Chapter VI

Conclusions
A better understanding of disease progression can improve the patient’s medical care. First, it allows for a more precise and earlier diagnosis and second, one can apply individual therapies. Figure 6.1 represents the different steps involved in disease formation especially cancer and inflammatory diseases. This figure also represents the involvement of natural resources in suppressing/ameliorating the steps/causes resulted from the various physiological and environmental factors. When human body gets exposed to the physiological factors like pro-inflammatory cytokines, produced during inflammation, leads to the release of different types of reactive oxygen and reactive nitrogen species in the body. These free radicals leads to diverse cellular phenomena, namely, damage of DNA-repair proteins and caspases, lipid peroxydation, DNA damage followed by mutation and NF-κB activation. All these phenomena give rise to wide range of diseases. Cancer is a multistage process defined by at least three stages: initiation, promotion, and progression (Schulte-Hermann et al., 1990; Ames and Gold, 1992; Guyton and Kensler, 1993).

Figure 6.1 Different Steps Involved in Disease Formation and Impact of Natural Resources

Generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is initiated by respiratory burst, which is set off by various physiological factors especially inflammation. The fabrication of an assortment of ROS and RNS from the molecular O₂ and L-arginine, respectively, carried on by different enzymes like MPO (myloperoxidase), NADPH oxidase, SOD (superoxide dismutase) and NOS (nitric oxide synthase) leads to diverse cellular phenomena, namely, damage of DNA-repair proteins and caspases, lipid peroxydation, DNA damage followed by mutation and NF-κB activation. All these phenomena give rise to wide range of diseases specifically cancer. Extracts of natural resources inhibit the generations of the free radicals by scavenging both the mother and the daughter products resulting the obstruction of oxidative stress and cancer manifestation. Extracts targets cancer cells to kill them and also possess anti-inflammatory potential to prevent cancer promotion.
Oxidative stress interacts with all three stages of this process. During the initiation stage, ROS may produce DNA damage by introducing gene mutations and structural alterations of the DNA. In the promotion stage, ROS can contribute to abnormal gene expression, blockage of cell to cell communication, and modification of second messenger systems, thus resulting in an increase of cell proliferation or a decrease in apoptosis of the initiated cell population. Finally, oxidative stress may also participate in the progression stage of the cancer process by adding further DNA alterations to the initiated cell population (Klaunig et al., 1998). In fact, initial experiments on the role of ROS in tumor initiation have assumed that oxidative stress acts as a DNA-damaging agent, effectively increasing the mutation rate within cells and thus promoting oncogenic transformation (Jackson and Loeb, 2001). However, more recent studies have revealed that in addition to inducing genomic instability, ROS can specifically activate certain signalling pathways and thus contribute to tumor development (Storz, 2005). Considerable laboratory evidence from chemical, cell culture, and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer. Hence, the first step i.e. inflammation can be considered as a target to prevent cancer, while; the last step can be targeted for curing of cancer. According to our previous studies, 70% methanol extract of Caesalpinia crista (CCME), Cajanus cajan (CLME), Hemidesmus indicus (HIME), Spondias pinnata (SPME), Terminalia belerica (TBME), Tinospora cordifolia (TCME), Pongamia pinnata leaf (PLME), Pongamia pinnata seed (PSME), Pongamia pinnata flower (PFME) displayed a potential to scavenge the free radicals and therefore possess antioxidant activity. These extracts along with one insectivorous plant, Drosera burmannii (DBME), were screened against human lung adenocarcinoma (A549), human breast adenocarcinoma (MCF-7), human cervical carcinoma (HeLa), human hepatocellular carcinoma (HepG2) and human glioblastoma (U87) cell lines for their anticancer activity. SPME and TBME exhibited profound anticancer activity against all of the malignant cells except HepG2 in case of TBME. SPME and TBME showed promising anticancer activities against human lung adenocarcinoma established through their ability to regulate the pro- and anti-apoptotic proteins. Both these extracts induce apoptosis in A549 cells through activation of intrinsic and extrinsic pathway of apoptosis. On the other hand DBME displayed promising anticancer activity against human breast adenocarcinoma by inducing G2/M arrest and apoptosis through modulation of cell cycle, pro- & anti-apoptotic protein expressions. Their effects are also reflected in the DNA damage, subsequent apoptosis and/or cell cycle arrest of the malignant cells, corroborated by the viability, flow cytometric studies. The remaining extracts showed negligible anticancer activity against all of the screened cancer cells. These extracts were then investigated for their potential as anti-inflammatory agents and hence their role in prevention of cancer. TBME and DBME exhibited their ability to inhibit inflammation in murine macrophage cell line, through suppression of nitrite formation. Both the protein and gene expression studies establish this fact for TBME and DBME; SPME did not show any signs of anti-inflammatory potential. The same trend is also observed in the effects of the extracts in regulating TNF-α which in turn mediates the induction of nitric oxide synthase. The extracts also reduce the level of COX-2, another pro-inflammatory protein, and intracellular ROS, which makes it significantly interesting for future studies.

Recently, along with the plants, much attention has been paid to several lichen species (Symbiotic products of algae and fungi) as a source of natural anticancer and anti-inflammatory agents. Lichens produce a varied range of secondary metabolites and also some of them are unique to lichen symbiosis. Lichens exhibit promising anticancer and anti-inflammatory properties. 70% methanol extract of a tropical lichen named Parmotrema reticulatum (PRME) displayed a promising anticancer activity against human breast adenocarcinoma by arresting
the cells at S and G2/M phases followed by induction of apoptosis through regulation of expressions of cell cycle regulatory proteins and pro- & anti-apoptotic proteins. Moreover, PRME showed promising anti-inflammatory activity through inhibition of induction in nitrite formation, production of intracellular ROS, downregulation of the expression of iNOS, TNF-α & COX-2 at protein and gene level comparatively better than TBME and DBME.

The present results demonstrate that SPME, TBME, DBME and PRME are rich source of phytochemicals which can be attributed to the potent bioactivities of the extracts. Moreover, when the identified compounds from PRME were applied individually, the specificity towards cancer cells was lost and the constituents attacked normal cells as well, indicating the advantage of using crude extract or mixture of compounds to treat of various diseases. Moreover, it cannot be ignored that the respective percentages in which the compounds co-exist in the plant likely have a vital synergistic role. This mechanism requires further in-depth investigations. Nevertheless, the natural formulation of PRME is a potential safe and effective drug for the treatment against breast cancer. Taken together, these inspiring results with the plant and lichen extracts provide the impetus to investigate active principle(s) from the extracts and elucidate several unrevealed molecular aspects behind these activities which may definitely bring about a new era in drug discovery for the benefit of mankind.