Summary
In the present study we evaluated the inhibitory effect of selected plant natural products on the breast cancer development and progression along with an analysis of the effect of these products on other allied aspects of tumour development. For the study on metastasis we selected the syngenic mouse 4T1 breast tumour model and the plant isolates used were punarnavine, harmine and an active fraction from the plant *Emilia sonchifolia* (γ-hum) enriched with the major sesquiterpene γ-humulene (γ-hum). From the results of the study it was clearly evident that punarnavine the quinolizidine alkaloid, isolated from the plant *Boerhaavia diffusa* significantly inhibited the primary tumour growth in the orthotropic site of induction and increased the life span of animals in the treated groups. The organ specific metastatic progression of tumour from the primary site to the distant organs via the lymphatic system was efficiently reduced by the punarnavine administration. The assessment of biochemical parameters and the analysis of inflammatory cytokines along with a gene expression study of the major genes involved in the metastatic progression undeniably proved the potential of punarnavine in combating breast tumour development and progression compared to the tumour control and the other two plant isolates used in the study.

When we tried to analyse whether this potent alkaloid punarnavine can be used in conventional radiotherapy the results obtained were quite promising. Here we find out the efficacy of punarnavine in down regulating the expression of the hypoxia inducible factor α that contribute immensely to the survival of hypoxic tumour cells and their retrieved growth and tumour development even after an episode of radiation therapy. The hypoxia induced vascularisation via the vascular endothelial growth factor was also significantly inhibited by the punarnavine administration along with reduced tumour vasculature as evident from the immunohistochemical analysis. The effect of punarnavine administration on the tumour development in X irradiated animals resulted with a high level of reduction in subsequent tumour development in the primary site of tumour induction after different doses of radiation exposure. Further the treatment efficiently inhibited the invasion of irradiated cells with negatively influencing the activation of matrix metalloproteinases and thereby inhibiting its effects on influencing the invasive capacity of surviving tumour cells. These
experiments for evaluating the potential of the usage of punarnavine in conventional radiotherapy using X irradiation in 4T1 tumour bearing mice once again proved the efficacy of this potent compound in the combinational therapeutic applications in conventional treatments.

The studies on the effect of the plant natural products on the other allied aspects of tumour growth and development revealed the effects of these isolates on the immune, inflammatory and angiogenic systems. The plant *E. sonchifolia* found to enhance the immune parameters, trigger stem cell proliferation as well as differentiation, and heighten antibody responses in a well-regulated way, which may be specifically mediated through various cytokine molecules. This immunomodulatory activity of the plant involved the combined action of humoral and cell-mediated immune responses as proven by the proliferation assay. Besides, the extract delivers enhanced CTL activity in tumour-bearing animals, which again signifies the augmented involvement of a cell-mediated immune system to defend against the tumour. Hence, it can be stated that the plant could definitely act as a nontoxic immunomodulator. As an immunomodulator, *E. sonchifolia* does not tend to overboost immunity but rather provides a stimulated and optimized immune response compared to the untreated group of animals. These results may be considered as a solid scientific evidence for its conventional and traditional medicinal uses for a number of ailments by regulating the body’s defense system against pathological manifestations.

The active fraction from *E. sonchifolia* containing the major sesquiterpene γ-humulene found to be quite promising with its anti-inflammatory, antiangiogenic and applications in conventional chemotherapy. The anti-inflammatory effects of γ-hum was evident with a reduction in the paw oedema induced by acute and chronic inflammatory agents. The lipopolysaccharide induced inflammatory response was also significantly reduced by the treatment as evident from the levels of proinflammatory cytokine, C-reactive protein and nitric oxide levels. Gene expression analysis of the cyclooxygenase (COX-2) and inducible nitric oxide (iNOS) also showed the effect of γ-hum in efficiently down regulating the genes involved in the process.
Antiangiogenic therapy that targets vascular growth within the tumor is now widely accepted to treat various tumors, because the agents used in this treatment modality have fewer side effects due to the quiescent nature of the blood vessels in adults. The present study clearly proved the inhibitory effect of \( \gamma \)-hum on tumor angiogenesis by efficiently decreasing MMPs, VEGF, and proinflammatory cytokines while at the same time increasing the level of TIMP. The retarding effect of \( \gamma \)-hum was clear-cut from the decreased capillary formation and prevention of microvessel outgrowth from the aorta. Additional evidence for the inhibitory effect of \( \gamma \)-hum is the impediment on endothelial cell proliferation, invasion, migration, and also hampering of the activation of proenzyme to active enzyme as evident by the gelatin zymographic analysis.

Harmful metabolites will be accumulated in the bladder than in any other areas and that makes this storage organ the most susceptible for the toxic effects of therapeutics. In this scenario we analysed the effect of \( \gamma \)-hum on the conventional chemotherapy using cyclophosphamide, the oxazaphosphorine cytostatic drug. The results obtained altogether proved the safeguarding effect of \( \gamma \)-hum in an in vivo experimental mice model. Current study revealed the protective effects of \( \gamma \)-hum by implicating reduced levels of urea nitrogen, total protein, creatinine and lipid peroxidation to almost normal. Revamping of GSH level, cellular antioxidants and marker cytokine levels towards positive amelioration. These results points towards the usage of \( \gamma \)-hum in combination for alleviating the toxic side effects of conventional chemotherapy.