Executive Summary of the Project entitled

Evaluation of the anticancer effects of the neem limonoids azadirachtin and nimbolide in hepg2 cells *in vitro* and in rat hepatocarcinogenesis model *in vivo*

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Hepatocellular carcinoma (HCC), is the fifth most widespread cancer and the third most leading cause of cancer-related mortality worldwide. Various etiological factors have been linked to HCC development, particularly hepatitis B or C (HBV, HCV) viral infections, alcohol abuse and aflatoxin B1 (AFB1) exposure. Medicinal plants rich in antioxidant phytochemicals particularly azadirachtin and nimbolide have attracted the focus of attention as potential chemopreventive agents. This study was designed to evaluate the protective effects of azadirachtin and nimbolide by analyzing a panel of markers involved in HCC progression in HepG2 cells *in vitro* and in the DAB-induced rat hepatocarcinogenesis model *in vivo*.

In vitro studies

The results of the present study provide evidence for the antiproliferative, antioxidant, anti-invasive, anti-angiogenic and apoptosis inducing potential of azadirachtin and nimbolide. The dose-dependent suppression of viability of HepG2 cells, together with the modulation of NF-κB and Wnt/β-catenin signaling pathway by azadirachtin (161 μM) and nimbolide (5 μM) underscore the growth inhibitory potential of these neem limonoids. In addition, both these compounds inhibited lipid peroxidation and enhanced glutathione-dependent antioxidants. Elucidation of the molecular mechanism underlying the cytotoxicity of these neem limonoids revealed apoptosis as the mode of cell death as evidenced by chromatin condensation, DNA fragmentation, depolarization of mitochondrial transmembrane potential, and appearance of subdiploid peak. The increase in pro-apoptotic Bax, apoptogenic molecules and caspases with decrease in the anti-apoptotic Bcl-2 and survivin also confirmed apoptosis induction by azadirachtin and nimbolide. In addition to genetic modifications, inhibition of DNMT-1 and HDACs by these limonoids revealed the association of epigenetic alterations in cancer and also the potential anticancer effects of HDAC inhibitors. Furthermore, nimbolide exerts protective effect against CHO cells deficient in BER and HR but does not exhibit protective effect against the toxicity induced by DBPDE and this might be due to the ability of nimbolide to induce cell death via apoptosis.

In vivo studies

Dietary administration of DAB induced well-differentiated HCC with increase in CYP450, GST and decreased QR activities together with enhanced lipid and protein oxidation, compromised antioxidant defense enzymes. DAB-induced HCC displayed aberrant NF-κB signaling accompanied by increased Bcl-2/Bax ratio and survivin with downregulation in the expression of the apoptogenic molecules Smac/diablo, cytochrome C and caspases indicating apoptosis evasion. Increased expression of MMP-2, MMP-9, VEGF and HIF-1α provided evidence for a pro-invasive and an angiogenic phenotype. In addition, DAB-induced hepatomas also exhibited aberrant HDAC-1 and DNMT-1 expressions. Intragastric administration of nimbolide effectively suppressed DAB-induced hepatocarcinogenesis as evidenced by reduced preneoplastic and neoplastic lesions, modulation of XME, amelioration of oxidative stress, inhibition of cell proliferation, invasion, and angiogenesis, and induction of apoptosis. Nimbolide exerted antiproliferative effects by inhibiting NF-κB signaling and inducing intrinsic apoptosis by modulating the expression of numerous molecules that endorses caspase-mediated cell death. Nimbolide also modulated the key molecules involved in invasion and angiogenesis. Moreover, nimbolide also inhibited the expression of HDAC-1 and DNMT-1 suggesting reactivation of epigenetically silenced genes. Thus the results of our study demonstrate that both azadirachtin and nimbolide are promising candidates for chemoprevention.