SUMMARY

1. The Eastern Himalayas, one of the two biodiversity hotspots in India, harbours a vast resource of medicinal plants which are often used by local inhabitants of their own experience. The people of Terai belt of the Eastern Himalayas use the leaf of one such plant *Eupatorium adenophorum* of Astaraceae (Compositae) family for treating mouth and skin sores. The healing property of *E. adenophorum* suggested to us the possible role of this plant could be anti-inflammatory and immunomodulatory.

2. *E. adenophorum*, a shrub was collected mainly from Kurseong hill at 1400 mt high slope of the Esatern Himalayas. Ten gms of freshly collected leaves were cleaned thoroughly with water, air dried, crushed and extracted in 10 ml absolute ethanol overnight. After sterilization through 0.22 μm cellulose acetate filter the ethanolic leaf extract of *E. adenophorum* (EEA) was stored at 4^0C and used at different doses for different experiments.

3. Toxicity of EEA, if any, was tested in reference to hematological parameters after injecting (i.v.) the extract in mice and *in vitro* culture of lymphocytes with EEA. There was no marked effect on hemoglobin percentage and RBC count. Rather it was promotional for WBCs and without any inimical effect for the lymphocytes.

4. EEA was in fact found to induce blastogenesis in both the B and T cell populations, and more efficiently for T cells. The stimulatory effect of EEA for lymphocytes was also reflected in DNA synthesis, assayed by ^{3}H-incorporation.

5. The cell cycle analysis by FACS showed that EEA could drive a notable percentage of lymphocytes towards S phase at 16 hrs and maintained the stimulation upto 48 hrs. Progression of the cells towards G_2-M phase is evident at 24 and 48 hrs. Thus, EEA can set the lymphocytes for cell division. The cell division is a prerequisite for the lymphocytes for functional differentiation.

6. Anti-inflammatory activity of EEA was judged by ability to inhibit delayed type hypersensitive (DTH) reaction set in mouse paw by subcutaneous injection of 25 or 50 μl 2,4-dinitrofluorobenzene (DNFB). Both topical and intravenous application of

EEA could inhibit the DTH induration and brought back normalcy of the paw much earlier than controls. Topical application was more effective in both the counts.

7. Differential counts of leukocytes in the oozing fluid from the DTH inflammation site in experimental and control mice revealed that EEA could restrict infiltration of inflammatory cells like neutrophils and monocytes in the afflicted site (paw). Inhibiting the number of these phagocytic cells likely lessen the ongoing inflammation reaction.

8. $CD4^+T$ cells participate in DTH reaction by secreting all different kinds of cytokines. Enumeration of $CD4^+T$ cells by labeling with paramagnetic probe and then separation in MACS revealed that treatment with EEA raised the number of $CD4^+T$ cells in splenic lymphocytes of DTH mice.

9. Serum level of the pro-inflammatory cytokine TNF-α increases with the progression of inflammation. It was apparently a paradox to find that EEA having anti-inflammatory effect, augmented TNF-α lev el. It was resolved that T NF-α li kely participates in tissue repair and growth in the aftermath of DTH reaction. This worthwhile finding has been discussed in view of others work. Subsequently, the effect of EEA on expression of *TNF-α* gene was studied by mRNA phenotyping; indeed, EEA induced higher level of expression of the gene.

   TGF-β encodes a growth factor and was found to be upregulated by EEA.This afctor might contribute in the growth process following inflammatory damage to the tissues.

10. Besides TNF-α, the regulatory role of EEA in expression of certain other pro- and anti-inflammatory cytokine genes was also analysed. Interestingly, EEA inhibited the expression of a pro-inflammatory cytokine gene *IL-1β*, but did not influence *IL-6* gene known to encode another pro-inflammatory cytokine. Neither EEA influenced the expression of *IL-10* gene known to participate in anti-inflammatory event. Again EEA acted differentially for expression of *COX1* and *COX2* genes encoding isoforms of cycloxygenase enzyme responsible for producing secondary mediators, leukotrienes and prostaglandins respectively for inflammatory reaction. EEA downregulated only the *COX2* gene.

Thus, genes encoding pro- and anti-inflammatory cytokines and mediators might not function or triggered all at a time.

11. EEA could not influence much the expression of *IKK* gene in relation to the controls indicating inability of EEA in activation of NF-κB pathway for contributing towards inflammation.

12. Topical application of EEA could heal up linear incisions and limited burnt area in skin of mice faster. Upregulation of *TNF-α* and *TGF-β* by EEA has been suggested to be likely reason for faster healing as it was found with repair of tissue following DTH reaction. The finding corroborates with the practice for ethnomedicinal use of the plant to treat skin and mouth sores.

13. Tumor initiation and progression bears similarities with the process of inflammation reaction in certain aspects, such as cytokine production, growth factor medited vascularization and induction of pro-inflammatory mediators that provide favourable environment for proliferation of cells. That is why EEA having anti-inflammatory properties was tested for its effect on tumor growth. EEA infact retarded growth of the solid tumor induced with Ehrlich ascitic carcinoma cells, to some extend at the initial phase and increased the longevity of the tumor bearing mice.

    In addition, EEA also induced cytotoxic differentiation of T lymphocytes capable of mounting lysis of ^{51}Cr-labeled tumor target cells. EEA in fact effectively induced expression of *perforin* gene, encoding the protein monomers in the cytotoxic T cells. Being secreted by cytotoxic T cells, perforin monomers polymerize on the membrane of the tumor target cells to create pores, and leading to the target cell death.

14. Enhanced production of reactive oxygen species (ROS) is hallmark to inflammation and tumor growth. EEA, capable of inhibiting DTH reaction and tumor growth to some extend, effectively quenches the generation of three deleterious ROS – superoxide ($O_2^{\cdot}$), hydrogen peroxide ($H_2O_2^{\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals.

15. EEA induced generation of $NO^{\cdot}$, a messenger molecule, from L-arginine in lymphocytes by activating the enzyme inducible nitric oxide synthase (iNOS). $NO^{\cdot}$ generation was found earlier to be conducive for cytotoxic differentiation of T cells.

The ability of EEA to quench deleterious ROS and induce $NO^{\cdot}$ generation seem to promote lymphocytes for function including cytotoxicity.

16. The anti-oxidant activity of an agent might depend on the presence of electrophile functional groups such as phenolic '-OH' on its structure. Chemical characterization and identification of active component(s) from an extract like EEA is likely to provide better understanding of its functional mechanism. Initial fractionation and TLC separation of the total extract revealed a major UV-active band and was identified by HPLC peak at 268 nm. IR spectroscopic analysis revealed the fraction to have hydroxyl and carbonyl functional groups and further analyses with NMR and mass spectroscopy suggested terpenoid nature of the fraction.

17. Comparison of biological effects such as blastogenesis and quenching of free radicals revealed the total EEA extract perform better than the major fraction (isolated through column chromatography). This could due to the presence of certain other minor components got lost in course of the purification of the major fraction.

18. Expression of genes related to anti-inflammatory and anti-tumor function under the influence of a herbal agent can be taken into consideration to screen for an effective agent from the herbl reservoir.