Chapter 6

Summary

- Skin cancer accounted for about 5% of deaths worldwide, but the recent empirical data showed an exponential increase in the number of people suffering from different types of skin cancer. Hence, there is an urgent need for developing a potent ethno-based therapeutic agent that can reduce/control the incidence of alarming skin tumors with comparatively lesser side effects.

- A recently discovered caspase (caspase 14), was found to be essential for normal skin development which is expressed suprabasally in normal human epidermal keratinocytes.

- The development of drugs from natural products that specifically targets caspase-14 by inducing terminal differentiation in skin cancer cells showed reduction in tumorigenicity in skin cancers, and could possibly be more effective with an improved safety profile.

- Hence, the present study investigated the properties of various plant compounds (BA, OA, PN, LUT, and vitamin D₃) for their potential to induce caspase-14 expression in immortalized human keratinocyte cells (HaCaT) and melanoma cells (A375).

- All the compounds were analysed for their abilities to scavenge free radicals by DPPH and FRAP assay. The results indicated that none of these compounds has significant (p<0.05) potential to scavenge the free radicals when compared to ascorbic acid (standard) used in this study.

- The compounds were analysed for its cytotoxicity on HaCaT and A375 cells. Most of the compounds exhibited significant (p<0.05) cytotoxicity on both the cells except OA which was found to be non-toxic even at higher concentrations. PN was found to be the most cytotoxic compound at a very low IC₅₀ values.

- The nuclear damage caused by the toxic effect of each compound was studied further using DAPI staining method and found to be higher for the cells
treated with PN which corresponds to the results of its cytotoxicity in both the cells.

- The apoptotic potential of the compounds was studied by DNA fragmentation ELISA method. All the compounds possess significant (p<0.05) levels of apoptotic induction with increasing concentrations. However, A375 cells were found to be resistant with moderate/minimum apoptotic potential when treated with these compounds.

- The ability of the compounds to arrest cell cycle was assessed by FACS analysis after treating the cells for a period of 24 h. All the compounds showed cell cycle arrest at different phases except BA in HaCaT cells. OA was not assessed since it possesses non-significant cytotoxic response in both the cells.

- Further, the compounds were screened for its ability to induce caspase-14 quantitatively in both cells by ELISA method. LUT showed elevated expression levels of caspase-14 in a concentration-dependent manner than positive control (vitamin D₃) in HaCaT cells. However, all the other compounds showed minimum response when tested and hence not studied further. A375 cells were found to be devoid for the expression of caspase-14, since it lacks the gene encoding for its expression and thus not taken for further investigation.

- The qualitative study, further confirmed the potential of LUT for inducing caspase-14 expression in HaCaT cells at mRNA level by RT-PCR. LUT showed an abundant expression of caspase-14 when compared to vitamin D₃, the positive control.

- Finally, the LUT induced caspase-14 mediated terminal differentiation was confirmed by investigating the expression of a marker gene called human involucrin in HaCaT cells. LUT showed elevated expression levels of human involucrin than untreated cells.

- The findings of the present study clearly indicated that LUT has the potential to induce caspase-14 mediated terminal differentiation and consequent apoptosis in HaCaT cells and hence it can be consider to develop a more potent and efficient chemotherapeutic drug to eliminate skin cancers in a target-based approach.