ABSTRACT

Cancer is a large and complex family of malignancies that arises from myriad of genetic and environmental events that elude and exploit normal cellular processes. Despite the impressive advances that have been made in cancer drug development, none of them is found to successfully control the tumor development once it undergoes metastasis. Since natural products find highest rank in anticancer drug designing, in the present study we have evaluated the effect of *A. paniculata* and its major component Andrographolide on different stages of tumor development including metastasis and angiogenesis.

The pathogenesis of cancer is strongly associated with chronic inflammation. Effect of *A. paniculata* and Andrographolide on carrageenan induced inflammation, LPS induced NO and TNF-α production were studied. We also evaluated the antitumor and antioxidant activity of *A. paniculata* and Andrographolide. It was found that *A. paniculata* and Andrographolide suppress the inflammation, production of free radicals such as superoxide lipid peroxides and hydroxyl radicals and tumor development.

The role of *A. paniculata* and Andrographolide on apoptosis was analyzed. *A. paniculata* and Andrographolide treated B16F-10 melanoma cells underwent apoptosis as observed by the morphological changes and DNA ladder formation. The expression of antiapoptoptic gene Bcl-2 and proinflammatory cytokines (IL-1β, IL-6, TNF-α, GM-CSF and IL-12p40) was also inhibited by *A. paniculata* and Andrographolide which is directly related to the induction of apoptosis.

It has been apparent that attacks of tumors by means of immune cells are efficient in curtailing tumor progression. We have analyzed the effect of *A. paniculata* and Andrographolide on the immune system and found that *A. paniculata* and Andrographolide increase the total WBC count, bone marrow cellularity, antibody titer, number of antibody producing cells and macrophage mediated phagocytosis. In addition, NK cell activity,
ADCC and ACC were also significantly enhanced by the administration of *A. paniculata* and Andrographolide in BALB/c mice while these compounds inhibited the Delayed type hypersensitivity (DTH) reaction. All these revealed the immunomodulatory effect of *A. paniculata* and Andrographolide.

Metastasis the hall mark of malignant transformation is a well coordinated sequential process. In the current study the antimetastatic activity of *A. paniculata* and Andrographolide was evaluated using *in vivo* as well as *in vitro* system. *A. paniculata* and Andrographolide treatment was found to significantly inhibit the lung tumor nodules when administered simultaneously as well as prophylactically. Biochemical analyses of lung tissues also showed reduced lung fibrosis. Since *A. paniculata* and Andrographolide enhanced the cell mediated immune responses in normal and EAC bearing animals we investigated the effect of *A. paniculata* and Andrographolide on cell mediated immune responses in metastatic tumor bearing animals and found to augment NK cell activity, ADCC and ACC which mediate tumor cell destruction. Gelatin zymography analysis showed that *A. paniculata* and Andrographolide inhibited the production of MMP-2 and MMP-9, major proteinases involved in extracellular matrix degradation during cancer cell metastasis. These compounds could also inhibit the tumor cell adhesion, motility and invasion in *in vitro* system. Gene expression profiling of metastatic tumor bearing animals showed inhibition of MMP-2, MMP-9, Erk-1, Erk-2, K-ras and VEGF expression and elevation of TIMP-1, TIMP-2 and antimetastatic gene Nm-23 by the administration of *A. paniculata* and Andrographolide. All these results contribute to the antimetastatic potential of *A. paniculata* and Andrographolide.

Adequate angiogenesis is essential for successful tumor metastasis. The inhibitory effect of *A. paniculata* and Andrographolide on tumor specific angiogenesis was investigated. Administration of *A. paniculata* and Andrographolide significantly inhibited the tumor directed capillaries and VEGF induced endothelial cell proliferation from rat aorta. In addition,
treatment with *A. paniculata* and Andrographolide down regulate the production of proangiogenic factor such as VEGF, NO and proinflammatory cytokines while upregulate the production of antiangiogenic factors like IL-2 and TIMP-1. All these results were positively linked to the antiangiogenic activity.

The possible role of *A. paniculata* and Andrographolide as an adjuvant during radiotherapy and chemotherapy was also assessed. *A. paniculata* and Andrographolide was effective in reducing the myelosuppression induced by radiation and chemotherapy without altering antitumor efficacy. *A. paniculata* and Andrographolide was also found to reduce the urotoxicity induced by the chemotherapeutic agent cyclophosphamide.

This study clearly demonstrates that *A. paniculata* and Andrographolide have significant effect on the inhibition of tumor development by blocking various events in tumor progression, metastasis and angiogenesis directly or through boosting the immune system and apoptotic machinery against the tumor. Moreover, *A. paniculata* can be used as adjuvant in cancer treatment.

*Key word: Metastasis, Angiogenesis, Immunomodulators, Inflammation, Proinflammatory cytokine, Chemoprotectors, Andrographis paniculata, Andrographolide.*