General discussions and conclusion

From overall findings of the present thesis work, it may be concluded that *Thuja occidentalis* extract had a great combating effect on NSCLC, which can help quite adequately in future anti-NSCLC drug development. Ethanolic leaf extracts of this plant, obtained in the form of homeopathic mother tincture, had shown significant anti-proliferative and apoptotic ability against a K-ras mutated NSCLC cell line, A549. The significant cytotoxic ability of TO was very much target-specific as it exhibited cytotoxicity in cancer cells, but spared the human normal lung embryonic cell line L-132. TO had anti-proliferative action against A549 cell line in a time-dependent manner. Moreover, TO activated caspase3 in a dose-dependent manner and altered the Bcl2/Bax imbalance which directed the cells into apoptosis.

However, highly diluted potentized forms of *Thuja occidentalis* (Thuja 5C, 9C and 30C) were not able to trigger lung cancer cell cytotoxicity. But the ultra-high diluted form, Thuja 30C, was able to ameliorate BaP intoxicated normal mice lung cell toxicity. Thuja 30C was found to be effective in ameliorating BaP toxicity, elevating cell viability when simultaneously intoxicated with BaP into the perfused normal mice lung cells. This ameliorating efficacy of Thuja 30C was possibly due to its ability of altering the BaP induced cellular stress responses, as lowered ROS activity and increased GSH activity were observed after treatment. In this context, down-regulation of Hsp90 after Thuja 30C employment also helped in ameliorating the BaP induced altered stress level that could render adequate protection to the cells towards their increased survivability. Besides this, DNA damaged by BaP could also be partially repaired and restituted by the Thuja 30C treatment. As Thuja 30C was unable to interact with calf thymus DNA in a cell free system, therefore it would not presumably be able to interact directly with cellular DNA. Thus, it may be extrapolated that this drug does not bear the self-reactive ability to damage DNA by interaction, rather bear the capability to reduce BaP induced DNA damage. So the protection rendered by Thuja 30C, an ultra-highly diluted remedy above Avogadro’s limit, showed quite convincingly was presumably through its action at the molecular level of gene regulation.
The flavonols-rich fraction (FRF), containing quercetin and kaempferol as predominant agents, was isolated from *Thuja occidentalis* leaf extract and its possible anti-cancer ability was determined against NSCLC both by *in vitro* and *in vivo* studies. For *in vitro* study, efficacy of FRF against A549, K-ras mutated NSCLC cell line, was ascertained. FRF was found to be apoptotic in A549 cells sparing the normal lung cell line L-132. However, the response was dual, as FRF was also able to block the cell cycle at both G2/M phase and sub-G1 stages. The FRF-induced apoptotic effect was mitochondria mediated but ROS independent and possibly occurred after intercalation with nuclear DNA at early hrs.

Further *in vivo* studies had been made to establish the isolated flavonols fraction as a putative anti-NSCLC agent. FRF was found to be effective against BaP induced mice lung carcinogenesis without putting significant impact on normal body physiology. FRF was able to combat BaP toxicity initially by down-regulating increased ROS level and up-regulating decreased antioxidants level. However, this ameliorative effect of FRF was found to be maximum during late phase of tumorigenesis (at 60 and 90 days after BaP induction) where the drug more precisely blocked the uncontrolled cell proliferation, down-regulating both constitutive and PI3K-induced Akt activities and ultimately induced apoptosis. Target-specific activity of this isolated fraction thus makes it an attractive candidate for formulating a potent anti-cancer drug capable of effectively combating NSCLC.

Quercetin, a major component of FRF was thereafter analyzed mechanistically against NSCLC *in vitro*. Quercetin was found to be able to raise apoptosis significantly in A549 cells, which was mitochondrial mediated and occurred through an imbalance in Bcl2/Bax response. The quercetin-induced apoptotic effect was ascertained to be due down-regulation of IL6/STAT3 signaling pathway which would otherwise help the cell to survive and proliferate in an uncontrolled manner. Quercetin was able to block NF-κB activity at early hours, which might be able to bring the down-regulation of IL-6 titer and in turn IL-6 induced STAT3 expression. Down-regulation of both STAT3 and NF-κB expression presumably occurred after quercetin exposure; this might therefore cause down-regulation of Bcl2 activity because both these are major upstream effector molecules of Bcl2. Instantaneously this alteration in Bcl2 responses might generate an imbalance in Bcl2/Bax ratio that ultimately brought about mitochondria mediated apoptosis in A549 cells.

As hydrophobic flavonoid molecule quercetin has poor solubility in water, an attempt was made to increase its bioavailability by encapsulation with PLGA nanoparticles by solvent
evaporation technique for improvement of its poor aqueous solubility. Maximum intensity of quercetin loaded PLGA nanoparticles was found to be at 110 nm of size with polydispersity index value of 0.186 with smooth and bright surface topology. The presence of quercetin characteristic peaks on PLGA-quercetin nanomolecule after FTIR analysis confirmed the successful encapsulation of quercetin in PLGA nanoparticles. The nano-PLGA encapsulated quercetin (NPEQ) was found to be effective in decreasing cell viability of two major NSCLC cell lines, A549 and H460, at relatively low doses. The reduction of viability was due to apoptotic cell death and might be through caspase3 mediated way. NPEQ was capable of down-regulating Hsp90 at early hrs which might have directed the cells towards caspase3 mediated apoptosis. Akt, being a major co-chaperone of Hsp90 was also down-regulated after NPEQ exposure at early hrs. This NPEQ-mediated early down-regulation of Akt presumably played a major role in inhibiting the Hsp90 and on the other hand in blocking the uncontrolled NSCLC cells proliferation which in turn put the cells towards apoptotic death.

Thus, from the overall results, it can be concluded that cancer is a dreadful disease, many aspects of which have been known by extensive research so far, but still the effective cure is not in sight after the disease has advanced to a critical point. Therefore research into other domain, like CAM, can be fruitful if there could be any answer from the nature for an effective cure. More research, particularly on the dose and combination of drugs, is warranted before any drug can be recommended for effective treatment of cancer. Further, results of meaningful animal trials and supportive in vitro studies should complement each other in determining the efficacy of a drug or group of drugs recommended for use, either solely or in combination with suitable and tested orthodox medicine, particularly to ameliorate the toxic effects of the latter. Hopefully, the overall results of this study would throw light on the efficacy of Thuja occidentalis, a medicinal plant already in use for aiding cancer treatment, and can be of help in anti-cancer drug design that can be effectively used against lung cancer in human cases after further suitable animal and human trials.