ABSTRACT

Title of the Thesis: SCREENING AND ISOLATION OF AN IMMUNOMODULATORY COMPOUND FROM PRUNUS-CERASUS FRUIT AND ITS BIOThERAPEUTIC POTENTIAL.

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In the rapidly evolving world of pharmacology, it is a continuing challenge to design a therapy which is both effective and has high specificity for the biology of diseases like cancer/ and immune related-disorders. The impact and the potential for immunologically–derived targets is becoming more and more evident, hence raising an interest in developing immunotherapeutic tools. A number of fruits used in the traditional medical system of remedies in India, have been shown to possess chemotherapeutic activities and emerging science shows that sour cherries (Prunus cerasus L.) is one among them. Sour cherry is a medicinal fruit claimed to possess number of therapeutic properties. In the present study fifteen extracts prepared from various parts of Prunus cerasus L., were screened for in vitro immunomodulatory potential employing in vitro tests: Nitroblue Tetrazolium Reduction test (NBT), Inducible Nitric Oxide Synthase test iNOS), Bactericidal activity (Phagocytosis) and lymphocyte proliferation assay. The extracts showed a significant difference in the immunomodulatory activities according to the parts of sour cherry plant. The Pc-methanolic fruit extract (PcMFE) showed the maximum immunopotential and hence was selected for further studies. The PcMFE was subjected to fractionation by partitioning the crude extract into polar aqueous (AQFR) and non-polar ethyl acetate (EAFR) fractions. In order to evaluate the role of PcMFE and its immunopotent ethyl acetate fraction (EAFR) in the modulation of immune responses, detailed studies were carried out using a panel of in vivo assays to see their effects on both humoral and cell mediated immune responses in SRBC immunized/treated BALB/c mice. Oral administration of PcMFE and its selected EAFR at different doses stimulated the IgM and IgG titre expressed in the form of haemagglutination antibody (HA) titre. Further, PcMFE and EAFR elicited a dose related increase in the delayed type
hypersensitivity reaction (DTH) after 24 and 48 h and enhanced macrophage activation. Besides augmenting the humoral and cell mediated immune response, the concentration of cytokines (IFN-γ, IL-4, and TNF-α) in serum with respect to T cell interactions, i.e. the proliferation of lymphocytes were significantly increased.

Bioactivity guided fractionation/chemical investigation of EAFR of PcMFE resulted in the isolation of four compounds, identified as quercetin, daidzin, rutin and chlorogenic acid. The in vitro immunomodulatory characterisation of four isolated molecules at different concentrations indicated that the marker molecules exerted significant but specific modulating effects on the functional ability of macrophages and lymphocytes in variable manner. Out of the four tested molecules, daidzin and chlorogenic acid showed immunostimulatory activity whereas quercetin and rutin were immunosuppressive in nature. Based on the in vitro data, daidzin showed the maximum immunomodulatory potential and hence was selected for the in vivo immunomodulatory studies to elucidate and characterize the role of daidzin in the immunostimulatory nature of the PcMFE and its EAFR as well. The results of the in vivo study indicated that daidzin induced a significant immunostimulatory effect on all tested immunological parameters as was evident by increased humoral and cell mediated immune responses, improved macrophage functional status and enhanced cytokine expression profile in SRBC immunized treated animals.

PcMFE and its isolated molecules were assessed for cytotoxic efficacy against five different cancer cell lines (A-549, THP-1, MCF-7, PC-3 and NCI-H322) by MTT assay. Results showed that PcMFE and one of its chemical constituents: quercetin showed significant inhibitory activity against almost all tested cell lines with maximum effect being observed against Lung cancer cell line NCI-H322. The in vivo anticancer activity of PcMFE and two selected bioactive molecules i.e. quercetin and daidzin was tested in EAC tumor mouse models. PcMFE treatment as well as quercetin showed maximum tumor growth inhibition in EAC bearing mice as compared to Daidzin. It is concluded that PcMFE played an important role in the modulation of the immune response as well as exhibited significant anticancer activity and thus may have applications as an effective immunotherapeutic agent. The isolated compounds: Daidzin can be employed as an immunomodulator whereas quercetin though immunosuppressive can be used in anticancer therapeutics.