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STANDARDIZED BLACK CHOKEBERRY (*Aronia melanocarpa* L.) EXTRACT SUPPLEMENTATION REDUCES THE SALIVARY ROS LEVELS IN ORAL LICHEN PLANUS PATIENTS

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ABSTRACT

The oxidative stress has a role in etiopathogenesis of oral lichen planus (OLP). Polyphenols renowned for their antioxidative, anti-inflammatory and immunomodulatory properties, have gained attention in autoimmune diseases management. Saliva, reflecting salivary gland health, offers promise for biomarker research and treatment monitoring. The aim of this study was to evaluate salivary levels of reactive oxygen species (ROS) in OLP patients using polyphenol rich, standardized *Aronia melanocarpa* extract (SAE). The ROS values were compared in pre- and post-treatment and significant differences were found, indicating the decrease of ROS after four-week supplementation with SAE.

INTRODUCTION

Plant foods, including fruits, vegetables and their juices, are primary source of exogenous antioxidant compounds that significantly contribute to the modulation of oxidative balance *in vivo*. A growing body of preclinical and clinical research has identified benefits associated with the consumption of berry fruits. They are a rich source of antioxidant constituents, and a number of evidence suggests that the consumption of berry fruits correlates with a lower risk of developing of broad range of pathological conditions mediated by uncontrolled oxidative processes [1]. Black chokeberry, *Aronia melanocarpa* (Michx.) Ell. (Rosaceae) is a rich source of phenolic substances, mainly flavonoids from the anthocyanin subclass which are responsible for its high antioxidant activity [2]. The results from both *in vitro* and *in vivo* studies indicate numerous health-promoting

activities of black chokeberry products: antioxidative, anti-inflammatory, immunomodulatory, antimutagenic, anticancer, cardioprotective, hepatoprotective, antidiabetic, antibacterial and antiviral [3,4].

Oral lichen planus (OLP) is a common affliction of the oral mucosa, affecting 0.5% to 2% of the population, primarily middle-aged women, with a 0.44% risk of malignant transformation. Its etiology involves immune dysregulation, microbial infections, endocrine imbalances, and microcirculatory disturbances. OLP pathogenesis is closely related to oxidative stress, evidenced by elevated oxidative damage markers and diminished cell antioxidants [5]. Standardized *Aronia melanocarpa* extract (SAE) is a commercial product in the form of a solution for oral use, rich in polyphenols and anthocyanins, with previously confirmed antioxidative and anti-inflammatory activity [6,7].

The study aimed to examine the effect of 4-week supplementation of SAE on reactive oxygen species (ROS) levels in saliva of OLP patients.

EXPERIMENTAL

Standardized Aronia extract (SAE) is the product of the pharmaceutical company Pharmanova, Belgrade, Serbia (A-LIXIR®400 PROTECT). The extraction procedure was done by the EU-Chem Company (Belgrade, Serbia). Black chokeberry used for extraction originated from Poland and was purchased from a licensed manufacturer. SAE is a solution for oral use rich in polyphenols (431 mg/30 mL), anthocyanins (120 mg/30 mL), potassium sorbate (35.1 mg/30 mL) and low content of ethanol (0.04% V/V).

Patients' data and collection of whole human saliva

In total, 12 patients diagnosed with OLP were recruited for the study. Supplementation of SAE was once *per* day, in a dose of 30 mL, for 28 consecutive days. Collection of saliva was performed using the passive drooling technique into a pre-chilled falcon. Saliva was then centrifuged, and the protease inhibitor was added to the sample followed by the estimation of protein concentration using the Bicinchoninic Acid (BCA Protein Assay kit) method according to the manufacturer's (ThermoScientificUSA) protocol, and the samples were then stored at -80°C until further use.

H2DCFDA assay (2',7'-dichlorofluorescin diacetate)

Salivary ROS levels were assessed using a fluorescent oxidation-sensitive probe, H2DCFDA (Merck Millipore, 2′,7′-Dichlorofluorescin Diacetate - CAS 4091-99-0 - Calbiochem). This reagent is cleaved by intracellular esterase enzymes, resulting in the formation of fluorescent 2,7-dichlorofluorescein, in the presence of ROS. The intensity of fluorescence is directly proportional to the ROS levels. For this experiment, saliva samples were mixed with 2 mM H2DCFDA and incubated in amber tubes in the dark at room temperature for 40 minutes. Following incubation, 2 ml of PBS was added to the tubes and 200 µl of each sample was added to a 96-well plate (PBS was used as blank). The generation of

salivary ROS level was determined by measuring the fluorescence on a fluorescent plate reader (Wallac 1420 multilabel counter Victor 3V) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. Data were expressed as relative fluorescence intensity (RFI) and the results were normalized to the protein concentration (RFI/μg protein).

Statistical Analysis

The Wilcoxon matched-pairs signed rank test was used as a nonparametric method to assess differences in pre- versus post-treatment samples, after data were tested for normality. All results are expressed as median with interquartile range (IQR). GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis, where $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

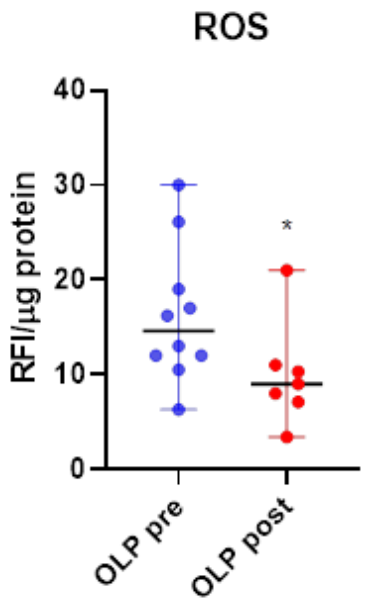

Figure 1. Effects of pre- and post-treatment with *Aronia melanocarpa* extract on the reactive oxygen species (ROS) levels (expressed as relative fluorescence intensity-RFI) in oral lichen planus (OLP) patients' saliva, * $p<0.05$.

Presented results (Figure 1) show that the median (IQR) ROS level was 17 (13 to 26.1) RFI/μg protein in salivary samples of OLP patients before the supplementation. Following the supplementation with SAE, in post-treated salivary samples the measured median (IQR) ROS level was 9 (7.1 to 11) RFI/μg protein. When the pre- and post-treatment values were compared, significant differences were found ($p< 0.0313$), indicating a decrease of ROS levels after treatment with SAE.

CONCLUSION

It could be concluded that four-week supplementation with standardized *Aronia melanocarpa* extract improves the antioxidant status in OLP individuals and reduces oxidative stress in saliva. Further studies with a large sample size are required for determining its usefulness. Also, these results indicate that salivary ROS levels could be measured as a putative non-invasive parameter for assessing oxidative stress levels after therapeutic interventions in autoimmune diseases with oral manifestation.

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