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PRAPINPAN SUNDARARJUN : INDUCTION OF APOPTOSIS BY THE FLAVONE ISOLATED FROM GARDENIA OBTUSIFOLIA: AN ANTIMITOTIC AGENT. THESIS ADVISORS: KULAWEE SUJARIT Ph.D., SAMAISUKH SOPHASAN Ph.D., SUKATHIDA UBOL Ph.D. 126 p. ISBN 974-664-015-1

Apoptosis, also known as programmed cell death, has been reported to be a major mechanism of action of various anticancer drugs. Recent evidence suggests that inactivation of the antiapoptotic function of Bcl-2 by phosphorylation occurs after treatment with the microtubule-damaging anticancer agents, such as taxol, vinblastine, and colchicine or the phosphatase inhibitor okadaic acid. During the screening of cytotoxic agents, 5,3′- dihydroxy-3,6,7,8,4′-pentamethoxyflavone (DPF) was purified from the extracts of *Gardenia obtusifolia*. This compound showed selective inhibition on the growth of various human cell lines. In addition, the cytotoxic effect of VR-3623 has proved to be due to disrupting microtubule formation. Further, it has been shown that this flavone bound to tubulin at colchicine binding site. Therefore, in the present study, induction of apoptosis by VR-3623 was investigated in the human lung (LU-1) cancer cell line. Treatment of LU-1 cells for 3-72 hours with three doses of DPF, GI_{50} (0.4 μM), $5xGI_{50}$ (2.0 μM), and $10xGI_{50}$ (4.0 μM) or colchicine, $5xGI_{50}$ (0.2 μM) used as a positive control, caused morphological changes characterized as nuclear condensation and apoptotic bodies formation detected by phase contrast microscope and 4′,6-Diamino-2-phenylindole (DAPI) staining consistent with the induction of apoptosis. The percentage of apoptosis induced by DPF was found to be dose-and time-dependent. The peaks of apoptosis seen after an addition of colchicine and DPF, $10xGI_{50}$, for 24 hours were approximately 59% and 51%, respectively. Furthermore, DNA strand breakage and fragmentation of the DNA into oligonucleosome-sized fragments was also clearly demonstrated by agarose gel electrophoresis after exposing cells to colchicine and DPF, $10xGI_{50}$, for 48 hours. Moreover, western blotting and analysis with monoclonal-Bcl-2 antibody demonstrated the expression of the phosphorylated form of Bcl-2 protein seen as the slower-migrating form of Bcl-2 ($\simeq$ 26 kDa) in cells treated with colchicine, $5xGI_{50}$ since 24 hours after incubation, but not in those cells treated with any dose of DPF studied. These data suggest that DPF may inhibit the growth of cancer cells by induction of apoptosis. Apoptosis induced by DPF in LU-1 cells is unlikely caused by phosphorylation of Bcl-2 as found in cells treated with colchicine. Therefore, this antimitotic agent initiates apoptosis by a different mechanism from colchicine. The exact mechanism is still not known. Further investigation is needed to identify this mechanism.