Abstract

Cancer has emerged as a second leading cause of disease related death worldwide accounting 8.2 million deaths and 14.1 million new cases in 2012. Surgery, radiation, and chemotherapy have been the mainstay of treatment for human malignancies for more than last 40 years. However, each of these treatment modalities are restricted by its own set of limitations owing to factors such as location, size, and stage of malignancy present, along with the age and medical condition of the patient. As the current preventive and treatment regime are inadequate to control this lethal disease, the research on anticancer drug development has been shifted towards a more realistic and holistic strategy termed as 'Chemoprevention', to inhibit or reverse or retard the 'biochemical' and 'molecular' process of carcinogenesis before the development of invasive cancer, using naturally occurring (plant derived) non-toxic chemical entities. Several studies have reported numbers of plant derived products that are being used in chemopreventive and anticancer therapies. However, a myriad of plant products exist that have shown promising chemopreventive and anticancer properties *in vitro*, but have failed in preclinical animal model. This necessitates the exploration of phytochemicals from the plethora of medicinal plants and to identify the amenable key biochemical and molecular pathways in a systematic way.

The north eastern region of India is a part of both Himalayan as well as Indo-Burma biodiversity hotspot and is inhabited by more than 200 tribal communities that have high diversity of indigenous knowledge on usage of medicinal plants. The rich medicinal plant knowledge can be a source of novel drug molecule for treating human diseases.With this background, in the present study, we have selected three medicinal plants, *viz. Nyctanthes arbor-tristis Linn.*, *Phlogacanthus tubiflorus Nees.* and *Phlogacanthus thyrsiflorus Nees.* on the basis of traditional knowledge from the north eastern region of India and validated their ethno-claimed potentials along with deciphering mechanism responsible thereof.

Animal systems are continuously exposed to various forms of environmental xenobiotics and some of them are potent active carcinogens and other are pre-carcinogens. However, the risk for disease manifestation depends primarily on the

ability to detoxify the xenobiotics. In the present study, hydroalcoholic root extract of *Phlogacanthus tubiflorus* Nees. (REPT) and flower extract of *Nyctanthes arbor-tristis* (FENA) exhibited significant protective functions against oxidative stress induced by H_2O_2 in isolated lymphocytes cultured *in vitro*. The significant increase in the levels of cellular reduced glutathione (GSH) might have decreased the level of nitric oxide (NO) that could have played role in protective function against oxidative stress induced by H_2O_2 through inhibition of lipid peroxidation. The ability of the extracts to scavenge free radicals were evaluated using cell free chemical based *in vitro* reaction system which provide direct evidence of radical scavenging property. From the present study, FENA was found to be prominent in scavenging ABTS, DPPH, superoxide, hydroxyl radicals as well as inhibition of Fe^{2+} chelation. The study also demonstrated that FENA possesses strong reducing potentials of Fe^{3+} to Fe^{2+}. Similar kinds of observation were also found for the leaf extract of *Phlogacanthus thyrsiflorus* Nees. (LEPT). The presence of high contents of phenolic compounds and flavonoids could have played role in antioxidant and free radical scavenging activities.

Understanding the importance of phytochemicals in cancer therapeutics, in the present investigation, FENA and LEPT were evaluated for their chemopreventive potentials using chemically induced carcinogenesis model. Oral feeding of FENA/LEPT prominently inhibited the papilloma incidence, multiplicity as well as papilloma yield and burden in 7,12-Dimethylbenz(a)anthracene (DMBA) induced and croton oil promoted skin papilloma model and in Benzo(a)pyrene [B(a)P] induced forestomach papilloma model. To understand the mechanism of chemoprevention, we have evaluated the status of cellular biochemical parameters related to carcinogenesis in hepatic, extra-hepatic as well as papilloma bearing tissues. The study clearly demonstrated that significant decrease in the activities of phase I enzymes probably block the biotransformation of pre-carcinogens into active form or they may be detoxified through elevated phase II systems. Moreover, increasing status of cellular redox system could have played role in scavenging reactive oxygen/nitrogen species (ROS/RNS) directly and/or eliminating their harmful consequences like peroxidation of biological macro and-micro molecules. The composite mixture of phytochemicals

such as phytol, n-hexadecanoic acid, oleic acid etc. present in the extract may have acted over multiple biochemical and molecular pathways that might have leads to chemoprevention of chemically induced cancer. Further ADME/Tox analysis validated these phytochemicals to possess druggable like characteristic pertaining to standard drugs.

Chemoprevention by virtue of action, inhibit or delay the process of carcinogenesis before cancer phenotype develops, however, already developed cancer phenotype cannot be targeted by chemoprevention. Therefore, a great deal of drug should have both the chemoprventive and anticancer potentials. In order to ascertain anticancer potentials, the plant extracts were further fractionated by differential solvent extraction method to enrich specific groups of molecule in a preparation. In the present investigation, the free flavonoid enriched fraction of *Nyctanthes arbor-tristis* flowers (F^3NAF) was found to be most active fraction as it exhibited strong anticancer activity against human lung cancer (H1299 and A549) and prostate cancer cell lines (PC3 and LNCap) as measured by MTT assay. The F^3NAF inhibited the growth of prostate cancer (PCa) cell lines (PC3 and LNCaP) in a dose and time dependent manner as ascertained from the trypan blue cell count assay. The results of MTT assay also showed that F^3NAF (6.25-200 μg/ml) decreases the viability of PC3 and LNCaP cells upto 51.8% and 31.4% after 48 hours of treatment respectively. In order to rule out the inhibition of total cell number, we performed replication based BrdU antiproliferation assay and the results clearly demonstrated the inhibition of proliferation of PC3 and LNCaP cells upto 31.3% and 56.7% respectively upon 24 hours of treatment. The arrest of cell cycle progression at G1 and G2 phase were due to the down regulation of expression levels of proteins involved in the transition of cell cycle from G1 to S phase (CDK4, CDK6, cyclin D1, cyclin D3, cyclin E) and G2 to M phase (cdc25c and cdc2) could have played role in antiprolifeartive efficacy of the F^3NAF. As the activated ERK1/2 is known to promote transition of cell cycle from G1 to S phase by enhancing Cyclin D1 accumulation and assembling and stabilizing D1–Cdk4/6 complexes, their downregulation by the F^3NAF treatment might be also responsible for the arrest of cell cycle progression at G1 phase and for observed antiprolifeartive efficacy.

Several molecular signaling pathways are implicated in the development and progression of PCa and disruption of critical signaling node has gained impetus as a therapeutic target in recent past. The phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signaling pathways are known to plays crucial role in cell survival, proliferation, and differentiation etc. Several studies have indicated up-regulated PI3K/AKT/mTOR signaling in 30-50% cases of PCa cases. In the present study, F^3NAF inhibited the expression level of mTOR and phospho-mTOR (Ser 2448) along with Rictor protein suggesting that the growth and survival of PC3 is inhibited upstream to Akt phosphorylation or downstream to mTOR. Additionally, F^3NAF also inhibited the migration and invasion of PC3 cells which was evident from the wound healing and Transwell invasion and migration assays through downregulation of MMP2 and MMP9 expression.

The finding of the present study clearly demonstrated chemopreventive and anticancer potentials of flower extract of *Nyctanthes arbor-tristis* (FENA) and leaf extract of *Phlogacanthus thyrsiflorus* Nees. However, there is a need for further investigation in order to identify and characterize the specific phytochemical responsible thereof and to precisely understand the molecular mechanisms using gene knock out/xenograft cancer models.