9 Chapter 9: Summary and conclusion

Plant-based substances (crude drug, extract, fraction, or isolated phytoconstituents) have shown their superiority in the therapy of numerous tumors in multiple molecular mechanisms. Further, such therapeutics can be used as an alternative as well as supportive therapy to the current chemotherapeutics. Among various skin cancers, melanoma is one of the most aggressive and deadly forms. Numerous plant extracts as well as isolated phytoconstituents, have been well exploited for melanoma therapy [2, 10]. Melanoma cells utilize various mechanisms (disordered replication and evasion of apoptosis, angiogenesis, tissue invasion, metastasis, modulation of the immune system, and oxidative reactions) for their rapid growth and development [2]. Hence, it is logical to use plant extract that will act synergistically in a multi-targeting manner rather than a single constituent or drug molecule. Plants have been reported to act against melanoma through the inhibition of the aforementioned mechanisms [2].

The taxonomical and DNA-based molecular authentication of fruits assured the identity of the received sample as fruits of *Piper longum* Linn. (Family: Piperaceae). The microwave-assisted cold maceration method was found to be effective. The GCHS analysis of PLFEE revealed the absence of ethanol in the extract, hence deprived of ethanol-related toxicities. The marker-based standardization was successfully carried out using a validated HPLC method. The overall results of validation reflected the suitability of the developed HPLC method for the quantification of PIP and PLGN accurately. The standardization of extract will ensure dosage uniformity and batch-to-batch consistency.

To improve the oral bioavailability and transdermal permeability, two novel formulations, such as SD and TFG of PLFEE, were developed and optimized using QbD. The optimized formulations were investigated by *in-vitro* and *in-vivo* tumor models in melanoma (B16F10) bearing C57BL/6 mice through oral and transdermal delivery for the treatment of melanoma.

The 4th generation solid dispersion was developed with the use of Soluplus® and Tween® 80. The solvent evaporation by rotary vacuum evaporation was utilized for the development of solid dispersion and optimized by the QbD approach. The SD showed amorphous properties with good drug content, content uniformity, wettability, and low moisture content. The ATR-FTIR and HPTLC results revealed the absence of any incompatibility among the extract and the excipients of the SD. The HR-SEM revealed the homogeneous irregular morphology of SD. The *in-vitro* dissolution study revealed the improved dissolution profile of SD compared to the physical mixture (PM) and PLFEE. The DLS and HRTEM results of the dissolution sample revealed the formation of micelle during the dissolution, resulting in micellar solubilization and improved dissolution. The stability study revealed the prolonged maintenance of physicochemical and pharmaceutical properties without any alterations. The *in-vivo* oral bioavailability result of SD reflected a significant ($p<0.05$) improvement of bioavailability (C_{max} and AUC) compared to neat extract and PM. The acute oral toxicity study (OECD 425) via hematological, biochemical, and histopathological observations revealed the nontoxic nature of the standardized PLFEE at the chosen dose. The effect of SD was studied in the syngeneic transplantation model in melanoma (B16F10) bearing C57BL/6 mice. The results of the tumor regression study revealed improved therapeutic activity of SD compared to neat PLFEE. Further, the SD also improved the anticancer activity of DTIC as an adjuvant therapy. The overall

result revealed the potential of developed PLFEE contained SD for melanoma cancer therapy either alone or as an adjuvant therapy with DTIC.

The thin-film hydration technique was employed for the development of standardized PLFEE-loaded ultradeformable vesicles (TFs) and optimized by the QbD approach. The response surface analysis and optimization were carried out by Design-Expert® software to obtain the best TFs with good % EE, acceptable vesicle size, and flexibility. The optimized TFs demonstrated amorphous nature, nano size, excellent % EE, drug loading, flexibility, low PDI, drug-excipient compatibility, prolonged stability, *in-vitro* cytotoxicity, and uptake in B16F10 melanoma cells. TFs were incorporated into Xanthan gum-based hydrogel to develop transgelosome for transdermal application. The transgelosome (TFG F2) showed excellent organoleptic possessions, consistency, homogeneity, spreadability, extrudability, rheological properties, syneresis, drug content, content uniformity, drug excipient compatibility, and stability. The *ex-vivo* skin permeability and CLSM revealed the improved skin permeability of TFG F2 compared to the plain gel. The acute dermal toxicity study (OECD 402) via skin irritation, biochemical, hematological, and histopathological observations revealed the nontoxic nature of the standardized PLFEE. The skin irritation study revealed the nonirritating nature of TFG F2. The *in-vivo* anticancer activity of standardized PLFEE-loaded plain gel was studied and compared with that of the PLFEE-loaded ultradeformable vesicular TFG F2 gel. The results of the tumor regression study in melanoma (B16F10) bearing C57BL/6 mice model revealed improved therapeutic activity of TFG F2 compared to plain gel. Moreover, the TFG F2 also enhanced the anticancer activity of DTIC as an adjuvant therapy. The overall outcome revealed the potential of formulated PLFEE-loaded vesicular transgelosome

(TFG F2) for melanoma cancer therapy either alone or as an adjuvant therapy with DTIC.

Both the SD and TFG F2 were found to be effective for melanoma therapy either alone or as adjuvant therapy with DTIC. The TFG F2 showed stronger antitumor activity compared to optimized SD at the equivalent dose. Also, the TFG F2 was found to be a good adjuvant with DTIC than that of SD for the effective treatment of melanoma due to the localized site-specific targeted anticancer activities and systemic effect of TFG compared to SD, which show only systemic activity. A combination of "SD + TFG F2" was found to be significantly more effective than SD alone at the same dose and less effective than TFG F2 alone.

The 50% survival in the case of TFG F2 alone as an adjuvant with DTIC was found to very close to that of the combination of formulations (SD+TFG F2) at the equivalent dose as adjuvant therapy. However, the overall life expectancy at the end of the study was found to be significantly higher in the case of TFG alone as adjuvant to DTIC than the combination of formulations (SD & TFG) as adjuvant therapy. The overall tumor regression outcomes and % survival at the end of the study was found to be significantly higher in the case of TFG alone as adjuvant to DTIC than in combination (SD & TFG F2) at same dose due to the localized site-specific targeted anticancer activities and systemic effect of TFG compared to SD, which reaches to throughout the systemic circulation instead of localization at specific site. In future detailed pharmacokinetic studies of other chemical markers may be studied.