsky and Walkhoff solutions and, also, SKH 1 and C57BL/6J mice. The mice were divided in 4 groups: control – no interventions, group 1 – Chlorhexidine solution was applied on the dorsal side of the mice, group 2 – mice were treated with Chlumsky solution and group 3 – mice that received Walkhoff. The treatment was applied daily for 5 days and skin parameters (melanin, erythema and hydration) were measured by the means of non-invasive techniques (mexametry and corneometry). After sacrification, samples of skin and internal organs were histopathological analyzed. Our data showed that topical administration of the three solutions was associated with changes in skin parameters values, such as: an increased erythema value and a decreased hydration status in all three groups as compared to control, but especially in the group of mice that received Walkhoff solution, results that were independent of mice strain. The toxicity of these solutions was confirmed by histological analysis.*

*Keywords: chlorhexidine, Chlumsky, Walkhoff, skin parameters*

In the dental practice are used several chemical compounds which exert some effects incompletely elucidated yet. Compounds used in this study are chlorhexidine digluconate, Chlumsky and Walkhoff solutions. Chlorhexidine is a strong base (fig. 1) that is commonly used due to the broad spectrum of action and it is known as the gold standard in the disinfectants class. In the dentistry field is used due to the broad antimicrobial/ antibacterial spectrum, for the treatment of endodontic and/or periodontal diseases [1], for gingivitis controlling, root canal disinfection and against plaque formation [2], because induces inhibition of the proteolytic activities of matrix metalloproteinase (MMP) [3, 4], is effective at low concentration and possess a very good absorption being retained in the oral cavity up to 12 h [5]. Digluconate salt is the most common preparation find in the domain of stomatology. Regarding the toxicity of this compound there are a number of studies both in vitro and in vivo that try to elucidate the mechanism of action, but so far this is incomplete unraveling. Faria et al. concluded that chlorhexidine exerts negative action on L929 fibroblasts via endoplasmic reticulum stress [6]. In another study, Gianelli et al. exposed some cell lines (osteoblastic, endothelial and fibroblastic) to different concentrations of chlorhexidine and found a high cytotoxic effect – induction of apoptosis, disturbance of mitochondrial function, an increase of calcium ions and oxidative stress [7]. The toxicity on Chinese hamster ovary cells was demonstrated by Li et al. using five different concentrations of chlorhexidine [8].

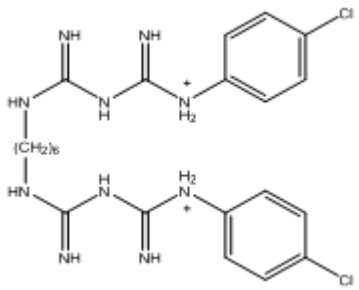

Fig. 1 Chemical structure of chlorhexidine

Chlumsky solution it is composed by phenol and camphor and it is found under the name of camphorated phenol being used as a disinfectant for the treatment of complicated caries and periodontal abscesses. The antibacterial effect of this was reported to be powerful in *vitro*, but somewhat inadequate when tested on infected channels demonstrating the need for prior calcium hydroxide in intracanal dressing station [9]. Walkhoff solution, whose name comes from the german dentist Otto Walkhoff, it has proven superior quality as disinfectant and is used as a reference standard in some research studies that tested the effectiveness of other types of disinfecting agents. In recent years were raised a number of questions related to the potential toxicity of this solution and its safe use, particularly because of the parachlorophenol found in his composition, although the original solution was described as weakly precipitating agent for proteins, non-teratogenic and non-carcinogenic [10,11].

So far no attention was paid to the effects of chlorhexidine, Chlumsky and Walkhoff solutions in cutaneous application, considering the fact they may come into contact with the skin. The objective of this study was to evaluate the effects of these solutions on the physiological parameters of the skin after topical application on two species of mice, SKH1 and C57BL/6J.

## Experimental part

### *Materials and methods*

The Chlumsky and Walkhoff solutions were purchased from SC Lucstar Prod SRL and chlorhexidine digluconat 2% (GLUCO-CHEX 2%) from PPH CERKAMED.

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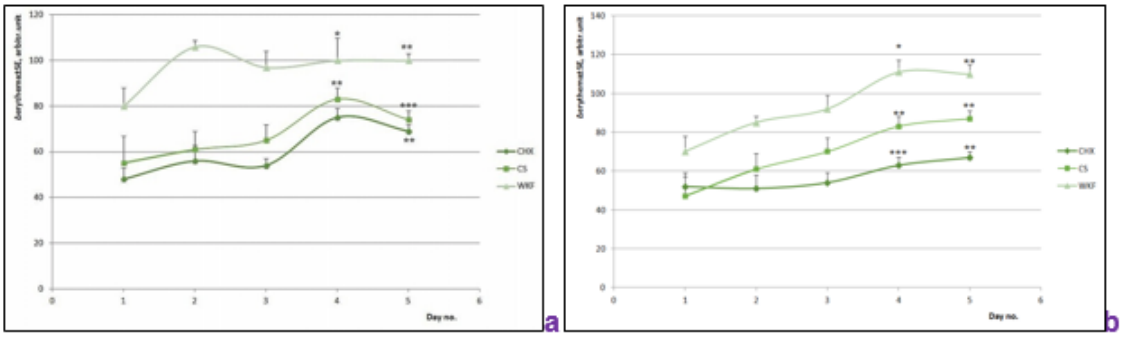


Fig. 2. Comparative evolutions of erythema at (a) SKH-1 mice, (b) C57BL/6J

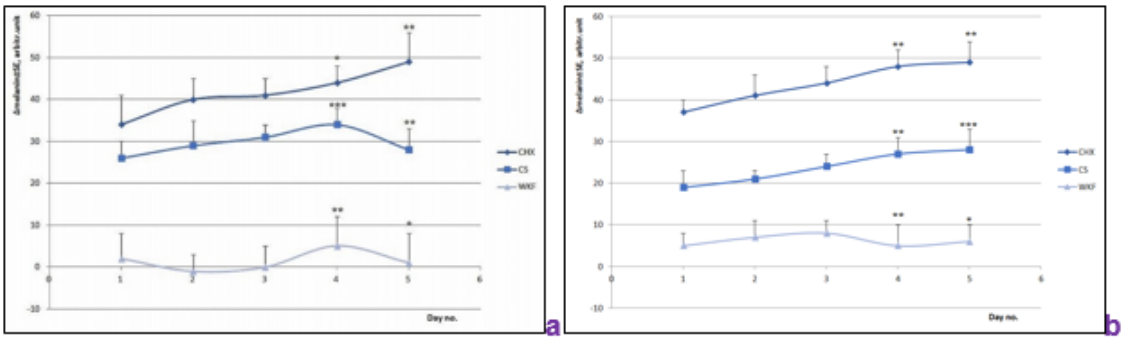


Fig. 3. Changes of melanin level at (a) SKH-1 mice, (b) C57BL/6J

SKH1 and C57BL/6J mice were obtained from Charles River Germany, female, 8 weeks. SKH1 mice were divided in 4 groups (4 mice/group): group 1 control group – no interventions, group 2 – mice treat with chlorhexidine, group 3 – mice treat with Chlumsky solution and group 4 – mice treat with Walkhoff solution. Each group of mice received topically the chemical agent, 1 application/day for 5 days. The same procedure was used for C57BL/6J mice.

At cutaneous level were determined skin's physiological parameter values: melanin, erythema and hydration using mexametry, tewametry and corneometry techniques. The measurements were carried out with a Multiprobe Adapter System (MPA5), Mexameter® MX 18 and Tewameter® TM300 all from Courage-Khazaka, Germany. The haemoglobin values for erytema were measured using 2 wavelengths: 560 and 660 nm [12-16]. We used their general units obtained by Mexameter soft evaluation and not the index as value. The applied area was between 4-5 mm diameters for 15 seconds.

The mice were sacrificed after 7 days. The biopsies from skin and internal organs were harvested. Specimens were fixed in 4% v/v buffered formalin and prepared in the routine histological technique. Three micrometres serial sections were cut and put on histological slides. For morphological evaluation we used hematoxylin and eosin stain (H&E stain) purchased by Merck Company. The histological evaluation was done using a Leica DM750 light microscope and the pictures were captured through Leica DMShare system.

*Statistical analysis*

Data were analyzed using paired Student's *t* tests or One-way Anova followed by Bonferroni's post-tests were used to determine the statistical difference between experimental and control groups; *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$.

## Results and discussions

Erythema, the most important parameter used to evaluate of the skin inflammation, presents slight increases of its values (fig. 2); the most important change was observed in the case of C57BL/6J mice treated with Walkhoff solution (around 40 units) and this is due to an important irritation effect.

Figure 3 shows the modifications of skin pigmentation. It can be seen that melanin values are almost constant for those mice which were treated with Walkhoff solution. In the other two cases, slight increases can be observed.

The increase of transepidermal water loss is a sign of a disruption of the barrier function of skin. No important changes of TEWL values were obtained in the case of SKH-1 mice, but a general ascendant trend of these values was seen in the case of C57BL/6J treated with these solutions (fig. 4).

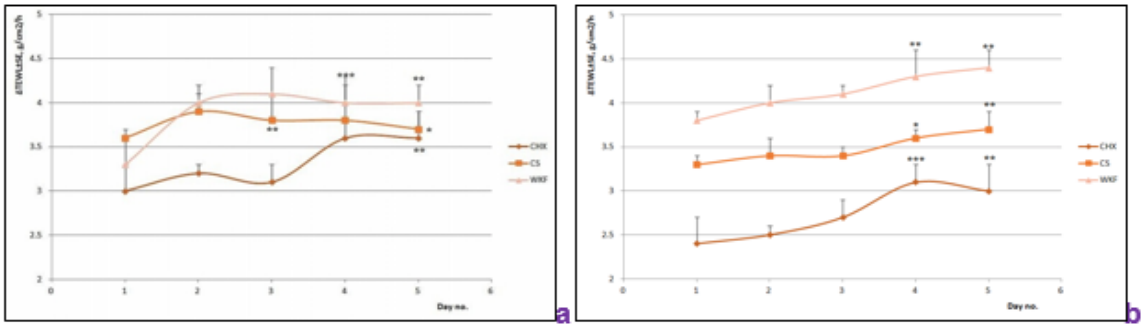


Fig. 4. Comparative evolutions of TEWL at (a) SKH-1 mice, (b) C57BL/6J

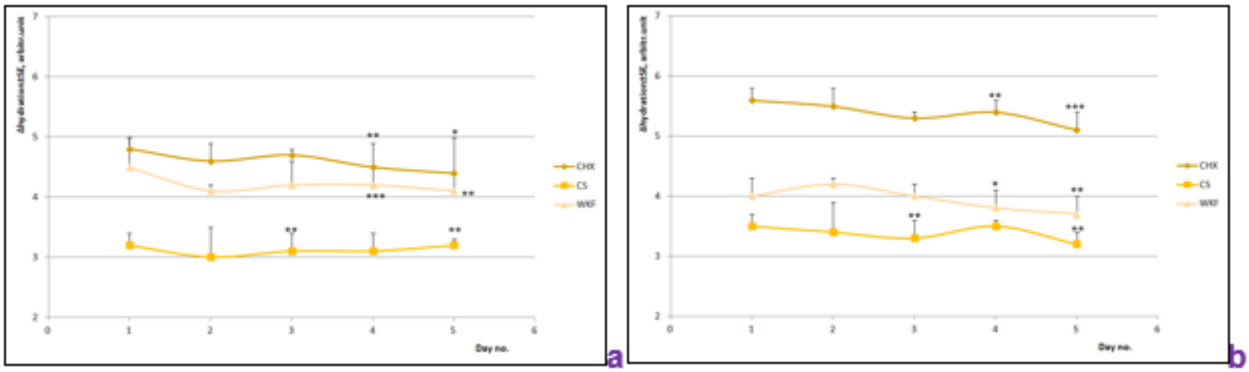


Fig. 5. Evolutions of hydration of *stratum corneum* at (a) SKH-1 mice, (b) C57BL/6J

Decreases of the hydration level of *stratum corneum* were recorded for almost all mice (fig. 5). It can be appreciated that decreases measured for C57BL/6J mice are more significant that decreases obtained in the case of SKH-1 and this is probably due to the difference of mice sensitivity.

## Conclusions

Based on the evaluations of skin parameters, it can be assumed that these solutions present a very slight pigmentation effect; Walkhoff solution seems to have the most irritation effects, and C57BL/6J mice present more clear changes of measured parameters.

*Aknowlegement: This work was supported by internal grant at UMFT Victor Babes PIII-C4-PCFI-2016/2017-04*

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Manuscript received: 23.10.2016