WNRF Technical Bulletin : 05

Antiproliferative activity of *Morinda citrifolia* L. on human retinoblastoma

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Published by	Edited by	Author (s)	Year of Publication
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Citation :
Mathivanan, N., Krishna Kumar. S and Surandiran. G. 2010. Antiproliferative activity of *Morinda citrifolia*. L., WNRF Technical Bulletin - 05, World Noni Research Foundation, University of Madras and Sankara Nethralaya, Chennai, p.36

Summary and Conclusion

The antiproliferative activity of *Morinda citrifolia* L. against human retinoblastoma Y79 cell line (children eye cancer) was evaluated in the present study. The IC50 of *M. citrifolia* fruit extract as determined by MTT assay was 0.8 mg/ml. *M. citrifolia* induced apoptosis in RB Y79 cells was visualized by light microscopy, which showed cell shrinkage, membrane blebbing and granule formation. Annexin V FITC staining showed translocation of phosphatidyl serine (PS) from the inner surface of plasma membrane to the cell surface, which also confirmed the apoptosis induce by *M. citrifolia* fruit extract. The apoptosis was further confirmed by analysis of DNA fragmentation using agarose gel electrophoresis. The apoptosis induced by fruit extract of M. citrifolia in RB Y79 cells was caspase-3 dependent, which was further confirmed by RTPCR analysis. *M. citrifolia* fruit extract treatment could inhibit Src activity at 400µg/ml concentration, which indicated the antiproliferative activity. The active principle was isolated from the fruits of *M. citrifolia* and purified by silica gel column chromatography. Among seven fractions obtained, the fraction M2 effectively induced apoptosis in retinoblastoma Y79 cells. Further, the IC50 of purified fraction was determined as 0.5 µg/ml at 48 h of

incubation. This active principle M2 was further identified as scopoletin by various spectral analyses and its structure was elucidated. The genome-wide expression profile (cDNA microarray) of scopoletin treated RB Y79 cells revealed that a total of 1236 genes were up-regulated and 1570 genes were down-regulated. The genes related to apoptosis were significantly up-regulated, which was accompanied by down regulation of genes related to proliferation. The cluster of genes located in chromosome 1 and 12 were up-regulated, and the clusters of genes in certain segments of chromosome 17 were down-regulated. The functional analysis using gene ontology revealed that the genes related to apoptosis, cell cycle, cell death and proteosome pathway were up-regulated and the genes involved in transcription, proliferation, kinase activity, cell cycle and differentiation were down-regulated.

The antiproliferative activity of *Morinda citrifolia* L. against human retinoblastoma Y79 cell line (children eye cancer) was established by MTT assay, light microscopy using Annexin V FITC staining and further confirmed by DNA fragmentation assay. The apoptosis induced by fruit extract of *M. citrifolia* in RB Y79 cells was caspase-3 dependent, which was further confirmed by RTPCR analysis. The active principle, scopoletin isolated from the fruits of *M. citrifolia* significantly induced apoptosis in RB Y79 cells with the IC50 of 0.5 µg/ml. The cDNA microarray of scopoletin treated RB Y79 cells revealed that the genes related to apoptosis were significantly up-regulated, which was accompanied by down regulation of genes related to proliferation. From the results it is concluded that *M. citrifolia* has promising antiproliferative activity against human RB Y79 cells.

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