ABSTRACT

A lectin was purified from *Morus alba* L. leaves by a two step chromatographic procedure namely, immobilized metal ion affinity chromatography and convective interaction media-based anion exchange chromatography. The purified mulberry leaf lectin (MLL - with a molecular weight of ~56 kDa) was specific towards galactose, galactosamine and N-acetyl galactosamine. The lectin retained agglutination activity up to 40°C and was independent of pH above 6.0. Hemagglutination activity of purified MLL was not dependent on any metal ions. However, with high concentration of trivalent metal ions, Fe$^{3+}$ and Al$^{3+}$ and the divalent metal ion Fe$^{2+}$, a threefold increase in agglutination activity was observed. The purified MLL showed an anticancer activity towards human breast cancer cells (MCF - 7) and colon cancer cells (HCT - 15) with a higher potency towards MCF - 7 cells.

Apoptotic cell death was induced when MCF - 7 and HCT - 15 were treated with GI$_{50}$ concentration (concentration of lectin required for 50% inhibition of cell growth - 8.5 µg/ml for MCF - 7 and 16 µg/ml for HCT - 15) of MLL for 24 h. The induction of apoptosis was studied by morphological analysis and DNA fragmentation. Cells that stained positive for annexin V and acridine orange/ethidium bromide indicated apoptosis induction by MLL. Up-regulation of caspase 3 activity was also found in HCT - 15 cells treated with MLL. Flow cytometry analysis showed an increase in the percentage of cells in sub G0-G1 phase confirming MLL induced apoptosis.

The *in vitro* anticancer activity of the methanol extract of *M. alba* L. was also studied. The extract induced apoptosis in breast cancer (MCF - 7) cells (IC$_{50}$ - 9.23 µg/ml) and human colon cancer (HCT - 15) cells (IC$_{50}$ - 13.5 µg/ml), resulting in significant morphological changes, fragmentation of DNA and caspase 3 activation. It down-regulated the amount of nitric oxide (NO) produced as a result of inducible nitric oxide synthase (iNOS) activation. The reverse phase - high performance liquid chromatography (RP - HPLC) analysis of the extract showed presence of some major and minor components in the extract that could have contributed to the antioxidant and anticancer effects.