ABSTRACT

Infectious diseases and cancer are potentially the largest threat to human race, with cancer being the second largest fatal disease in the world. Key aspects of cancer biology are cell division and apoptosis and are essential targets of new anti-cancer therapies. Cyclin Dependent Kinases (CDK 1, 2, 4 and 6) play essential role in intracellular control of cell cycle which form complexes with specific regulatory proteins called cyclins (A, B, D and E). Activation of cyclin/CDK is counterbalanced by CKI (P16 and P27) that serves as negative regulator of the cell cycle, stopping the cell from advancing to the next phase of the cell cycle.

Leukemia is characterized by abnormal proliferation and can lead to dedifferentiation at any point. The fate of both cell-cycle arrested and differentiated cells is apoptosis. Apoptotically dying cells activate a set of degradative enzymes, the caspases which mediate the controlled disassembly and degradation of the cell. Lung cancer remains the leading cause of cancer-related deaths. Inflammation has been suggested to promote lung cancer via several possible path ways, which is activated by a regulatory protein called NF-κB that promotes the production of various growth factors and protects pre-malignant cells from surveillance mechanisms. Thus, there is an increasing level of interest in developing inhibitors of NF-κB for novel treatment strategies of human cancers.
In recent times, plants have been considered as an untapped reservoir of novel drugs as the compounds isolated from traditional plants serve as a platform for new drug synthesis. The crude and the pure compound isolated from *Centrosema pubescens* were studied on cancer cell lines (A549 and HL-60) and evaluated on various key anti-cancer targets. Three objectives were framed for carrying out the present study, (a) assessment of the anti-proliferative activity of *C. pubescens* Benth using A549 as an *in vitro* model (b) isolation of bioactive molecule from active crude extracts and elucidation of the mechanistic action exhibited by the active crude extracts and bioactive molecule in A549 in an *in vitro* and *in vivo* model (c) The role of crude *C. pubescens* extracts in differentiation therapy using HL 60 as an *in vitro* model.

An assessment of dichloromethane (CPDE) and methanol (CPME) extracts of *C. pubescens* showed high anti-proliferative activity in A549 cells. These extracts were further taken for bioactivity guided purification to isolate the bio-active molecule(s) responsible for anti-proliferative activity. It was observed that the isolates obtained from CPDE did not exhibit activity on par with that of the crude extract in both leukemic and lung cancer cell lines. Thus the bioactivity might have been due to the compound level synergy present in the crude extract, and can be used as a nutraceutical for treating leukemic and lung cancer. In the case of CPME, purification yielded a pure compound, Lupenone, which exhibited significant anti-proliferative effect on A549 lung cancer cell line.
As cell cycle arrest and apoptosis are two crucial facets of proliferation of cancer cells, the role of CPME, Lupenone and CPDE in the regulation of cell cycle in lung cancer cells were studied. Their inhibitory effect on proliferation was due to G1 arrest of cell cycle at 24h and G2/M phase arrest at 48h which was corroborated using flow cytometric analysis. These were further confirmed by examining the cell cycle regulatory markers wherein, down regulation of cyclin D, cyclin E, CDK 2, CDK 4, CDK 6, pRb and E2F-1 and an up regulation of CKI like p16 and p27 was observed. Apoptosis was also found to be induced at 24 h which was manifested by the activation of cleaved poly (ADP-ribose) polymerase (PARP). Flow cytometry revealed an increased level of apoptotic cells in the sub G1 phase.

Apoptosis inducing property was observed by the suppression of NF-κB activation and down regulation of the anti-apoptotic gene expression. Reduced levels of COX-2, PLA2, and AKT and increased level of NO production indicates the inhibition of NSCLC survival. An Electrophoretic Mobility Shift Assay (EMSA) assessed the NF-κB DNA binding activity. The genes involved in the intrinsic pathway of apoptosis (P53, Bax, Bcl-2, Cyt C, Caspase 3 and Ras) was activated which was confirmed by the disruption in the mitochondrial membrane potential. Our results thus suggested that CPME, Lupenone and CPDE may be a promising alternative because of their high anti-proliferative activity in the treatment of lung cancer via cell cycle arrest and inflammation mediated apoptosis.

These results were further confirmed by performing *in vivo* studies in swiss albino male mice. Lung cancer induced mice treated with CPDE and
CPME showed inhibition of cell cycle markers involved in G1 phase that confirmed cell cycle arrest found from *in vitro*. Thus the study carried out on *Centrosema pubescens* using both *in vitro* and *in vivo* models confirmed its role in lung cancer treatment.

Further, study was conducted to assess the effect of CPDE and CPME on leukemic cancer using HL-60 cells as *in vitro* model. [$^3$H]-thymidine incorporation assay revealed the anti-proliferative activity of CPDE and CPME in HL-60 cells at IC$_{50}$ concentration at 24h. At 1 μg/ml it was also found to induce differentiation at 72 h and 96 h respectively. The occurrence of differentiation was observed by NBT reduction assay and confirmed with superoxide dismutase assay (SOD). Western blot analysis revealed an increase in the levels of p27 and p16. Flow cytometry analysis inferred a G2-M phase arrest. The fate of the differentiated cells in apoptotic process was evaluated by DNA fragmentation and Propidium iodide staining. RT-PCR showed an up-regulation of Bax, TNF and Cas 3 and down regulation of Bcl2. Thus, *C. pubescens* can be used for inducing differentiation in HL-60 cells through cell cycle arrest leading to apoptosis. These data show that *Centrosema pubescens* exhibits three distinct anti-cancer activities: induction of cell cycle arrest, redifferentiation and apoptosis.