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Preparation and Characterization of Silymarin Vesicular System Customized for Wound Healing

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List of Abbreviations

DL	Drug loading
DMSO	Dimethylsulfoxide
EE%	Entrapment efficiency
HPLC	High performance liquid chromatography
HMWT	High Molecular Weight
LMWT	Low Molecular Weight
PS	Particle size
SIL	Silymarin
SIL-PEVs	Silymarin loaded penetration enhancer containing vesicles
SD	Standard deviation
TEM	Transmission electron microscope
UV/Vis	Ultraviolet/Visual
ZP	Zeta potential

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Abstract

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The cascade of healing is divided into four overlapping phases: Hemostasis, Inflammatory, Proliferative, and Maturation phases. Wound healing aims to repair the injured tissues, replace the damaged structures and prevent invasion of pathogens into the damaged tissues.

Conventional treatment of wounds depends mainly on moisture intake in order to permit moist treatment of wounds. They often require long duration; do not provide optimal conditions to permit recovery of the wounds, and dressings usually cause injuries upon removal. Moreover, they require frequent application and wound coverage to ensure sterility of wound, therapeutic action of drugs and avoid wound drying; hence, they decrease patient compliance and acceptance.

The therapeutic efficacy of a locally applied drug depends mainly on its ability to penetrate and permeate the skin. PEVs are deformable vesicles which improve *ex-vivo* cutaneous drug deposition more effectively than conventional vesicles. They are able to squeeze themselves through the intercellular regions of the stratum corneum so they are able to achieve higher skin penetration and permeation than conventional vesicles.

Silymarin (SIL) is the active component of Milk thistle which was shown to suppress inflammation and oxidation, which are important in the process of wound healing. Additionally, lactic acid helps collagen synthesis, angiogenesis and stimulates endothelial cell proliferation and migration. CaCl2 has an effective role in wound healing as it augments the production of granulation tissue in early stages of wound healing and it stimulates clot formation. High molecular weight hyaluronic acid has anti-inflammatory and antioxidant activities. Also, it is able to increase

fibroblast cell proliferation and stimulate keratinocyte migration, proliferation and differentiation. Chitosan has antibacterial activity; it is able to absorb wound exudates, and to protect the wound.

The purpose of the first chapter was to formulate these novel families of deformable vesicles; called penetration enhancer containing vesicles (PEVs) as carriers for enhanced topical delivery of silymarin for wound healing. Thin film hydration method was used to prepare SIL loaded PEVs consisting of soybean phosphatidylcholine (PC) as bilayer forming lipid and Transcutol® as penetration enhancer. The PEVs formulations were evaluated for their particle size, polydispersity, entrapment efficiency and zeta potential.

Three formulae from the preliminary study were chosen in order to augment their wound healing activity through addition of functional additives like low molecular weight chitosan, high molecular weight hyaluronic acid, lactic acid and calcium chloride. All the optimized PEVs formulae were characterized for their particle size, polydispersity, zeta potential, entrapment efficiency, viscosity. Assessment of the physical stability of the vesicles was performed by monitoring the change in particle size, span index, zeta potential and entrapment efficiency when stored at (2-8°C) after six months. *Ex vivo* deposition on rat skin was conducted on the chosen formulae (P1, P5, and P11) and compared with silymarin suspension. Then, the selected formulation (P11) was tested for its morphology, antibacterial activity against *staphylococcus aureus* and *pseudomonas aeruginosa* compared to pure silymarin in DMSO and its plain counterpart formulation using DMSO as negative control.

Results revealed that SIL could be successfully incorporated within PEVs. The prepared PEVs were nearly spherical, in the size ranged from 3.08-5.73 µm, depending on the type and amounts of additives added and all the prepared PEVs were charged (hyaluronic acid containing vesicles were negatively charged while chitosan containing vesicles were positively charged.

PEVs were able to entrap SIL with percentages reaching 76.15%. The PEVs dispersions were found to be of higher viscosity than water and they displayed good

storage properties as manifested by the generally insignificant changes in particle size, SPAN index, zeta potential and EE% values compared to freshly prepared formulations after 6 months of storage. *Ex vivo* deposition of such vesicles proved their superiority in accumulating the drug in different skin layers with deep penetration potential (in the stratum corneum, epidermis and dermis) in comparison with the silymarin suspension. In the antibacterial assay, the zone of inhibition produced by formulation P11 against *staphylococcus aureus* was comparable to that produced by gentamicin, and was higher than both the plain formulation and silymarin drug. The zone of inhibition produced by formulation P11 against *pseudomonas aerugionsa* was larger than its plain counterpart.

Finally, in the second chapter the selected PEVs formula (P11) was tested for its wound healing activity on rats with incisional wounds. In vivo studies were done in comparison to its plain counterpart formulation and healosol®. A three weeks (21 days) wound healing assessment study was carried out on different rat groups at 3,5,7,10,14 and 21 days after wound induction, rats were photographed using a digital camera and the diameter of the wound was measured in mm. Then, staining using hematoxylin and eosin (H&E) was performed for the obtained sections, followed by histopathological examination. A semi-quantitative method was used to evaluate the following: epithelization, PMNL (polymorphonuclear leucocytes) formation, fibroblasts formation, new vessels and collagen production. Measurement of dermal content of collagen at day 14 which expressed as MTC area % and measurement of angiogenesis through measuring VEGF expression by immune staining technique at day 21.

The in vivo study revealed that P11 formulation showed a significantly faster onset of wound healing than healosol® spray and higher anti-inflammatory effect, epithalization, angiogenesis and collagen deposition than its plain formulation counterpart. Hence, PEVs can be considered a promising approach for improving cutaneous SIL deposition, which can further aid in wound healing and overcome the side effects encountered with conventional healing agents.

The proposed approach adopted in this thesis of using PEVs for wound healing has shown very promising results, and if well implemented, it is expected to overcome the drawbacks of local wound healing agents.

Keywords: Silymarin, vesicles, PEVs, wound healing, *In vivo* wound healing activity.

General Introduction

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1. The topical route

Topical preparations are formulations which are applied directly to an external body surface by spreading **(Singla et al., 2012)**, rubbing, spraying or instillation such as creams, ointments, lotions and gels **(Buhse et al., 2005)**. The topical route has gained much interest for many years, owing to its impressive advantages compared to other routes of drug delivery. Topical formulations have high patient compliance **(Singla et al., 2012)** as they are non-invasive, can be self-administered **(Singla et al., 2012)**, and they offer the flexibility to terminate the drug administration through removal of formulations from the skin**.** Topical treatment of various skin infections as fungal infections **(Fasolo et al., 2020)** has proven to be quite advantageous due to various factors like targeting the site of infection, minimizing systemic side effects, and enhanced efficacy of treatment. The most important problem encountered with topical drug delivery is the low penetration of most drugs, owing to the barrier function of the skin **(Herkenne et al., 2008; Kreilgaard, 2002; Manca et al., 2019; Nagula and Wairkar, 2019)**. Conventional formulations for topical drug delivery have weak percutaneous permeability and poor deposition in the skin. In search of improved topical products, scientists either design new vehicles or explore novel carriers to ensure adequate penetration, and more importantly, localization of the drug within the deeper layers of skin **(Bernal-Chávez et al., 2019**; **Foldvari et al., 1990; Nada et al., 2018; Pachuau, 2015).**

2. The skin: structure and barrier properties

The skin is the largest organ in the human body with the highest surface area about 1.7 m^2. The skin provides an excellent effective barrier **(Bouwstra and Honeywell-Nguyen, 2002)** that controls water and electrolyte loss. Being impermeable to most substances, it prevents the entry of detrimental substances