ABSTRACT

The antineoplastic potential of Indigofera tinctoria Linn. was evaluated in various cancer cell lines in-vitro and in-vivo. The pharmacological evaluation of the plant resulted in the isolation of the antitumor agent, indirubin. Indirubin was characterized by NMR and electron-impact mass spectrometry methods. Acute, chronic studies of indirubin revealed that indirubin did not produce any abnormal toxicity at normal doses. The in-vitro cytostatic activity of indirubin on 26 cancer cell lines showed a potent activity on lung, colon, central nervous system and breast cancer cells. Morphological changes of A549 cells was observed after treatment with indirubin identified by propidium iodide and Hoechst staining. A brighter and longer tail in the COMET assay of indirubin showed the higher level of DNA damage exhibited by indirubin. DNA fragmentation assay on HCT116 cells showed the DNA fragmentation as well as the condensation of chromatin and fragmentation of nuclei after 48 hour. Flow cytometry analysis of indirubin in MCF7-cells showed an increase of 10% in population of cells in G1 phase with a concominant decrease in the percentage of cells in the S and G2/M phase of cell cycle compared to the control cells suggesting a cell cycle arrest at G1 phase. Among the array of cell cycle proteins tested CDK4 and CDK6 protein levels were significantly reduced in dose dependent manner in response to indirubin treatment. The proportions of TUNEL-positive cells were strongly increased in TUNEL assay. Cell viability (MCF7-cells) was decreased following treatment with indirubin after 72 hrs. Indirubin induced the activation of pro-caspase-8 into caspase-8, activation of pro-caspase-9 into caspase-9 and cleavage of pro-caspase-3. Lactate dehydrogenase (LDH), Aspartate amino transferase (AST), Alkaline phosphatase (ALP) and Alanine amino transferase
(ALT) could be used as tumor markers in the management of malignancy of the head, neck, breast and the uterine cervix. There was no significant increase in enzyme level of ALT, AST, LDH where as ALP showed significant increase indicating onset of breast cancer but enzyme level reduced once treated with indirubin at a dose level of 100mg/kg bt.wt. The *in-vivo* studies of indirubin on animals carrying the MCF7 and NCI-H460 cells showed a marked reduction in the growth of tumor and the effective dose was found to be 100 mg/kg and there is no body weight loss.

Key words: *Indigofera tinctoria* Linn.; Indirubin