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Short communication

Spray-dried snail mucus as raw material with potential for chronic wound treatment

Teodora Tasevska[1*], Marija Glavas Dodov[1], Dushko Shalabalija[1], Ljubica Mihailova[1], Radmil Polenakovic[2], Maja Simonoska Crcarevska[1]

[1]*Institute of Pharmaceutical Technology and Center of pharmaceutical nanotechnology, Faculty of Pharmacy, Ss. Cyril & Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, R. North Macedonia*
[2]*Institute for production engineering and management, Faculty of Mechanical engineering, Ss. Cyril & Methodius University in Skopje, Rudger Boshkovikj 18, 1000 Skopje, R. North Macedonia*

Introduction

Snail slime is externally secreted mucus from the snails that produces different effects depending on the variety of species. The garden snail's *(Helix Aspersa)* slime has antibacterial, antifungal, anti-aging, wound healing, anti-inflammatory and regenerative properties (Dhiman & Pant, 2021). Components of garden snail mucus are 90-99% water (depending on the species), proteoglycans, glycosaminoglycans (hyaluronic acid, glycoprotein enzymes), copper peptides, antimicrobial peptides and metal ions (zinc, iron, copper, and manganeseiron), mitamycin AF, mucin, allantion, collagen, glycolic acid, achacin and elastin (Waluga-Kozlowska et al, 2021). Garden snail's mucus also contains vitamin E, polyunsaturated fatty acids and steroids that help in the regeneration of epidermal cells (Dhiman & Pant, 2021).

Different effects that snail slime produces originate from the various individual effects of active ingredients.

Allantoin promotes cell proliferation and healing of the affected area of the damaged skin and glycolic acid helps in synthesis of collagen-elastin fibers, which is the reason for using the snail slime in skin treatment (Dhiman & Pant, 2021).

Regenerative properties and antimicrobial effect on gram positive and gram negative bacteria's comes from glycoproteins such as mucin and the antimicrobial proteins in mucin.

The garden snail mucus has antioxidant properties of superoxide dismutase (SOD) and glutathione S-transferase (GST) and its antioxidant effect protects the cells of damage caused by free radicals and reactive oxygen species (Waluga-Kozlowska et al, 2021).

Despite the data about the snail slime's compounds there is not much information for the mechanism of its different actions.

The liquid extract of snail slime is not very stable and has to be kept in special conditions such as temperature of -20 °C.

The aim of this short paper was to prepare dried powdered snail slime extract with preserved biological/pharmacological properties.

Materials and methods

Snail slime was mechanically extracted from garden snails and was strained through sieve to remove the mechanical contaminants. Liquid mucus was subjected to spray-drying (inlet temperature 105 °C, 2.5 mL/min flow, 100% aspiration, Buchi Mini Spray dryer B-290, Switzerland) (Patent application MK/P/2021/000853).

Particle size and particle size distribution of spray-dried snail mucus were determined by laser diffractometry using Mastersizer 2000 equipped with Hydro 2000S, Malvern Instruments, UK.

The protein content was determined spectrophotometrically (540 nm, Cary 60UV-VIS, Agilent Instruments, USA) by Biuret protein assay test (Sapan et al., 1999).

Assessment of cytotoxic activity was conducted by MTT test carried out on keratinocytes cell culture (HaCaT, CLS Cell Lines Service GmbH). The readings

*teataso@gmail.com

were performed at 570 nm on microplate reader VICTOR Perkin Elmer, USA.

Dynamic light scattering (Zetasizer Nano Series, Nano-ZS, Malvern) was used for zeta potential and electrophoretic mobility determination in distilled water and artificial wound exudate.

Antioxidative capacity was characterized by ORAC (oxygen radical absorbance capacity) assay test (microplate reader VICTOR Perkin Elmer, USA) (Markova et al., 2021)

Results and discussion

Spray-dried snail slime was in form of dry powder with light brown-yellow color. Mean particle size expressed as Dv50 was 4.691±0.01 µm with a Span value of 1.46 thus indicating narrow and uniform particle size distribution. The protein content was 83.46±5.2%. High percentage of protein content was in favor of its quality related to potential biological/pharmacological efficacy. Results from MTT test showed that proliferation of HaCaT cells in tested concentrations (0.01; 0.1 and 1 mg/mL) increased at 72h (80.15±0.026; 70.63±0.018; 69.38±0.027%, accordingly) compared to 48h (73.11±0.039; 61.31±0.023; 59.38±0.017%, respectively) indicating concentration and time dependent mechanism.

Average zeta potential measured in distilled water was -2.3±0.12 mV indicating its positive influence in wound healing process most likely due to the interactions with different proteins in the wound matrix (Santos-Vizcaino et al., 2020; Xie et al., 2013). This hypothesis was supported by the results determined for zeta potential value measured in artificial wound exudate (0.07±0.02 mV) i.e. lower zeta potential in artificial wound exudate compared to distilled water is presumably result of the interaction of snail mucus components with components of exudate.

Results from ORAC assay showed that snail mucus possess antioxidative effect. Namely, increased snail mucus concentrations (0.05; 0.1; 0.5 and 1 mg/mL) resulted with increased fluorescence (103.3; 103.5; 104.1 and 106.4%, respectively) after 2 hours thus favoring anticipated faster wound healing rates through antioxidant and free radical regulation (McDermott et al., 2021).

Conclusion

Snail slime due to its composition is very attractive raw material and recently become a subject of scientific interest and research in the treatment of chronic wounds and other skin related conditions. Prepared spray-dried snail mucus was characterized by high protein content and physicochemical and biological/pharmacological properties that favors faster and more efficient wound healing. However, there is need for more in depth research in order to determine the exact mechanisms behind its activity.

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References

Dhiman, V. and Pant, D., 2021. Human health and snails. J. Immunoassay Immunochem. 42(3), 211-235. https://doi.org/10.1080/15321819.2020.1844751.

Markova, E., Taneska, L., Kostovska, M., Shalabalija, D., Mihailova, L., Glavas Dodov, M., Makreski, P., Geskovski, N., Petrushevska, M., N. Taravari, A. and Simonoska Crcarevska, M., 2022. Design and evaluation of nanostructured lipid carriers loaded with Salvia officinalis extract for Alzheimer's disease treatment. J. Biomed. Mater. Res. Part B Appl. Biomater. 110(6), 1368-1390. https://doi.org/10.1002/jbm.b.35006.

McDermott, M., Cerullo, A.R., Parziale, J., Achrak, E., Sultana, S., Ferd, J., Samad, S., Deng, W., Braunschweig, A.B. and Holford, M., 2021. Advancing Discovery of Snail Mucins Function and Application. Front. Bioeng. Biotechnol. 9, 734023. doi: https://doi.org/10.3389/fbioe.2021.734023.

Santos-Vizcaino, E., Salvador, A., Vairo, C., Igartua, M., Hernandez, R.M., Correa, L., Villullas, S. and Gainza, G., 2020. Overcoming the inflammatory stage of non-healing wounds: in vitro mechanism of action of negatively charged microspheres (NCMs). Nanomaterials 10(6), 1108. https://doi.org/10.3390/nano10061108.

Sapan, C.V., Lundblad, R.L. and Price, N.C., 1999. Colorimetric protein assay techniques. Biotechnol. Appl. Biochem., 29(2), 99-108. https://doi.org/10.1111/j.1470-8744.1999.tb00538.x.

Xie, X., Chen, L., Zhang, Z.Q., Shi, Y. and Xie, J., 2013. Clinical study on the treatment of chronic wound with negatively-charged aerosol. Int. J. Clin. Exp. Med. 6(8), 649-654.

Waluga-Kozlowska, E.W.A., Jasik, K., Wcislo-Dziadecka, D., Pol, P., Kuznik-Trocha, K., Komosinska-Vassev, K., Olczyk, K., Waluga, M., Olczyk, P and Zimmermann, A., 2021. Snail mucus-A natural origin substance with potential use in medicine. Acta Pol. Pharm. 178, 793-800. https://doi.org/10.32383/appdr/145377.