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Combination Therapy of 5-Fluorouracil and 4′-Bromoflavone Enhances Cytotoxicity Through Modulation of Apoptotic Genes in HCT-116 Colorectal Cancer Cells

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Colorectal cancer remains a leading cause of cancer-related mortality worldwide, with current therapies often limited by toxicity and drug resistance. This study evaluates the potential of combining 4′-Bromoflavone (4-BF), a flavonoid with reported anticancer activity, with 5-fluorouracil (5-FU), a standard chemotherapeutic agent, to improve therapeutic efficacy and reduce side effects in colorectal cancer treatment. HCT-116 colorectal cancer cells were treated with 5-FU and 4-BF individually and in combination across a concentration gradient, and cytotoxicity was assessed using an MTT assay. The Combination Index and Dose Reduction Index (DRI) were calculated using CompuSyn software, while isobologram analysis and synergy determination were performed with Combenefit. The synergy score was further evaluated using SynergyFinder 2.0. Apoptotic features were analysed via acridine orange/propidium iodide (AO/PI) double staining and Annexin V flow cytometry. Additionally, gene expression analysis of key apoptotic markers was performed to assess the regulation of pro- and anti-apoptotic genes. Combined treatment of 4-BF and 5-FU produced pronounced synergistic cytotoxicity, evidenced by significantly reduced IC50 values and Combination Index values consistently below 1, allowing a three-fold reduction in the effective dose of 5-FU. Apoptosis was the primary mode of cell death, with limited necrosis, as confirmed by Annexin V-FITC staining and increased caspase activity. Gene expression analysis further demonstrated downregulation of anti-apoptotic genes and upregulation of pro- apoptotic genes. These results indicate that 4-BF enhances 5-FU efficacy in colorectal cancer cells by promoting apoptosis and reducing necrosis, suggesting this combination as a potentially safer and more effective therapeutic strategy.

Keywords: Colorectal cancer, Combination index, Dose reduction index, Synergistic effect,4-Bromoflavone; 5-Fluorouracil

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