Cancer is a cellular disease of uncontrolled growth. It has become the most fatal disease worldwide, accounting for 13% of all deaths in 2008 (Jemal et al., 2010). Lung cancer is the leading cause of cancer-related deaths worldwide. Among lung cancers, NSCLC account for approximately 80% of lung cancer. Despite improvements in survival rate of NSCLC diagnosed patients through early detection and treatment, the major therapeutic scope for NSCLC is limited, as it bears a very narrow range of chemo- or drug-sensitivities (Fong et al., 2003, Neel et al., 2013). Thus, most cancer therapeutic approaches, especially the chemosynthetic compounds, are made to inhibit cancer cell progression and survivability by inducing apoptotic cell death (Sikdar and Khuda-Bukhsh, 2013a; Saha and Khuda-Bukhsh, 2013; Elomre, 2007). These chemosynthetic drugs have the ability to make them bio-available to the target cells, but they also affect the normal cells as well and produce similar cytotoxicity. Therefore, the search for new therapeutic approaches against NSCLC is still important and urgently needed in clinical oncology.

Using complementary and alternative medicine (CAM)’s for treatment of NSCLC has now become popular for relatively less side-effects. One way through which CAM act is through activating apoptosis process to kill cancer cells sparing the normal ones. Moreover, it acts also in those cancer cells which bear several genetic mutations and thus produces drug/chemo resistance. Homeopathy is a major arm of CAM therapy, in which either plant extract (as mother tincture) or ultra-low doses of ultra-highly diluted remedies (potentized forms) are prepared to ameliorate and cure several diseases (Boericke 2004; Khuda-Bukhsh 1997, 2006) including cancer (Boericke 2004; Biswas et al., 2005; Banerjee et al., 2010; Preethi et al., 2012). However, very few plant extracts, their potentized forms and bioactive fractions/components isolated from it had been reported earlier to be effective particularly against NSCLC. However, so far as the author is aware, no in-depth mechanistic study of any noble plant extract having anti-NSCLC properties has yet been made.

We have tried to fill up this lacuna in knowledge and evaluated *Thuja occidentalis* extract, its potentized forms and isolated flavonols-rich fraction against NSCLC. Ameliorating effect of highly potentized form, Thuja 30C, was verified against BaP (lung carcinogen) induced lung cells. However, quercetin, a noble bioactive component of this isolated fraction, was difficult to separate from the isolated flavonols-rich fraction. Therefore, quercetin was procured from Himedia, India and efficacy of it was verified on NSCLC cell
cell line, in vitro. As quercetin is a hydrophobic molecule, an effort was made to increase its cellular availability by encapsulating it in nano-PLGA form and efficacy of this quercetin loaded PLGA nanoparticles was verified against two major NSCLC cell lines, A549 and H460.

With this background scenario, the main objectives of this study are -

- Evaluation of anti-cancer potential of ethanolic leaf extract of *Thuja occidentalis* and its potentized forms against NSCLC in vitro.

- Isolation of flavonols-rich fraction from ethanolic leaf extract of *Thuja occidentalis* and evaluation of its anti-cancer potential against NSCLC both by in vitro and in vivo studies.

- Evaluation of anti-cancer potential of quercetin, a major component of flavonols-rich fraction of *Thuja occidentalis* and its PLGA nano-encapsulated form against NSCLC in vitro.

Accordingly, the total work has been subdivided into three chapters and all the chapters have been subdivided into several sub-sections.

**Chapter 1. Evaluation of anti-cancer potential of ethanolic leaf extract of *Thuja occidentalis* and its potentized forms against NSCLC in vitro**

A. Evaluation of anti-cancer and anti-proliferative potentials of ethanolic leaf extract of *Thuja occidentalis* (TO) against NSCLC cell line, A549.

B. Cytotoxic efficacy of potentized forms of *Thuja occidentalis* against A549 cells.

C. Amelioration of Benzo(a)pyrene (BaP) induced normal mice lung cell toxicity by ultra-high dilution of Thuja (Thuja 30C).

**Chapter 2. Isolation of flavonols-rich fraction from ethanolic leaf extract of *Thuja occidentalis* and evaluation of its anti-cancer potential against NSCLC both by in vitro and in vivo studies**

A. Apoptotic potential of flavonols-rich fraction (FRF), isolated from ethanolic leaf extract of *Thuja occidentalis* against NSCLC cell line, A549.

B. Apoptotic and anti-proliferative potentials of flavonols-rich fraction (FRF), isolated from ethanolic leaf extract of *Thuja occidentalis* against Benzo(a)pyrene induced mice lung carcinogenesis.
Chapter 3. Evaluation of anti-cancer potential of quercetin, a major component of flavonols-rich fraction of *Thuja occidentalis* and its PLGA nano-encapsulated form against NSCLC *in vitro*

A. Apoptotic potential of quercetin against NSCLC cell line, A549.

B. Apoptotic and anti-proliferative potentials of nano-PLGA-encapsulated quercetin against NSCLC cells, A549 and H460.

To examine these aspects, several parameters were studied. Firstly, for *in vitro* evaluation, MTT assay was done to check the potentiality of the drug to raise cellular cytotoxicity. Thereafter according to the mode of drug-induced cell death several assays like-morphological analysis, DNA fragmentation assay, AnnexinV assay, cell cycle analysis were done. After getting the preliminary confirmation of apoptosis, as drug-induced cell death type further analyses were done to find out the background mechanism behind the drug-induced apoptosis. Studies were done emphasizing on anti-proliferative activity, apoptosis related gene or protein expression modulation, drug-DNA interaction, ROS activity, mitochondrial membrane potential, IL-6/STAT3 signaling pathway and Hsp90 modulation. To find out the ameliorating effect of potentized drug on BaP-intoxicated mice normal lung cells stress modulation pathways like activities of cellular ROS, GSH and Hsp90 were analyzed. Furthermore, to evaluate the efficacy of the isolated fraction of *Thuja occidentalis* extract, assessment was also made in lowering BaP induced mice non-small cell lung carcinogenesis *in vivo*. In this study, drug-induced anti-proliferative activity, constitutive and PI3K-induced Akt signaling pathway modulation, oxidative stress activities were verified.

Overall results indicated *Thuja occidentalis* extract as a potential agent that reduced the viability of a K-ras mutated NSCLC cell line-A549 in a dose-dependent manner. The reduction was done apparently by blocking uncontrolled cell proliferation and was apoptotic in nature. The TO-induced apoptosis process in A549 cells occurred via caspase3 mediated pathways.

However, potentized forms of Thuja i.e. Thuja 5C, 9C and 15C were not able to be cytotoxic on A549 cell line. But, the ultra-highly diluted form Thuja 30C was found to be ameliorative in function against BaP-induced normal mice lung cell cytotoxicity by lowering the cellular stress levels.
Isolated flavonols-rich fraction of Thuja extract was ascertained to be apoptotic against A549 cell line in a ROS-independent, mitochondria dependent pathway and probably by interacting with nuclear DNA at early hrs. In BaP-induced non-small cell lung carcinogenic mice body, this isolated fraction was also found to be a noble agent that lowered the BaP induced lung tumor load, proliferation and toxicity. The drug was also able to down-regulate BaP-induced oxidative stress level. Beside this, it also exerted anti-proliferative activity, presumably through down-regulation of constitutive and PI3K-induced Akt expressions which in turn induced caspase3 mediated apoptotic process in BaP induced lung tumor cells. Experimental observation revealed that quercetin, major flavonols of this isolated fraction was effective to initiate mitochondria mediated apoptosis in A549 cells line via down-regulation of IL-6/STAT3 signaling pathway and NF-κB activity. Furthermore, when this hydrophobic flavonol quercetin was encapsulated in PLGA nanoparticles, cellular availability was improved as this exerted anti-proliferative and apoptotic efficacy on two major NSCLC cell lines, A549 and H460 at minimal doses. This nano-PLGA loaded quercetin molecule presumably exerted such anti-proliferative and apoptotic activities through down-regulation of Akt. However, this Akt down-regulation on the other hand presumably helped in down-regulation of Hsp90, major protein related to cell survivality. This drug-induced inhibition of Hsp90 ultimately led the cells into apoptosis via caspase3 activation.

In summary, this detailed and systematic study revealed that Thuja occidentalis extract, isolated flavonol-rich fraction, quercetin (major component of flavonol-rich fraction) and quercetin loaded PLGA nanoparticles as potential agents for treatment of NSCLC. However, ultra-high diluted potentized form of this plant extract i.e. Thuja 30C was also found to have ameliorative action against of BaP induced lung cell damage. This signifies that the potentized remedy can also be used as a supportive remedy in lung cancer.

The present findings provide pre-clinical data suggesting that the plant Thuja occidentalis has the potential to be developed as a pharmacologically safe agent either alone or as supportive medicines for the treatment of NSCLC in human. Further research is warranted to be conducted in other animal models before it can be recommended for human trial for the development of a suitable strategy for effective control and management of lung cancer.