Chapter 3

Rationale and Objectives

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3.1 Rationale

Melanoma, a type of skin cancer originating from melanocytes, poses significant health risks due to its aggressive nature and potential for metastasis if not detected and treated early. Caucasians are particularly susceptible to melanoma compared to other racial groups, with a lifetime risk estimated at 2.6% for whites, 0.1% for blacks, and 0.6% for Hispanics. An estimated total of 325,000 new melanoma cases were diagnosed, and 57,000 deaths occurred in 2020. The current treatment regimen relies heavily on chemotherapeutic agents such as dacarbazine (DTIC), a first-line treatment for melanoma. However, severe liver toxicity and decreased production of blood cells in the bone marrow are major drawbacks. Moreover, DTIC is delivered via the parenteral route, which causes feelings of burning, pain, and sometimes bleeding. Furthermore, the parenteral route is not patient-compliant and needs to be given under direct supervision. Such circumstances urge the development of a cost-effective drug or drug delivery system with fewer or no adverse effects.

Drug repurposing, or repositioning, is an innovative strategy involving taking existing medications and exploring their potential use in cancer treatment. Drug repurposing has been gaining much attention due to the availability of complete clinical trial reports regarding drug safety, pharmacokinetics, and pharmacodynamics profiles. On the other hand, Flavonoids, plant-derived polyphenolic secondary metabolites, have gained significant attention for their potential as therapeutic agents, driven by their versatile mechanisms of action, cost-effectiveness, and minimal adverse effects. Hence, in the current work, we selected two different drugs, i.e., dihydroartemisinin (drug repurposing) and hesperidin, for the treatment of melanoma.

Both dihydroartemisinin and hesperidin inhibit melanoma growth via different mechanisms of action such as apoptosis, generation of free radicals, and modulation of various anti-

inflammatory factors. Despite the good anti-cancer activity of both drugs, their clinical effectiveness is hampered by solubility, low oral bioavailability, and side effects; hence, a drug delivery system is indispensable for delivering the drugs to improve solubility and bioavailability and reduce toxicity.

Exosomes have recently attracted significant attention in drug delivery as naturally derived, lipid-based nanocarriers. These membrane-bound extracellular vesicles are released by all cell types through the fusion of the multivesicular body (MVB), an intermediate endocytic compartment, with the plasma membrane. Exosomes play a crucial role in intercellular communication and are present in various body fluids, including milk. Bovine milk-derived exosomes, in particular, are emerging as promising drug delivery systems due to their favorable physicochemical and biological properties, as well as their easy accessibility. Therefore, in our current study, we utilized bovine milk-derived exosomes for the delivery of both drugs.

The exosomal formulations developed in this study were characterized based on particle size, zeta potential, and morphology. Their *in vitro* anti-cancer efficacy was evaluated using B16F10 melanoma cell lines, followed by *in vivo* testing in a murine model of B16F10-induced melanoma in Swiss mice. Additionally, the *in vivo* anti-cancer efficacy of dihydroartemisinin and hesperidin-loaded exosomal formulations was compared to the standard anti-cancer drug, Dacarbazine.

3.2 Objectives

Isolation, characterization, *in vitro* and *in vivo* investigation of dihydroartemisinin-loaded bovine milk exosomes

Isolation, characterization, *in vitro* and *in vivo* investigation of hesperidin-loaded bovine milk exosomes