ABSTRACT

Inflammatory diseases, involving multiple processes mediated by activated immune cells are becoming common throughout the world. Epidemiological and recent studies have indicated chronic infections and inflammation as a major risk factor in various types of cancer and many of the cancer deaths are linked to underlying infections and inflammatory reactions. Breast cancer is the common malignant neoplasm and is the second major cause of cancer-related death in women world-wide.

Pleiotropic agents, such as phytoprinciples have been reported to exhibit significant impact on breast cancer prevention and/or therapy by targeting both receptor positive and negative cancer cells. A lower incidence of breast cancer has been observed with high consumption of phytoestrogens, which are biologically active plant-derived phenolic compounds structurally mimicking the mammalian estrogen, 17-β Estradiol. Pharmacologically, *Alpinia officinarum* (lesser galangal) belonging to Zingibeeaceae family possess a very complex mixture of compounds such as gingerols, caffeic acid and curcumin. Diarylheptanoids (DAHs), are naturally occurring phytochemicals in the rhizome part of *Alpinia officinarum* and are known to exhibit multifunctional bioactivity for various diseases. DAHs are used as traditional herbs for relieving stomach ache, treating colds, invigorating the circulatory system, and for reducing swelling in China.
In the current study, the rhizome of *Alpinia officinarum* was subjected to sequential extraction with solvents ranging from non-polar to polar. The *Alpinia officinarum* hexane extract (AOHE) exhibited 85% anti-proliferative potential in comparison to the other extracts. Fractionation and purification of the AOHE led to the isolation of a pure molecule. Structural characterization of the molecule using NMR, Mass spectroscopy led to the identification of the pure bioactive molecule as Diarylheptanoid (DAH).

Unraveling the molecular mechanism of action of the DAH was attempted using MCF-7 (ER +ve), MDA-MB-231 (ER-ve) breast cancer cells and RAW264.7 cells. Estrogen receptor binding assay and Estrogen induced cell proliferation assay in MCF-7 cells showed an anti-estrogenic activity of AOHE and DAH. A downregulation in the expression of the pS2 in comparison to ER antagonist ICI182780 confirmed the anti-estrogenic potential of AOHE and DAH. Docking analysis clearly suggested binding of DAH with the estrogen receptor in comparison to ER antagonist tamoxifen.

The transcription factor NF-κB is involved in regulation of genes responsible for cell proliferation and survival. In this study, an overexpression of IκBα and downregulation of NF-κB was observed confirming the inhibition of NF-κB activation by AOHE and DAH. TNFα induced NF-κB reporter gene assay revealed an inhibition of NF-κB transcriptional activity in both MCF-7 and MDA-MB-231 breast cancer cells. A downregulation in the expression of NF-κB signaling genes such as Akt, PI3K, COX2, iNOS and cyclin D1 were observed confirming the inactivation of NF-κB signaling.
Since deregulated NF-κB expression is also a characteristic phenomenon in several inflammatory diseases, the anti-inflammatory effect of AOHE and DAH was investigated in Lipopolysaccharide (LPS) induced RAW264.7 macrophages. Release of nitric oxide, an indicator of inflammation process, measured by the Griess reaction, showed the anti-inflammatory potential of AOHE and DAH. Cell cytotoxicity, evaluated by MTT assay in PBMCs and RAW264.7 cells showed no toxicity. TNFα induced NF-κB reporter gene assay revealed an inhibition in the transcriptional activity of NF-κB in RAW264.7 cells.

Lipopolysaccharide stimulated macrophages generate a variety of inflammatory mediators, such as nitric oxide, IL-1β, TNF-α. Other members of TNFα superfamily such as IL-6 and IL-8, COX-2 and iNOS also contribute to systemic inflammatory response and tumorigensis, regulated by activation of NF-κB. An upregulation in the protein level expression of IκBα and a down regulation of NF-κB, was observed upon treatment with AOHE and DAH indicating an inhibition in the translocation of NF-κB to the nucleus due to degradation of I kappa Bα (IκBα). A downregulation of the NF-κB signaling markers such as IL-8, TNF-α, iNOS and COX-2 were observed, with a decrease in NO production confirming the anti-inflammatory property of AOHE and DAH.

Cyclin D1 an NF-κB-regulated gene, is overexpressed in breast cancer and is observed to play a significant role in cell proliferation through activation of cyclin-dependent kinases. Eukaryotic cell cycle progression
involves the sequential activation of cyclin-dependent kinases, whose activation is dependent upon their association with cyclins. In this study, FACS analysis showed an induction in cell cycle arrest at G0 phase in MCF-7 and an S-phase arrest in MDA-MB-231 cells at 48 h respectively. Cell cycle progression is also regulated by a relative balance between the cellular concentrations of CDK inhibitors, including P27/KIP and p21/WAF1. Thus, anticancer agents may alter regulation of cell cycle machinery, resulting in the arrest of cells in different phases, thereby reducing the growth and proliferation of cells, even inducing apoptosis in cancerous cells. Hence, cell cycle regulatory markers such as CDKs, cyclins and CDK inhibitors related to G0 and S-phases were analysed by western blot analysis. The results suggested a downregulation of CDKs, cyclins whereas, an upregulation in the CDKs inhibitors was observed confirming the cell cycle arrest in both receptor positive and receptor negative breast cancer cells. To summarize, DAH isolated from *Alpinia officinarum* through bioactivity based column chromatography was observed to exhibit anti-proliferative, anti-estrogenic and anti-inflammatory activity inhibiting NF-κB transcriptional activity and inducing cell cycle arrest in breast cancer cells leading to the conclusion that DAH can act as a therapeutic modality for breast cancer treatment.