CHAPTER 5: SUMMARY AND CONCLUSIONS

Rasagenthi Lehyam contains 38 different botanicals, many of which have been shown to possess therapeutic efficacy, and also 8 inorganic compounds, some of which are considered in the Indian traditional medicine literature as therapeutic, all prepared into a paste in a palm sugar and hen’s egg base. The efficacy of RL in killing prostate and lung cancer cells in vitro / in vivo, as the case may be, was investigated in this study to determine whether RL could be recommended as a CAM for prostate and lung cancers.

To begin with, in the first phase of the study, in order to scientifically validate the anticancer activity of RL on prostate cancer, the methanolic extract of RL was serially extracted with four organic solvents, and the extracts were tested for clonogenic inhibition/affecting viability and induction of apoptosis in different prostate and lung cancer cell lines so as to identify the most potent extract for further testing. This is because, it is non-practical to test the whole medicine in the in vitro test models, and also to eliminate the apparently toxic inorganic, and also to aid in standardizing the dosage for the in vitro testing and optimizing the concentrations for the in vivo testing. Among the five fractions (four organic solvent extracts and the last residue in water) n-butanol extract didn’t show any effect and the final residue remaining in water extract is all cellulosic material and the inorganics which makes them obvious for the purpose of extraction and elimination. Among all the cell lines tested PC-3 cells from prostate cancer and A-549 and H-460 cells from lung cancer showed the greatest response. In view of the broadest range of possibilities and the wide variety of outcome probabilities, considering the funding and time limitations the above cell lines were chosen to explore the RL.

In the preliminary study conducted on prostate cancer cell lines PC-3, the IC-50 values of n-hexane, ethyl acetate and chloroform extracts of RL were found to be 3.84 μg/ml, 3.68 μg/ml, 75 ng/ml, respectively. All the three extracts induced apoptosis in PC-3 cells. Further, all the three extracts, showed a significant radio-sensitizing effect among which chloroform extract proved to be the most potent. In order to recommend the RL or its extract as CAM with their anti-cancer potential and to exploit the radio-sensitizing property to treat cancers resistant to
radiation therapies, it is important to explore the mechanism of radiation resistance and apoptosis caused by RL extracts.

In the study conducted on lung cancer cell lines A-549 and H-460 including one normal bronchial epithelial (BEAS-2B) cell line were used to test the chemotherapeutic effect of RL. Out of five fractions of RL, the chloroform fraction (cRL) demonstrated a significant inhibition of cell proliferation and induction of apoptosis in A-549 and H-460 cells but not in normal BEAS-2B cells, which is positive signal for the safety of the extract even though the RL was made with all natural ingredients and all the inorganics were removed through organic solvent extraction of the active ingredients. It also showed significant radio-sensitizing effect on both the lung cancer cell lines. When an attempt was made to explore the molecular mechanism of cRL-induced apoptosis and radio-sensitization effect it was found that cRL up-regulates pro-apoptotic genes \( p53 \) and \( Bax \) and induces caspase-3 activation, and down-regulates the pro-survival gene \( Bcl-2 \) in both the lung cancer cell lines in addition to the nuclear export of \( p53 \). cRL also caused a significant G2/M arrest of the cell cycle. All these molecular effects explain the apoptotic and radio-sensitizing effect of cRL since there is considerable literature explaining the down regulation of pro-survival \( Bcl-2 \), and the cell cycle arrest would lead to radio-sensitization and up-regulation of pro-apoptotic pathways could lead to apoptosis.

Thus the study so far suggest that cRL may prove to be a potent anti-cancer agent that may be used for the treatment of radio-resistant prostate and lung cancer cell lines with limited or no toxicity on normal cell lines, offering a selectivity of the extract towards the cancer cell lines though the mechanisms of the selectivity might need to be explored for a strong recommendation as a CAM.

The cRL was subjected to HPLC-MS separation and analysis for individual molecules coupled with bio-assays like MTT assay and also subjected to structural elucidation with intent to identify the individual molecules that are responsible for the cRL effects and with a hope to find the molecular mechanisms responsible for the apoptotic and radio-sensitizing effects. Fortunately, the last hope came into reality with curcumin (compound I) showing the radio-sensitization property for the first time and the mechanism that Withaferin-A (compound-II)
induces a par-4 dependent apoptosis which is independent of $p53$ and pTEN apoptotic pathways in androgen refractory prostate cancer cell PC-3. Restoration of wild type AR abrogated par-4 mediated apoptosis by withaferin-A.

Thus, this study adequately substantiates, from the most modern scientific perspectives, the potential of RL as therapeutic in prostate and lung cancers. The study also leads to the conclusion that the cRL could be a potential CAM for these cancers. The therapeutic efficacy would lie in the active principles Withaferin A and Curcumin, although further studies might add to the list of the potentially therapeutic compounds in RL.