

Pro-apoptotic activity of the phytochemical Indicaxanthin on colorectal carcinoma cells (Caco-2) and epigenetic CpG demethylation of the promoter and reactivation of the expression of p16

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Phytochemicals play prominent roles in human diet and nutrition as protective redox-active substances in prevention of several disorders and chronic diseases in humans. Today, their function as potent modulators of the mammalian epigenome-regulated gene expression is rapidly emerging. In the present study antiproliferative effects of Indicaxanthin (Ind) from the fruits of *Opuntia ficus-indica* (1), and potential influence on DNA methylation has been investigated on Caco-2 cells, a human cell line of colorectal carcinoma. Ind caused a clear dose- and time-dependent decrease of the cell proliferation ($IC_{(50)} 50 \mu\text{M}$) associated to apoptosis as demonstrated by phosphatidylserine externalization and depolarization of mitochondrial membrane. Ind decreased the G₀-G₁ phase whereas increased S and G₂-M phases of the cell cycle. The phytochemical did not alter the intracellular ROS levels but decreased the $[Ca^{2+}]_i$. Investigation on DNA methylation using MESAP-PCR (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction) (2), showed that 100 μM Ind induced a slight global demethylation after a 48 h treatment. Analysis of epigenetic changes in the DNA methylation pattern at CpG promoter of p16 (INK4a), using MSRE (Methylation-Sensitive Restriction Endonucleases Multiplex-Polymerase Chain Reaction), showed that Ind caused CpG demethylation. Western blotting analysis carried out with p16 monoclonal antibody, confirmed the reactivation of the protein expression. Present data, suggesting that a long-term exposure to indicaxanthin in diet might potentially affect epigenetic machines of the intestinal cells, preventing or repairing initial derangements/disorders, encourage studies on the mechanism involved.

1. Livrea M.A and Tesoriere L. in *Herbal and Traditional Medicine*, 537-556, (eds. Parker L., Ong C, Halliwell B) Marcel Dekker
2. Caradonna F, Barbata G, Sciandrello G (2000). Nova Science Publishers, Inc. New York USA 4th quarter, ISBN.1-60021-875-1.