Cancer is a group of diseases of higher multicellular organisms characterized by alterations in the expression of multiple genes, leading to deregulation of normal cellular program for cell division and differentiation. This results in an imbalance of cell replication and cell death that favours growth of a tumour cell population. Cancer incidence data generated by population-based cancer registries in India predicts that total cancer cases are likely to be 979,786 in the year 2010.

The link with chronic inflammation and cancer has been recognized for certain cancers several decades earlier. The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases, including cancer. Tumour growth can lead to the suppression of immunity; both systemically and in the microenvironment of the tumour.

Despite improvements in diagnosis, surgical techniques, local and systemic adjuvant therapies, most deaths from cancer result from the progressive growth of metastases that are resistant to conventional therapies.

Angiogenesis is a physiological process by which capillaries sprout from preexisting blood vessels. Neovascularisation is a requirement for solid tumour growth beyond 1–2 mm in diameter and play an important role by supplying nutrients and allowing metastatic spread.

Important safeguards like DNA repair mechanisms and check-points exist within the cells to prevent replication of cells bearing mutations. As the final safeguard, there is apoptosis, causing death of defective cells and thus prevent mutations that cause cancer. Apoptosis is a terminal cell fate, a highly regulated "suicide" process that is distinct from necrosis.

In current clinical scenario, radiation therapy alone is often used with curative intent for localized tumours, and is synergistic with chemotherapy in susceptible cancers. Unfortunately, this therapy is not tumour specific and has other serious side effects, most important of which is myelosuppression and there is probability of developing metastatic disease in distant organ sites. Chemotherapy is the most effective modality of managing metastasis of cancer cells. It is also a non specific mode of treatment with serious adverse effects like bone marrow suppression, mucositis and hair loss.

Immunostimulation via natural substances is considered to be a most promising way for the prevention and cure of neoplastic diseases. *Aerva lanata* is an important medicinal plant belonging to the family *Amaranthaceae*. 10-Methoxycanthine-6-one (10-MC) is a β carboline alkaloid from the plant *Aerva*
lanata. Thujone, a monoterpenoid ketone, occurs in nature as a mixture of α/β diastereoisomers, was obtained from Sigma Aldrich (Bangalore, India).

Anti tumour activity of A. lanata, 10-MC and Thujone was assessed using in vivo and in vitro models. In vivo anti tumour study was determined by studying the effect on tumour development in DLA induced solid tumour model and life span of EAC induced ascites tumour bearing mice. Animals treated with test compounds had significant reduction in tumour volume when compare to untreated control animals. The anti inflammatory activity of A. lanata, 10-MC and Thujone was studied using acute (carrageenan and dextran models) and chronic (formalin model) inflammation models by analysing paw thickness at various time points and serum levels of pro inflammatory cytokines.

The immunomodulatory activity of A. lanata, 10-MC and Thujone was analysed using BALB/c mice. Treatment of animals with A. lanata, 10-MC and Thujone was found to enhance the total WBC count, relative weight and cellularity of lymphoid organs, bone marrow cellularity and the number of α-esterase positive cells. The test compounds had stimulatory effect on the circulating antibody titre and number of antibody producing cells in spleen. Effect of A. lanata, 10-MC and Thujone on the cell mediated immune response in normal and tumour bearing animals were analysed by determining the natural killer cell activity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent complement-mediated cytotoxicity (ACC) and the generation of cytotoxic T lymphocytes (CTL).

Anti metastatic effect of A. lanata, 10-MC and Thujone was analysed using in vitro as well as in vivo model. For in vivo studies, lung metastasis was induced in C57BL/6 mice by injecting B16F-10 melanoma cells through lateral tail vein (Fidler, 1978; Liotta, 1986). Treatment of metastatic tumour bearing animals with A. lanata, 10-MC and Thujone significantly reduced the number of lung tumour nodules and increased the life span. Biochemical parameters in lungs and serum as well as expression levels of genes involved in metastatic process also correlated with the in vivo study. In vitro anti metastatic activity was determined using B16F-10 melanoma cells. The effect of test compounds on the rate of proliferation of B16F-10 cells was analysed by ³H-thymidine incorporation assay. Inhibition of tumour cell motility, tumour cell adhesion to collagen matrix and invasion of Type I collagen coated polycarbonate membrane were determined. The test compounds inhibited the production of matrix metalloproteinase (MMP-2 and MMP-9).
Antiangiogenic activity of *A. lanata*, 10-MC and Thujone were analysed using *in vivo* and *in vitro* models. Treatment with test compounds was found to inhibit the tumour associated capillary formation in C57BL/6 mice. The levels of serum NO, VEGF, TIMP-1, TNF-α, IL-1β, IL-2 and IL-6 were estimated using specific ELISA kits. *In vivo* vascular density during angiogenesis was determined by matrigel plug assay using anti-CD31 antibody. Mean vascular density in *A. lanata*, 10-MC and Thujone treated animals was much lower than the control group. The *in vitro* antiangiogenic activity of *A. lanata*, 10-MC and Thujone was determined by analysing microvessel outgrowth from rat aortic ring and experiments using isolated human umbilical vein endothelial cells (HUVECs). HUVEC treated with test compounds was found to have significant inhibition in proliferation, migration, invasion and tube formation in dose dependent manner.

Effect of *A. lanata*, 10-MC and Thujone on induction of apoptosis in B16F-10 melanoma cell line was studied by analysing morphology of cells, DNA laddering pattern, cell cycling pattern and TUNEL assay. 10-MC and Thujone was found to inhibit the activation and nuclear translocation of NF-κB in tumour cells.

The effect of 10-MC on immunosuppression during radiation therapy and chemotherapy was analysed using *in vivo* system. Mice were treated with gamma radiation/cyclophosphamide along with the administration of 10-MC. Haematological parameters, relative weight of lymphoid organs, bone marrow cellularity and serum cytokine levels were monitored. There was significant augmentation of therapeutic benefits of radiation by 10-MC.

In summary, the present study reveals the immunomodulatory activity of *A. lanata*, 10-Methoxyxanthin-6-one and Thujone; augmenting both humoral and cell mediated immune responses and also curtail the inflammatory responses. The test compounds also exhibited a significant anti metastatic potential in both *in vivo* and *in vitro* systems. *A. lanata*, 10-MC and Thujone could induce cell cycle delay and apoptosis in B16F-10 melanoma cells through p53 dependent mechanism; both may contribute to anti tumour and anti metastatic activities. Treatment with these compounds was found to effectively block the tumour specific angiogenesis; *in vivo* and *in vitro*. 10-MC treatment was found to reverse radiation therapy and chemotherapy induced immunosuppression and augment therapeutic efficacy of lower dose radiation therapy.

**Key words:** Immunomodulation, Metastasis, Angiogenesis, Apoptosis, NF-κB, Pro inflammatory cytokines, *Aerva lanata*, 10-Methoxyxanthin-6-one and Thujone.