

USE OF ANTHOCYANIN EXTRACT FROM WINE PRODUCTS IN THE PREVENTION OF EXPERIMENTAL DENTAL CARIES

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Research objective: evaluation of the effectiveness of the use of anthocyanin extract obtained from wine products in the prevention of experimental dental caries. Materials and methods. The experimental model has included three series of experiments, dental caries has been modeled according to the method described by Muller K. P. et al, 2007 (Group 2 and 3). In order to prevent dental caries in the rats from Group 3, there has been topically applied an anthocyanin extract obtained from wine products. The animals from Group 1 were fed with the standard vivarium ration. The frequency of dental caries, the number of cavities and the surfaces affected by caries have been evaluated per animal, and there has been determined the surface roughness index of the dental enamel. The dental caries reduction index (IR) in the studied group (Group 3) was evaluated in comparison to Group 2. The experiments were conducted in accordance with the recommendations of the Committee of Research Ethics of SUMP "Nicolae Testemițanu". Results. The modelling of dental caries had a significant cariogenic impact, the number of carious cavities per animal in Group 2 was 4.10 ± 0.366 , $p < 0.001$, in comparison to Group 1 (0.03 ± 0.033). In Group 3, the number of carious cavities has decreased by 1.62 times ($p < 0.01$) in comparison to Group 2. The three-dimensional scanning of the enamel samples has revealed a decrease in the surface roughness index by 2.13 times ($p < 0.001$) in comparison to Group 2. The reduction index (RI) in Group 3 represented 56.1% in comparison to Group 2, indicating a sufficient cariopreventive effect. Conclusions. Use of topical applications of anthocyanin extract from wine products represents an important step in reducing the aggressiveness of the dental biofilm and in dental caries control. Therefore, it is necessary to conduct further studies regarding the opportunities of increasing the effectiveness of the preventive measures that can provide a superior carioprotective effect.

Keywords: *dental caries prevention; anthocyanins; experimental dental caries; enamel.*

Preclinical studies are an important step for assessing the efficiency of new methods of dental caries control which will then be used in clinical trials. Experimental modeling of pathological processes, including dental caries is commonly used in current biomedical research, taking advantage of the possibility of investigating the tissues and the subcellular structures. According to multiple researchers, the modeling of experimental dental caries under the influence of the most common risk factors (high number of acetogenic bacteria in the dental biofilm, lack of a proper oral hygiene and a diet high in refined carbohydrates) conducted in a laboratory, is in fact able to create similar conditions to those observed during the development of dental caries [1-5]. The wine polyphenol, especially anthocyanins are responsible for the beneficial physiological effects, such as immunomodulatory and antioxidant actions, intracellular metal chelation, the capacity of the enzyme modulation, inhibition of transcription factors and many others [6, 7]. A large number

of studies report that in the oral cavity the anthocyanins prevents biofilm formation, inhibit the proteolytic activity, inhibit glucosyltransferases activity, shows the inhibition of adherence on hard surfaces and inhibition of acid production from glucides. All of these activities together generates a good anti-cariogenic properties [6-8].

It has been shown that the grape extracts inhibits the synthesis of insoluble glucans from sucrose controlled by glucosyltransferase (GTF) produced by *Streptococcus mutans* and *Streptococcus sobrinus* and the activity of F-ATP-ase, and in addition to that it affects the production of acids by *Streptococcus mutans*. Moreover, anthocyanins cause a decrease of the hydrophobic properties of cell surface and diminishes the aggregation properties for several *Streptococcus* species: *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus gordonii* and *Streptococcus sanguinis* [9-14]. A large variety of polyphenolic compounds, including anthocyanins, that are able to control dental caries have been studied, however only a limited number of compounds are readily available from natural products, mainly due to the efficiency and stability of the compound, organoleptic properties (smell, taste) and economic feasibility of its production [15]. The purpose of this paper is to study the anti-cariogenic effect of an crude anthocyanins extract from marc rapes of the traditional winery varieties grown here.

Materials and methods. In line with the proposed research objectives, the study was conducted on a sample of 90 white rats (*Rata albicans*). An experimental model was developed that included three series of experiments. In each group there were included 30 rats, which is the minimum number required for achieving the proposed objectives and for obtaining statistically significant results. In the first series of experiments, the dental caries was modelled by administering daily a particular cariogenic food ration, with a 54% refined carbohydrates content [5]. In order to increase the cariogenic effect, the rats were given a solution of 10% sucrose *ad libitum* [4]. During the first three consecutive days, the animals were orally infected with a suspension containing *Streptococcus mutans* $\times 10^7$, based on the method described by Muller K. et al. (2007) [5]. The cariogenic diet was administered throughout the experiment. Animals from all experimental groups except the control group (L₀) were deprived throughout the study of food that may favor self-cleaning of the teeth (wheat grains, fruits and vegetables) and weren't given any supplements that may prevent the development of dental caries. For the prevention of dental caries in rats from the Group 3 there has been topically applied a 0.5 ml of 5.0% anthocyanins extract obtained from wine byproducts [14]. The experimental protocol was following the „3R” principle (replacement, reduction and refinement) [16], that expresses the concerns of the worldwide community of researchers regarding the standardization of animal testing. Throughout the study, the animals were maintained at the Vivarium of State University of Medicine and Pharmacy of the Republic of Moldova “Nicolae Testemițanu”. The vivarium spaces allow division based on activity type: maintenance, quarantine and conducting the experimental procedures. Animals were maintained under standard conditions, food and water being available *ad libitum*. The impact of experimental modelling of dental caries and the preventive efficiency of topic use of the anthocyanins extract was evaluated by determining the dental caries rate, the number of cavities and areas

affected by caries for each animal and determining the surface roughness of the enamel. In order to study the microstructure and morphology of enamel in the rat molars, there was used the scanning electron microscopy (SEM) method by means of the VEGA TESCAN TS 5130 MM equipment. We examined the surface roughness of the enamel samples by performing three-dimensional scanning by means of atomic force microscopy – AFM, the results being expressed as values of average surface roughness. The study was approved by the Committee for Research Ethics of the State University of Medicine and Pharmacy of the Republic of Moldova “Nicolae Testemițanu” (positive approval from 14.11.2011). Data analysis was performed using the functions and modules of the SPSS v. 16.0 (IBM, USA) and Excel (Microsoft, USA) software.

Results. After 60 days of observation, in $96.67 \pm 3.28\%$ of rats from the Group 1, there weren't observed any carious cavities or areas of the demineralization of the dental enamel. After modeling dental caries by administering a cariogenic diet and by infecting the oral cavity with a suspension containing a culture of cariogenic strains, after 60 days in the majority of animals there were detected carious cavities or areas of demineralization of the dental enamel. Thus, the frequency of dental caries in animals from the Group 2 was $93.33 \pm 4.55\%$, contrasting with the detection of a single cavity in the control group (Group 1) – $3.33 \pm 3.28\%$. The electro-optic research of the enamel surface in laboratory animals from the Group 1 shows a lesser pronounced granular surface. In some sectors there can be found micro-craters, micro-protrusions, crystals of different shapes and sizes and microfissures. In some cases, there can be observed isolated and grouped micro-craters and micro-protrusions, which correspond to the ends of enamel prisms. In the enamel samples taken from the experimental animals, there were found in the control group numerous areas of demineralization of the enamel, which have an irregular topography, characterized by the abundance of micropores and the disorganization of hydroxyapatite crystals, on some electronograms there can be observed an increase in the number of craters, which proves the increase in the porosity of the enamel. On the three-dimensional scanning of the enamel samples there was found a significant increase of the surface roughness, from an average roughness value of $1.17 \pm 0.084 \mu\text{m}$ in Group 1, to an increase of the index in Group 2 – $6.47 \pm 0.481 \mu\text{m}$ ($p < 0.001$).

The experimental modeling of dental caries has a significant cariogenic impact, the number of cavities for each animal in Group 2 is 4.10 ± 0.366 , $p < 0.001$, the number of decayed surfaces/animal – 5.2 ± 0.530 , $p < 0.001$, in contrast to the values observed in the control group. According to several authors, the dental caries modeling has had a sufficient cariogenic impact if the frequency of dental caries exceeds 80-85% in the group of studied animals [3, 4]. Therefore, the estimated high degree of dental caries in animals from Group 2 is equated with the high/extreme caries risk and is sufficient for comparing the efficiency of preventive methods that were studied in further experimental series. In Group 3, after 60 days of observation, as a result of the preventive treatment that was carried out, the number of cavities was reduced significantly by a 1.62 fold ($p < 0.01$) in comparison to the control group (Group 2). Three-dimensional scanning of enamel samples has shown a significant reduction of surface roughness by a 2.13 fold ($p < 0,001$) in comparison to Group 2. Following the topical application of the anthocyanins extract, there have not been

found any specific changes of the surface enamel by using electro-optic investigation (SEM) and three-dimensional scanning (MFA), thus there is observed an anti-adhesion and anti-biofilm activity against *Streptococcus mutans*, as it was proven in several other studies [7, 8, 10-14, 17-20].

The preventive efficiency of the topical application of the anthocyanins extract 5.0% (pH 8.0-9.0) in experimental animals (Group 3) was measured by estimating the dental caries reduction index (RI) in comparison to the control group (Group 2), which constituted 56.1%, which indicates a sufficient preventive effect. As a result of topical application of the anthocyanins extract in laboratory animals, there has been observed a significant reduction of dental caries and in the enamel roughness in comparison to Group 2, as well as there are significant differences of the aforementioned indices in comparison to the non-experimental group (Group 1).

Conclusions. In this study, there has been experimentally modelled dental caries and there was evaluated the efficiency of experimental use of an anthocyanins extract for preventing dental caries in animals. As a result of topical application of the anthocyanins extract in laboratory animals, there has been observed a significant reduction of dental caries and in the enamel roughness in comparison to the control group, as well as there are significant differences of the aforementioned indices in comparison to the non-experimental group. The use by local applications of the anthocyanins extract with anti-bacterial and anti-adherence effects on dental biofilm, antioxidant and immunomodulatory actions, is an important step in reducing of the risk factors and dental caries control. Therefore, there are needed further studies in order to research opportunities of increasing the efficiency of preventive measures that can ensure a higher anti-cariogenic effect.

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