Abstract

Breast cancer is the topmost malignancy found in women and is responsible for most of cancer-related deaths in women worldwide. The foremost challenges are resistance to the drug and its metastatic characteristics which, accounts more than 90% of cancer related deaths. The physiological conditions leading to the origin, metastasis and relapse of cancers is still not fully understood, making its prevention, diagnosis and treatment a difficult task. Therefore, to curb the cancer associated mortality, the quintessential requirement is a drug with specificity towards cancerous cells and less side effects resulting in improved cancer survival rate. The tumor microenvironment of the solid tumor including breast cancer also had high level of TNF-α which promote the tumorigenesis. Breast cancer cells in high TNF-α conditions inflammatory breast carcinoma show correlation with tumor grade and nodal metastasis. Further, TNF-α regulates the metastatic behavior of breast cancer cells including MCF-7 and MDA-MB-231 breast cancer cells. There is evidence that indicates that TNF-α is involved in the transformation, proliferation, angiogenesis, invasion and metastasis of many cancers. TNF-α has emerged as a key cytokine, whose chronic levels influences cellular metabolism by increasing ROS generation and affecting mitochondrial structure and function. However, the role of TNF-α induced signalling, immune response and mitochondrial metabolism in controlling the cancer pathogenicity is not well known. Mitochondria is known for its major role in energy metabolism, however there are evidences which strongly demonstrates its involvement in inflammatory and cell death pathways.

There are limited therapeutic options available for the breast cancer patients and intense efforts are being made to explore phytocomponents present in various medicinal plants for different types of cancer, including breast cancer. The current study focuses on a medicinal plant, *Bauhinia variegata* L. which is known for plethora of potentials including anti-oxidant, anti-inflammatory and anti-cancer properties. The molecular mechanisms of aqueous extract of *Bauhinia variegata* leaves induced cell death in the breast cancer cell lines have not been explored. In the present study, *Bauhinia variegata* leaves extracts were explored for their anti-tumorigenic potential on hormonally distinct breast cancer cell lines, ER/PR +ve, Luminal subtype MCF-7 and ER/PR -ve, Her2 -ve, Basal subtype MDA-MB-231. Two breast cancer cell lines (with difference

in molecular characteristics) MCF-7 and MDA-MB-231 were used in this study with the purpose better understanding of their differential response to extract and phytocomponents from *Bauhinia variegata* leaves. The crude aqueous extract displayed high level of cytotoxicity. Different fractions obtained from aqueous extract by Flash chromatography were also possessed cytotoxic potential against breast cancer cell lines MCF-7 and MDA-MB-231. HRLC-MS of aqueous extract of *Bauhinia variegata* leaves showed presence of three anti-cancer phytocomponents, two alkaloids: Berbamine and Papaverine; and a stilbene glycoside, Rhapontin.

Systematic investigation of aqueous extract of *Bauhinia variegata* leaves on cell proliferation, cell migration, cell invasion, and mitochondrial function and cell death in the presence of TNF-α on human breast cancer cells lines was performed. The aqueous extract of *Bauhinia variegata* leaves shows anti-proliferative, anti-migratory and anti-invasive effect against MCF-7 and MDA-MB-231 cells. The aqueous extract of *Bauhinia variegata* leaves could restrict the cell invasion in MCF-7 spheroids (3D) even in the presence of TNF-α and Estradiol. Thus, the phytocomponents present in the aqueous extract of *Bauhinia variegata* leaves may modulate TNF-α regulated cell migration and invasive properties. The aqueous extract was explored for their role in modulation of TNF-α-regulated mitochondrial function and regulation of cell death. The aqueous extract altered ROS levels, decrease the mitochondrial membrane potential and cause necroptosis type of cell death. The anti-cancerous property of the extract might be due to synergistic effects of these compounds or either individual effect along with other phytocomponents with different levels of bioactivity.

To our knowledge till date this is the first study showing inhibitory effect of aqueous extract of *Bauhinia variegata* leaves on cell proliferation and cell migration in presence of TNF- α and estradiol on breast cancer cell lines and spheroids. Thus, *B. variegata L.* having rich repertoire of bioactives can be a good source for anti-cancer phytocomponents which can be explored for pharmaceutical development of drugs for therapy against breast cancer.