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Title: Differential control of growth, apoptotic activity and gene expression in human colon cancer cells by extracts derived from medicinal herbs, Rhazya stricta and Zingiber officinale and their combination

Author(s): Elkady, AI (Elkady, Ayman I.); Hussein, RAEH (Hussein, Rania Abd El Hamid); Abu-Zinadah, OA (Abu-Zinadah, Osama A.)

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Abstract: AIM: To investigate the effects of extracts from Rhazya stricta (R. stricta) and Zingiber officinale (Z. officinale) on human colorectal cancer cells.
METHODS: Human colorectal cancer cells (HCT116) were subjected to increasing doses of crude alkaloid extracts from R. stricta (CAERS) and crude flavonoid extracts from Z. officinale (CFEZO). Cells were then harvested after 24, 48 or 72 h and cell viability was examined by trypan blue exclusion dye test; clonogenicity and soft agar colony-forming assays were also carried out. Nuclear stain (Hoechst 33342), acridine orange/ethidium bromide double staining, agarose gel electrophoresis and comet assays were performed to assess pro-apoptotic potentiality of the extracts. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR), using gene-specific primers and Western blot analyses were performed to assess the impact of CAERS and CFEZO on the expression levels of key regulatory proteins in HCT116 cells.
RESULTS: Treatment with a combination of CAERS and CFEZO synergistically suppressed the proliferation, colony formation and anchorage-independent growth of HCT116 cells. Calculated IC50, after 24, 48 and 72 h, were 70, 90 and 130 mu g/mL for CAERS, 65, 85 and 120 mu g/mL for CFEZO and 20, 25 and 45 mu g/mL for both agents, respectively. CAERS- and CFEZO-treated cells exhibited morphologic and biochemical features of apoptotic cell death. The induction of apoptosis was associated with the release of mitochondrial cytochrome c, an increase in the Bax/Bcl-2 ratio, activation of caspases 3 and 9 and cleavage of poly ADP-ribose polymerase. CAERS and CFEZO treatments downregulated expression levels of anti-apoptotic proteins including Bcl-2, Bcl-X, Mcl-1, survivin and XIAP, and upregulated expression levels of proapoptotic proteins such as Bad and Noxa. CAERS and CFEZO treatments elevated expression levels of the oncosuppressor proteins, p53, p21 and p27, and reduced levels of the oncoproteins, cyclin D1, cyclin/cyclin-dependent kinase-4 and c-Myc.
CONCLUSION: These data suggest that a combination of CAERS and CFEZO is a promising treatment for the prevention of colon cancer. (C) 2014 Baishideng Publishing Group Inc. All rights reserved.

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Addresses: [Elkady, Ayman I.; Abu-Zinadah, Osama A.] King Abdulaziz Univ, Dept Biol Sci, Fac Sci, Jeddah 21589, Saudi Arabia.
[Elkady, Ayman I.] Univ Alexandria, Fac Sci, Dept Zool, Alexandria, Egypt.
[Hussein, Rania Abd El Hamid] King Abdulaziz Univ, Dept Clin Nutr, Fac Appl Med Sci, Jeddah 21589, Saudi Arabia.

Reprint Address: Abu-Zinadah, OA (reprint author), King Abdulaziz Univ, Dept Biol Sci, Fac Sci, Jeddah 21589, Saudi Arabia.

E-mail Addresses: oaboznada@kau.edu.sa

Author Identifiers:

Author	ResearcherID Number	ORCID Number
Fac Sci, KAU, Biol Sci Dept	L-4228-2013	

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