NATURAL COSMETICS: HARNESSING THE ANTIGENOTOXIC EFFECTS OF ELDERBERRY IN *DROSOPHILA MELANOGASTER* AND HUMAN LYMPHOCYTES USING COMET ASSAY

Sara Gonçalves and Isabel Gaivão, CECAV and Department of Genetics and Biotechnology, Trás-os-Montes and Alto Douro University, Vila Real, Portugal; Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Vila Real, Portugal

sgoncalves@utad.pt

RESUMO

Elderberry is known for its antioxidant and anti-inflammatory properties and has been suggested to have potential genoprotective effects. In this study, we evaluated the antigenotoxicological effects of elderberry using the Comet assay in *Drosophila melanogaster* and human lymphocytes. Four concentrations of elderberry extract (1%, 5%, 10%, and 15% w/v) were tested in both cell types. Hydrogen peroxide (H_2O_2) and streptonigrin (SN) were used as DNA-damaging agents. In the *in vitro Drosophila* assay, the results showed a reduction in DNA damage in the elderberry extract-treated groups compared to the SN group. In the *in vivo* human lymphocyte assay, the elderberry extract-treated groups showed lower levels of DNA damage compared to the H_2O_2 and SN groups. These findings shows that elderberry has potential antigenotoxiological effects in both *Drosophila melanogaster* and human lymphocytes. The use of elderberry in cosmetics may provide a natural alternative for genoprotection. Further studies are needed to investigate the mechanism of action and the long-term effects of elderberry on genoprotection.

Palavras-chave: Antigenotoxicity; Comet assay; Cosmetics; Elderberry; Human lymphocytes

INTRODUTION

Natural cosmetics are becoming increasingly popular among consumers who are concerned about the potential negative effects of synthetic chemicals on their health and the environment. Natural cosmetics are formulated with ingredients sourced from natural, renewable resources and are free from synthetic chemicals, such as parabens, phthalates, and synthetic fragrances. The use of natural ingredients in cosmetics has been shown to have several benefits, such as providing antioxidants, vitamins, and minerals that nourish and protect the skin, and reducing the risk of adverse skin reactions. Natural ingredients, derived from plants, minerals, and other natural sources, are believed to be safer and more environmentally friendly than synthetic

chemicals commonly used in cosmetics. The use of natural ingredients in cosmetics has gained popularity due to consumer demand for safer and more sustainable products (Liu, 2022). The demand for natural cosmetics has increased due to the growing awareness of the potential health risks associated with the use of synthetic chemicals in cosmetics, such as allergic reactions, hormone disruption, and even cancer. The use of natural ingredients in cosmetics has gained attention due to their therapeutic properties, such as anti-inflammatory, antimicrobial, and antioxidant activities (Mukherjee et al., 2011).

Natural ingredients not only provide benefits to the skin but also support the sustainable use of natural resources, reduce the environmental impact of cosmetics production, and support local communities. The development of natural cosmetics requires a deeper understanding of the biological and physicochemical properties of natural ingredients, as well as their interactions with skin cells and microorganisms. The aim of the study was to evaluate the potential genoprotective effects of elderberry in two different model systems, *Drosophila melanogaster* and human lymphocytes, using the Comet assay.

METODOLOGIA

The Trás-os-Montes region in Portugal is abundant in natural ingredients such as elder flowers and berries (*Sambucus nigra*). This area is known for its favorable microclimate and agricultural diversity, making it the region with the most organic farmers in Portugal. The natural ingredients used in the experiments were obtained from organic farmers and ground into particles <2 mm before being frozen at -18 ºC until further analysis.

D. melanogaster Oregon K (Ok) strain was chosen since it has a low antioxidant enzymatic activity and is, therefore, more sensitive for this study (Gaivão & Comendador, 1996). For the genotoxicity assay, crossings were made to obtain heterozygous offspring (w/w^+). Flies were kept in an incubator at 24°C and were anesthetised by etherisation when necessary. The comet assay utilized a previously described method to evaluate the genotoxic effects of various natural ingredients (Sierra & Gaivão, 2014). Larvae were exposed to different quantities of the ingredient, and their brain ganglia were extracted for analysis. After neuroblast maceration and agarose solidification, the slides were immersed in a lysis solution and electrophoresed. Slides were then stained and analyzed using fluorescence microscopy, and the percentage of tail DNA and tail length were scored. 50 cells per gel were observed, and the final score (expressed as

"arbitrary units" in a range of 0—400) was obtained by multiplying the mean percentage of nucleoids in each class by the appropriate factor This method provides a reliable way to assess the genotoxicity of natural ingredients.

As for the assay in human lymphocytes, four different concentrations (1%, 5%, 10%, and 15% w/v) of elderberry were chosen based on previous research. Two treatments were prepared: one with H_2O_2 and the other with Streptonigrin (SN). For the H_2O_2 treatment, 50 µL of H_2O_2 was dissolved in 0.5 L of PBS, and six Coplin jars were prepared. One jar contained only PBS, the second only H_2O_2, and the remaining four had different elderberry concentrations with the H_2O_2 treatment. Two slides were prepared for each condition, with one following normal protocol and the other undergoing enzymatic incubation. For the SN treatment, a final concentration of 20 µM was achieved by dissolving SN in PBS. Two slides were prepared for each condition, with one following normal protocol and the other undergoing enzymatic incubation. These methods provide a reliable way to assess the effects of elderberry and SN treatments on DNA damage. The comet assay followed the described method (Sierra & Gaivão, 2014).

RESULTADOS E DISCUSSÕES

As for the assay in *D. melanogaster*, the streptonigrin-challenged group exhibited overall increased DNA damage. Flies fed with C and challenged with streptonigrin presented the highest levels of DNA damage, while flies fed with Eb showed the lowest levels in both unchallenged and streptonigrin-challenged groups (Figure 1).

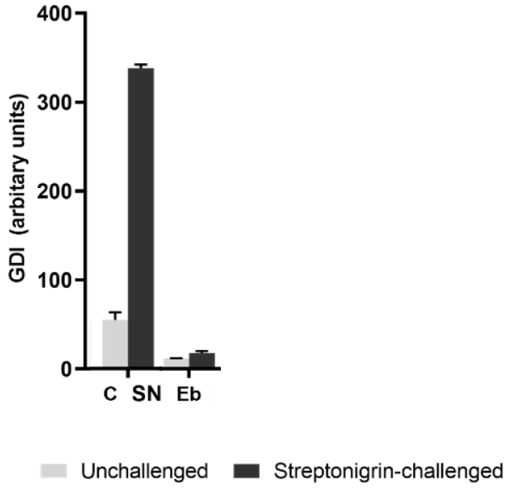

Figure 1. Mean values of DNA damage (GDI) in drosophila neuroblasts measured by the *in vivo* comet assay in unchallenged and streptonigrin-challenged groups. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry;). Grey bars correspond to unchallenged groups, and black bars correspond to streptonigrin-challenged groups. Values are mean ± SEM (n = 2).

Regarding the assay in human lymphocytes, in the analysis of Genomic Damage Index (GDI), the group challenged with H_2O_2 exhibited a significant increase in DNA damage compared to the control group (C) and the H_2O_2 group (C+), as depicted in Figure 2 (GDI). However, treatment with elderberry resulted in lower levels of DNA damage. Notably, the H_2O_2 - challenged groups treated with elderberry showed a significant reduction in GDI relative to the C+ group. The GDI with Fpg data (Figure 2 (ENZ)) revealed that the 5% elderberry treatment produced the most effective result.

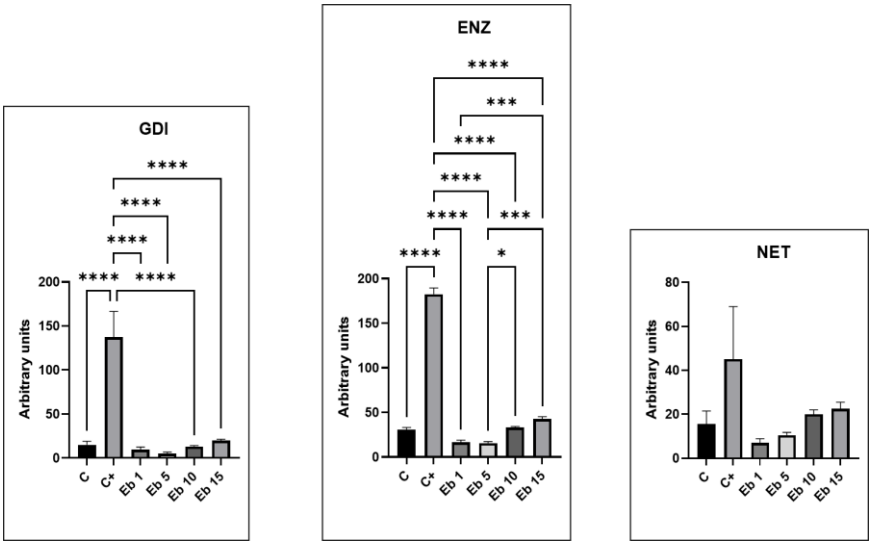

Figure 2. GDI: Mean values of DNA damage (GDI, expressed as arbitrary units) in human lymphocytes measured by the *in vivo* comet assay in unchallenged and H_2O_2-challenged groups. C corresponds to the control group, with only PBS treatment, and C+ corresponds to the group treated only with H_2O_2. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry) and the percentage of elderberry used (1:1%, 5:5%, 10: 10%, and 15: 15%). Bars represent the standard error. ENZ + NET: Mean values of oxidative purine DNA damage, measured by the improved comet assay in human lymphocytes after elderberry treatment (four levels of supplementation – 1, 5, 10 and 15%, transposed to the group identifier abbreviation as the number preceding the letters Eb). C corresponds to the control group, with only PBS treatment, and C+ corresponds to the group treated only with H2O2. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry). (ENZ) Overall damage using Fpg. (NET) Net Fpg-sensitive sites. Bars represent the standard error.

The SN-challenged group exhibited increase DNA damage (Figure 3). Human lymphocytes in the control group (C) and in the SN group (C+) presented the highest levels of DNA damage, while human lymphocytes with the elderberry treatment showed lower levels of DNA damage. SN-challenged groups treated with elderberry showed a reduction on GDI relatively to the C+ group. Regarding the GDI with Fpg data (Figure 5 (ENZ)), it was noticeable that the best result was obtained with a 1% treatment.

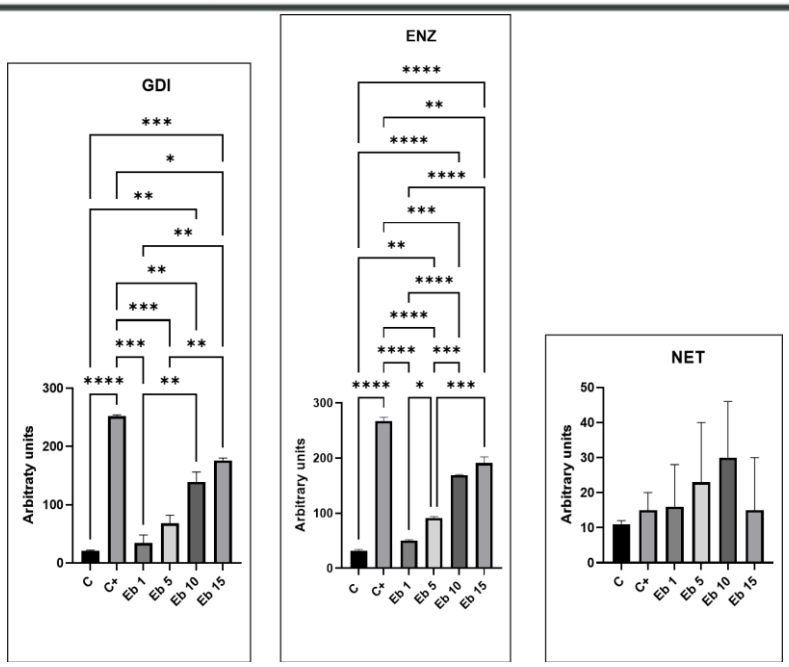

Figure 3. GDI: Mean values of DNA damage (GDI, expressed as arbitrary units) in human lymphocytes measured by the *in vivo* comet assay in unchallenged and SN-challenged groups. C corresponds to the control group, with only PBS treatment, and C+ corresponds to the group treated only with SN. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry) and the percentage of elderberry used (1:1%, 5:5%, 10: 10%, and 15: 15%). Bars represent the standard error. ENZ + NET: Mean values of oxidative purine DNA damage, measured by the improved comet assay in human lymphocytes after elderberry treatment (four levels of supplementation – 1, 5, 10 and 15%, transposed to the group identifier abbreviation as the number preceding the letters Eb). C corresponds to the control group, with only PBS treatment, and C+ corresponds to the group treated only with SN. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry). (ENZ) Overall damage using FPG. (NET) Net FPG-sensitive sites. Bars represent the standard error.

CONSIDERAÇÕES FINAIS

The use of natural ingredients and plants in cosmetics has a long history, and there is growing interest in their potential benefits for skin health. The present study demonstrated the genoprotective effects of elderberry in both *Drosophila melanogaster* and human lymphocytes using the comet assay. The results showed that elderberry was able to reduce DNA damage induced by H_2O_2 and Streptonigrin in both models. These findings suggest that elderberry could be used as a natural ingredient in cosmetics to protect the genome from damage caused by oxidative stress. Further studies are needed to investigate the underlying mechanisms of elderberry's genoprotective effects and to evaluate its potential as a natural ingredient in cosmetics. Overall, elderberry showed excellent potential as a natural genoprotective agent. The results of this study could provide valuable insights into the benefits of natural ingredients for skin health and inform the development of safer and more effective cosmetics.

REFERÊNCIAS

Gaivão, I., & Comendador, M. A. (1996). The w/w+ somatic mutation and recombination test (SMART) of Drosophila melanogaster for detecting reactive oxygen species: Characterization of 6 strains. *Mutation Research/Environmental Mutagenesis and Related Subjects*, *360*(2), 145–151. https://doi.org/10.1016/0165-1161(96)00003-9

Liu, J.-K. (2022). Natural products in cosmetics. *Natural Products and Bioprospecting*, *12*(1), 40. https://doi.org/10.1007/s13659-022-00363-y

Mukherjee, P. K., Maity, N., Nema, N. K., & Sarkar, B. K. (2011). Bioactive compounds from natural resources against skin aging. *Phytomedicine*, *19*(1), 64–73. https://doi.org/10.1016/j.phymed.2011.10.003

Sierra, L. M., & Gaivão, I. (Eds.). (2014). *Genotoxicity and DNA Repair*. Springer New York. https://doi.org/10.1007/978-1-4939-1068-7