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Short communication

Evaluation of the *in vitro* bee venom release and skin absorption from bioadhesive gel formulation

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Introduction

Topical and transdermal drug delivery are one of the most suitable alternative, non-invasive routes for administration of drugs in clinical practice mainly due to the increased patient compliance and reduced systemic drug side effects. Many drug products applied to the skin surface may penetrate to some extent into the skin layers, where their effects are expected, as for example, topical formulations for the treatment of different local skin disorders. Also, significant concentrations of drug could be absorbed by the body regions close to the site of delivery, where regional effects are expected, for e.g., in the muscles, local blood vessels and articulations (Ruela et al., 2016).

Arthritis is a systemic, autoimmune disease characterized by inflammation of joints. Inflammatory cytokines cause activation of the macrophages which leads to swelling of joints, damage to cartilage, bone erosion , functional impairment and stiffness (Mohanty et al., 2019). Bee venom (BV) contains a variety of peptides, including melittin, apamin, adolapin, the mast-cell degranulating peptide, enzymes (phospholipase [PL] A2), biologically active amines (histamine and epinephrine) and nonpeptide components with anti-inflammatory, anti-arthritis, anticoagulant, antimicrobial, anticancer and anti-nociceptive properties. Melittin, a major peptide component of BV shown to have anti-inflammatory and anti-arthritis properties and inhibitory activity on nuclear factor kappaB which is involved in the synthesis of inflammatory mediators and may be essential for the treatment of arthritis using BV (Son et al., 2007).

The aim of this study was to evaluate the stability of crude BV as an active ingredient, as well as to evaluate the *in vitro* release and skin absorption of BV from a designed topical gel formulation.

Materials and methods

BV sample was supplied from Kozarac, BiH, during July 2019. BV samples were stored at 25 ˚C and 60% RH, 2-8 ˚C and -20 ˚C for stability period of 3 months. The stability of BV was determined by a modified HPLC method (Rybak-Chmielewska and Szczêsna, 2004) using melittin (Sigma, USA) as an external standard (Agilent Technologies 1200 Series; Restek Ultra C18 column; gradient elution

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with 0.1% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.1% TFA in acetonitrile-water 80:20 (mobile phase B)), flow rate of 2.5 mL/min, 20 µL injection volume, λ of 220 nm.

The gel was prepared by dissolving 1.75% of chitosan (CTS, low-molecular weight; Sigma–Aldrich, USA) in 1% of lactic acid solution with 0.5% of poloxamer 407 (Pluronic F127, BASF Chemtrade GmbH, Germany), 5% of propylene glycol (Alkaloid, N. Macedonia), 0.2% of potassium sorbate (Apac Chem. Corp., USA) and 0.3% m/m of BV (300 rpm, ambient temp.; Variomag, Germany). *In vitro* BV release from the prepared gel (1.5 g) was performed using membrane diffusion cells (MEMBRA-CELL dialysis tubing; Serva Feinbiochemica GmbH, Germany) (32±0.5 ˚C, 15 mL of distilled water as a dissolution medium, 300 rpm). At predetermined time intervals (after 1, 2, 4, 6, 8 and 22 h) aliquots were taken and analyzed by HPLC (n=3). *In vitro* permeation studies were performed by using pig skin obtained from local slaughterhouse (dermatomed, stored at -20 °C). Before the experiment, the skin was thawed in 0.9% NaCl sol. at 37 °C and rinsed twice to remove any adherent blood or other material from the surface. The test was carried out under identical conditions as described for *in vitro* release studies with the difference of pig skin between donor and receptor compartment (n=2).

Results and discussion

Freshly obtained BV sample contained 43.54% of melittin. Stability studies of crude BV showed that the sample stored at 2-8 ˚C was the most stable one (~93% of the initial amount) during the period of 3 months. Samples stored at -20 ˚C and at 25 ˚C/60% RH for 3 month stability period showed 78.2% and 77.1% of the initial BV amount, respectively. Therefore, BV sample which was stored in refrigerator was used for further studies.

During the period of 22 h, ~90% of BV was released from the prepared gel, following the Peppas-Sahlin kinetic model. The skin absorption studies and penetration of BV trough pig skin was found to be 77.1% for 22 h and best fitted to the Peppas-Sahlin kinetic model with T_{lag} of 0.6 h. The steady state diffusion flux (Jss) value was 0.0319 mg/cm^2/h and the permeability coefficient (Kp) was 0.1063 mg/cm^2/h. Compared to *in vitro* BV release, the permeation was slower, most likely related to the time required for release, penetration and participation of BV through different skin layers before it reaches the acceptor compartment. High correlation coefficient (r=0.97) was found between the data from release and skin permeation studies of BV from the prepared gel.

Conclusion

According to the results obtained from this study it could be concluded that freshly obtained BV could be stored at a temperature of 2-8 °C for prolonged time period. Based from *in vitro* performed studies, gel formulation prepared with 1.75% CTS and 0.5% PL could be a promising candidate for efficient topical delivery/treatment of BV for arthritis. Further clinical studies should be conducted.

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