Chapter - VI

Summary & Conclusions
6 Summary & Conclusion

- Natural products are the foundation of the most complex and fascinating chemical structures available from Mother Nature. They are considered as part of natural wealth of a geographical region. They exhibit biological activity either as single compounds or often as complex mixtures. They serve as important source of existence beginning with agriculture and food, yield pharmaceuticals, coloring agents among others. Since time immemorial, natural products have served as anticancer drugs and have remained in native or traditional medicine practice.

- Among several such single compounds, Berberine is a wonder molecule with potent biological activity and is widely available from different plant sources across different ecological zones. Cancer is a devastating disease with high mortality rates and debilitating treatment outcomes. Hence there is always a quest for search of new natural products with potent cancer cell specific activity and sparingly toxic effect on normal cells and tissues. It is in this direction, the present study was formulated to gain insights into molecular mechanistic basis of Berberine’s anticancer activity on human breast cancer cells as a single agent and in combination with well known chemotherapeutic drug, Epirubicin.

- The study design involved systematic cell biology, biochemistry and in-silico based approaches to decipher the effects of Berberine on human breast cancer cells. Detailed analysis of the results obtained showed that Berberine was very effective in killing breast cancer cells namely MCF-7 & MDA MB 231, and the morphology based assay provided the first line of evidence that it probably induced an apoptotic mode of cell death.

- Further biochemical assays, In-vitro clonogenic assay confirmed the cell death & quantified the pharmacological effects on MCF-7 & MDA MB 231. These assays also were done in combination with the chemotherapeutic agent Epirubicin. Since malignant cancer cells are endowed with the capacity to migrate, the anti metastatic potential of Berberine was evaluated by the In-vitro scratch assay; the results of which essentially showed that Berberine alone and in combination with Epirubicin inhibited the migration of MCF-7 & MDA MB 231 cells.
To decipher the molecular basis of cell death induced by Berberine, apoptotic gene expression using the sensitive quantitative PCR was performed, which showed dysregulated expression and suggesting an apoptotic mode of cell death. Some of the critical protein players involved in apoptosis was also determined by immunoblot analysis, which showed deregulated expression designating the trigger of apoptosis in Berberine treated MCF-7 & MDA MB 231 cells.

A novel aspect of the present study is the combination experiments carried out on MCF-7 & MDA MB 231 using specified concentration of Berberine and the chemotherapeutic agent Epirubicin. Results of the combination chemotherapy assays suggests that Berberine acts with Epirubicin in a synergistic manner on MCF-7 & MDA MB 231 cells and holds promise for future preclinical and clinical evaluation.

In-silico docking studies were performed using the Berberine’s structure and a well known human breast cancer deregulated cellular target PI3 kinase. Docking studies showed that Berberine had superior docking scores as compared to Epirubicin or other known kinase inhibitors.

In conclusion, the present study has given vital evidence for molecular basis of cancer cell death induced by a natural product Berberine and its synergistic effects with Epirubicin on human breast cancer cells MCF-7 & MDA MB 231 using sensitive molecular techniques and paved the way for future pre clinical investigations.
6.1 Future Directions

With burgeoning technology, awareness about chronic disorders and management is increasing. Present understanding about the molecular basis of cancers has shown good targets for efficient management with minimal damage to non target tissues. Despite these positive trends, lacunae exist in medical management of several cancer types including Breast cancers, due to increasing costs of therapy, poor quality of life post treatment among others. It is in this focused arena, the present study was designed to propose a novel phytocompound, Berberine as a therapeutic entity, which is presently under clinical trial evaluation. The current study has thoroughly shown that Berberine is very effective in killing breast cancer cells, namely, MCF-7 & MDA MB 231. Berberine has been shown to induce morphological changes akin to apoptosis and was further confirmed using gene and protein expression of key players involved in the phenomenon. Besides, these cytotoxicity assessments, the study results have also shown that Berberine potently inhibits migration of breast cancer cells, MCF-7 & MDA MB 231. A notable aspect in this work is that the study has shown undeniably the combination of Berberine with Epirubicin is synergistic and holds promise for clinical translation. Further in-silico analysis has shown that Berberine can effectively inhibit an intra cellular signaling PI3 kinase in comparison to known small molecule inhibitors. These investigation results have paved the way for future studies, which are:

i. Development and testing of Berberine alone and in combination with Epirubicin in a clinically relevant pre clinical mouse model.

ii. Determine the combinatorial effects of therapy in pre clinical orthotopic models

iii. Perform pre clinical toxicity studies on mice to design dosing regimens

iv. Determine drug - drug interaction and dosing regimen in Berberine and Epirubicin combination therapy

v. Optimize dosing regimen suitable for probable clinical translation.

Based on the original observations from the study, and correlating with existing literature, Berberine can serve as a useful therapeutic agent for treatment of breast cancers and has large scope for future studies.